The Marine Nitrogen Cycle

Marine nitrogen fixation is a key focus for researchers studying ocean biogeochemical cycles and biological diversity

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Nitrogen fixation is an integral component of the global nitrogen cycle, providing the link between the largest reservoir of nitrogen on earth, gaseous N₂, and the pool of biologically fixed nitrogen. The first suggestions that nitrogen could be biologically “fixed” occurred in the early 1800s with the realization that some types of leguminous plants thrive in nitrogen-poor soils. Researchers later identified the rhizobial symbionts that are responsible for this activity.

In the late 1800s, marine researchers from Kiel, Germany, were cognizant that this terrestrial work with leguminous plant roots had identified the biochemical pathway whereby N₂ gas becomes fixed and enters the biological nitrogen cycle. Their own research suggested that nitrogen availability can limit phytoplankton growth in the ocean and that nitrogen available to such marine species derives in part from runoff from land sources, and therefore from terrestrial nitrogen fixation (Fig. 1). Furthermore, the complementary process of denitrification appeared to take place in oceans, returning some of that fixed nitrogen to the gaseous reservoir. Nitrogen fixation was not then considered a major process in the oceans.

However, as research on macronutrients in the sea gained momentum in the early 20th century, patterns emerged from the accumulating data. Alfred Redfield from Woods Hole Oceanographic Institution in Woods Hole, Mass., astutely observed in 1934 that the ratio of nitrogen and phosphorus in marine plankton was strikingly similar to their ratio in the deep sea. Phytoplankton in the euphotic zone, the upper, sunlit layer of the oceans where photosynthesis occurs, produce biomass with an average stoichiometry of carbon to nitrogen to phosphorus of 106:16:1. This value is now referred to as the Redfield ratio. He also concluded that the biology of the ocean dictates those ratios, noting that the biomass that forms in the upper layers of the sea sinks into the deep where organically bound forms regenerate and are released.

Redfield also helped frame a great and continuing debate over what is the primary limiting nutrient in the sea. He and his contemporaries knew that marine phosphorus largely derives from weathering and runoff from terrestrial sources, that there are no de novo sources of phosphorus in the sea, and that phosphorus entering the sea from land ultimately is removed to deep sea sediments.

With advice from G. E. Hutchinson of Yale University, who worked on lakes, Redfield rea-

Summary
• Early during the 20th century, Alfred Redfield determined that marine phytoplankton help to set the ratio of nitrogen and phosphorus in the deep sea; he also framed a still-unsettled debate over which one of them is the primary limiting nutrient in the sea.
• Many marine diazotrophs, including long-recognized *Trichodesmium* and also more recently identified diazotrophs that are smaller than 10 μm, are globally important contributors to nitrogen input.
• Limiting nutrients for nitrogen fixers appear to differ in different ocean regions and depend on the relative supply of iron versus phosphorus.
• Some experts believe that there are feedback processes that maintain overall fixed global nitrogen within relatively strict limits.

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soned that in situ biological nitrogen fixation could make up for any excess of phosphorus over nitrogen to meet the stoichiometric requirement for phytoplankton growth. Hence, he concluded, the ultimate limiting nutrient for primary marine production is phosphorus. Nonetheless, quantitative work on the biogeochemical importance of nitrogen fixation had to wait until the early 1960s and the advent of biochemical and geological techniques to sensitively quantify and detect this process in marine environments.

New Nitrogen-Fixing Players

The earliest research on marine nitrogen fixers ironically occurred without the scientists realizing that they were indeed studying diazotrophs. For example, the German biologist Christian Ehrenberg in 1830 identified the filamentous cyanobacterium, *Trichodesmium erythraeum*, growing with characteristic red surface blooms in the Red Sea.

*Trichodesmium*, which occurs in macroscopic colonies and often forms large surface slicks, is very conspicuous. Indeed, members of early oceanographic expeditions kept close track of how these slicks were distributed in major ocean basins. Since then, microbiologists brought numerous other marine diazotrophs, including aerobic and anaerobic heterotrophs and photoautotrophs as well as lithotrophs from diverse environments, into culture.

In 1961, Richard Dugdale, who is now at Tiburon Laboratory of San Francisco State University in San Francisco, Calif., and his collaborators exposed samples of Sargasso Sea plankton (anticipating recent microbial metagenomic studies in the Sargasso Sea) to nitrogen gas that was enriched with the isotope $^{15}$N and “traced” its uptake into particulate nitrogen (Fig. 2).

They noted much higher uptake in those samples that contained *Trichodesmium* (Fig. 3). This report was met with skepticism because the other then-known diazotrophic cyanobacteria possessed heterocysts, which are specialized cells for fixing nitrogen. Later studies confirmed that *Trichodesmium* is diazotrophic, dispelling that skepticism.

