Emerging patterns of marine nitrogen fixation

Jill A. Sohm, Eric A. Webb and Douglas G. Capone

Abstract | Biological N_2 fixation is an important part of the marine nitrogen cycle as it provides a source of new nitrogen that can support biological carbon export and sequestration. Research in the past decade has focused on determining the patterns of distribution and abundance of diazotrophs, defining the environmental features leading to these patterns and characterizing the factors that constrain marine N_2 fixation overall. In this Review, we describe how variations in the deposition of iron from dust to different ocean basins affects the limiting nutrient for N_2 fixation and the distribution of different diazotrophic species. However, many questions remain about marine N_2 fixation, including the role of temperature, fixed nitrogen species, CO_2 and physical forcing in controlling N_2 fixation, as well as the potential for heterotrophic N_2 fixation.

Upwelling
Wind-mediated movement of deep water to the surface.

Physical forcing
The effect of physical conditions and processes in the ocean on biological properties.

Diazotrophs
Organisms that can fix N_2 by converting it to ammonia.

Department of Biological Sciences and Wrigley Institute for Environmental Studies, University of Southern California, Los Angeles, California 90089, USA. Correspondence to J.A.S. e-mail: sohm@usc.edu
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Microbial N_2 fixation in the ocean is globally important because inputs of new N to surface waters (as opposed to N that is regenerated from existing organic material in surface waters) controls the export of organic matter to the deep ocean in many regions[1,2], a process commonly referred to as the 'biological pump' (REF. 3) (FIG. 1). Through the biological conversion of CO_2 to organic matter and its subsequent sinking, the deep ocean becomes a reservoir where CO_2 can be sequestered from the atmosphere for hundreds to thousands of years. Although it was initially acknowledged that N_2 fixation was a potential source of new N, the relative importance of newly fixed N was considered to be minor compared to the upwelling or diffusional flux of dissolved inorganic N (DIN) from deep waters[2]. About a decade ago this view began to change, as it became apparent that marine N_2 fixation was a globally significant process that could equal the input of DIN to surface waters in areas of the tropical and subtropical ocean[4,5]. Moreover, because upwelling and diffusion of DIN into surface waters brings with it an approximately stoichiometric amount of CO_2 (relative to the Redfield ratio (BOX 1)), N_2 fixation and atmospheric deposition of N are the only sources of new N that can lead to a net sequestration of atmospheric CO_2 in the deep ocean[2].

There are many factors that could limit and control marine N_2 fixation. In this Review, we specifically focus on the role of the nutrients P and Fe, as well as the potential effects of temperature, CO_2, fixed N and physical forcing on the distribution of N_2 fixation and diazotrophs in the ocean water column (although N_2 fixation also occurs in various marine sedimentary environments; see BOX 2). We also highlight the potential of heterotrophic N_2 fixation in surface waters as an unrealized source of new N.

Nitrogen fixation — the basics

N_2 fixation is the process by which N_2 gas is reduced into two molecules of ammonia. The reaction consumes cellular energy, and the overall reaction is:

$$N_2 + 8H^+ + 16ATP + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16\ P_i$$

This reaction is catalysed by the nitrogenase enzyme complex, which is extremely oxygen sensitive. The most common form in the oceans is made up of two distinct proteins: dinitrogenase reductase (the Fe protein) and dinitrogenase (the Fe molybdenum, or FeMo protein)[6]. It is encoded by three genes (*nifH*, *nifD* and *nifK*); however, the *nif* operon can in some species contain up to 20 genes that are also involved in the synthesis and regulation of nitrogenase and its cofactors[6]. The nitrogenase proteins are highly similar among diazotrophs, and the well-conserved *nifH* gene is commonly used for phylogenetic and ecological studies[6]. Several 'alternative' nitrogenases are also known that contain a third subunit in the FeMo protein that is encoded by *nifG*[6]. The capacity for N_2 fixation occurs in bacteria and archaea of diverse physiologies (including anaerobes, facultative aerobes, aerobes and phototrophs), and although nitrogenase is thought to be an ancient enzyme, it is not uniformly distributed and is present in perhaps a few hundred cultivated species[6].