When *Trichodesmium* trichomes aggregate, they become macroscopic and thus easy to collect with and isolate from other plankton for experiments (Fig. 3). During the 1970s and 1980s, *Trichodesmium* was the object of extensive open-ocean studies, taking advantage of a
very convenient field assay for nitrogen fixation, the acetylene reduction method. This assay exploits the ability of the nitrogenase enzyme, which reduces nitrogen gas (N₂) to ammonium, to reduce other chemical entities, including acetylene (C₂H₂) which, like N₂, is triply bonded. Nitrogenase preferentially reduces acetylene to ethylene, which is readily measured by gas chromatography (Fig. 2).

These studies established that *Trichodesmium* is a globally important contributor to nitrogen input in the sea. However, parallel geochemical studies were uncovering evidence for a more substantial input of fixed nitrogen than could be accounted for by *Trichodesmium* alone, implying that the input by *Trichodesmium* was underestimated, that other agents were missed, or both.

Although researchers knew of other diazotrophs in the sea, no one had measured their abundance or knew how much nitrogen they contributed. Among those diazotrophs were the heterocystous cyanobacteria of the genus *Richelia* that are found as endo- or ecto-symbionts associated with eukaryotic algae that synthesize siliceous outer frustule (Fig. 3). Field studies, including satellite remote-sensing efforts, established that these organisms are abundant in some ocean areas.

Perhaps the most exciting recent finding was the existence of diazotrophs that are smaller than 10 μm among plankton of the tropical and subtropical oceans. Marine microbiologists suspected that some pelagic bacteria might be diazotrophic. However, because culture methods can be highly selective, most marine microbiologists now accept that the large bulk of oceanic bacteria are not culturable by traditional approaches. Nonetheless, some researchers suspected that microorganisms in this size fraction with nitrogenase activity, even if relatively low in numbers, could produce considerable amounts of nitrogen across the large volumes of the ocean. Their early attempts to measure nitrogen fixation produced ambiguous results.

In 2001, Jon Zehr of the University of California, Santa Cruz, and his colleagues successfully amplified the *nifH* gene, the structural gene for the dinitrogenase reductase component of the nitrogenase enzyme, as well as its transcript from the less-than-10-μm fraction of the plankton, identifying both coccoid cyanobacteria and proteobacteria as potential contributors to this activity. He later focused on cyanobacteria in this fraction, identifying at least three distinct phylogenetic groups engaged in nitrogen fixation. Two groups cluster with known cyanobacteria. For example, members in Group B are related to *Crocosphaera watsonii*, an earlier marine isolate, while those in Group C cluster with *Cyanotheca*. Members belonging to the third group, Group A, have resisted culturing attempts to date. Other efforts are under way to determine their diversity, distributions, and degree of nitrogen contribution in the oceans. Even though their abundance varies greatly, they contribute significant nitrogen inputs.

Other marine sites of nitrogen fixation have also been identified. Bacteria within the gland of
DeShays of marine wood-boring shipworms, which are mollusks, are diazotrophic and provide nutrients to worms. The gut flora of copepods may also host nitrogen fixers. Moreover, both diazotrophic bacteria and archaea are found in the deep sea, including near hydrothermal vents.

**Constraints and Controls**

Redfield’s question about the primary limiting nutrient for phytoplankton growth remains a matter of debate. Various physical, chemical, and biotic factors influence the extent of diazotrophy and the composition of this community in the ocean. With regard to nutrient factors, the importance of phosphorus and iron may be key. To help address such questions, several strains of both *Trichodesmium* and *Crocosphaera* are being studied in culture.

The limiting nutrients for nitrogen fixers appear to differ in different ocean regions, and their availability depends on the relative supply of iron.
versus phosphorus in different locations. Photosynthetic diazotrophs have an increased cell quota for iron because of the demands of the nitrogenase enzyme system relative to heterotroph diazotrophs or nondiazotrophic photoautotrophs.

The input of iron to the sea varies from one region to another. For example, in tropical sections of the Atlantic, atmospheric dust, mainly from the Sahara and Sahel regions of northern Africa, is the principal source of iron, sometimes raising concentrations of dissolved iron to high nanomolar levels. Experimental studies indicate that diazotrophs in the surface waters of the northern Atlantic tend to be more phosphorus stressed than iron stressed because high iron concentrations drive the drawdown of phosphorus until it becomes limiting. In oceanic areas farther away from land masses, iron concentrations drop to sub-nanomolar levels, making this nutrient much more critical for diazotroph success.

Genomic sequence data for two marine diazotrophs, *Trichodesmium* and *Crocosphaera*, which is a coccoid cyanobacterium, are providing valuable insights about how these organisms survive and grow in the open ocean where many nutrients are in vanishingly short supply. For instance, *Trichodesmium*, but not *Crocosphaera*, has the capacity to use phosphonates, which are part of the organic phosphorus pool of the upper ocean.

A surprising very recent finding is that dissolved CO₂ concentrations apparently affect rates of marine nitrogen fixation. Surface seawaters contain about 2 mM inorganic carbon, mostly as bicarbonate ions. However, the enzyme carbonic anhydrase facilitates exchanges between these ions with the gaseous dissolved pool of CO₂. Three separate research groups reported in late 2006 and early 2007 that doubling the partial pressure of CO₂ in culture—as might occur globally during the next 100 years—increases the growth rate and nitrogenase activity of *Trichodesmium*.

**Questions about Geochemical Nitrogen and Phosphorus Balances**

A major current question in oceanography is how close to balance are the processes that add and remove combined nitrogen in the sea. There is no a priori reason that the nitrogen cycle is in balance at any particular instant. Because the deep ocean mixes on the time scale of 2,000–3,000 years, nitrogen cycle feedbacks could be operating on that scale. In any event, data collected during the last few decades suggest that denitrification and the recently discovered anammox reaction, two biological nitrogen-removal processes in which fixed nitrogen is converted to a gaseous form, are operating in excess of once-estimated inputs (Fig. 1).

While the Redfield ratio for nitrate and phosphate remains robust for much of the deep ocean, analysis of nutrient distributions during the past several decades reveals departures from this ratio in some ocean areas (Fig. 4A and B). For instance, in the western tropical northern Atlantic (Fig. 4B), waters below the euphotic zone are strongly enriched in inorganic nitrogen relative to phosphorus. Diazotrophs generally have a higher N:P ratio (or a positive N*) than do other phytoplankton, and some experts attribute that enrichment to the decay of diazotroph biomass in surface layers.

Meanwhile, other areas of the ocean, including the Arabian Sea and the eastern tropical North and South Pacific, display a deficit in nitrogen relative to phosphorus (Fig. 4C). These areas are low in oxygen, referred to as oxygen minimum zones (OMZs) and typically are found at depths below the euphotic zone down to 500 m. Recent results suggest that anammox is the predominant process converting combined nitrogen back to gaseous forms in these zones.

OMZs are generally associated with upwelling regions of the sea, areas where surface winds force deep waters to the surface. Waters that pass through OMZs and that are subsequently upwelled are depleted in combined nitrogen relative to phosphorus. Nonetheless, there are sufficient concentrations of nitrate in these waters to fuel diatom blooms and to support fisheries such as the anchovy-rich areas off the coast of Peru. However, blooms near the source of upwellings draw down the remaining nitrate, leaving residual phosphorus (largely as phosphate). As upwelled waters subsequently advect offshore at the surface, the residual phosphate is drawn down in the absence of any available nitrate (Fig. 4D).

This process was recently interpreted as a “smoking gun” for nitrogen fixation. Curiously, the areas of the ocean implicated as involved in nitrogen fixation by this P* diagnostic are different than those using the N* parameter, a dichotomy yet to be reconciled.
The global marine input of nitrogen, based on a variety of direct estimates scaled to the ocean basins as well as integrative geochemical methods that depend on N* and P* diagnostics or on measured distributions of stable nitrogen isotopes, yield values in the range of 100 and 200 TgN y\(^{-1}\) \((Tg = 10^{12} g)\), considerably less than some of the recent estimates of denitrification of 400 Tg N y\(^{-1}\). Such an imbalance would deplete the oceans of nitrate in a few thousand years.

The Case for Global Nitrogen Homeostasis

In parallel with field efforts, experts using geochemical models of the ocean nitrogen cycle conclude that there are feedback processes that maintain overall fixed nitrogen within relatively strict limits—in effect, forming a global nitrogen homeostat. There is some paleoecological evidence to support this notion. Moreover, P* distributions provide a mechanism for a closer temporal coupling between the processes removing fixed nitrogen in OMZs and those that fix nitrogen.

If nitrogen is fixed “downstream” of the OMZs, as P* distributions suggest, nitrogen deficits may be compensated for during periods of months to several years rather than longer periods of thousands of years during which oceans mix. Experts now believe that we are either underestimating biological nitrogen inputs or overestimating processes that remove this nutrient.

The oceans are vast and are vastly under-
sampled microbiologically. However, new methods to detect specific diazotrophs, including microarrays and quantitative PCR systems that can be automated for in situ sampling on moored arrays and in autonomous sampling vehicles, are under development. Additionally, some diazotrophs, such as *Trichodesmium*, have unique optical signatures that can be detected from space by sensors on satellites.

Scientists who model the carbon cycle and its potential responses to increasing CO₂ levels and global temperatures along with decreasing pH of the oceans have come to recognize the critical importance of marine nitrogen fixation as an important biogeochemical processes linked to the carbon cycle. Indeed, microbiological nitrogen fixation may itself be affected by anticipated increases in upper ocean temperatures and CO₂ concentrations as well as pH decreases. Moreover, the global nitrogen cycle itself is experiencing a major human perturbation as a result of the steady increases in nitrogen fertilizer production to support a growing human population. Much of this fertilizer nitrogen ultimately finds its way to the ocean.

Hence, we can expect to combine new sampling and measurement technologies with refined approaches to modeling as they try to resolve some of these outstanding questions about global nitrogen and CO₂ balances and the response of these cycles to climate change. Indeed, marine nitrogen fixation is now a key focus of ocean biogeochemical and biological diversity research.

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SUGGESTED READING


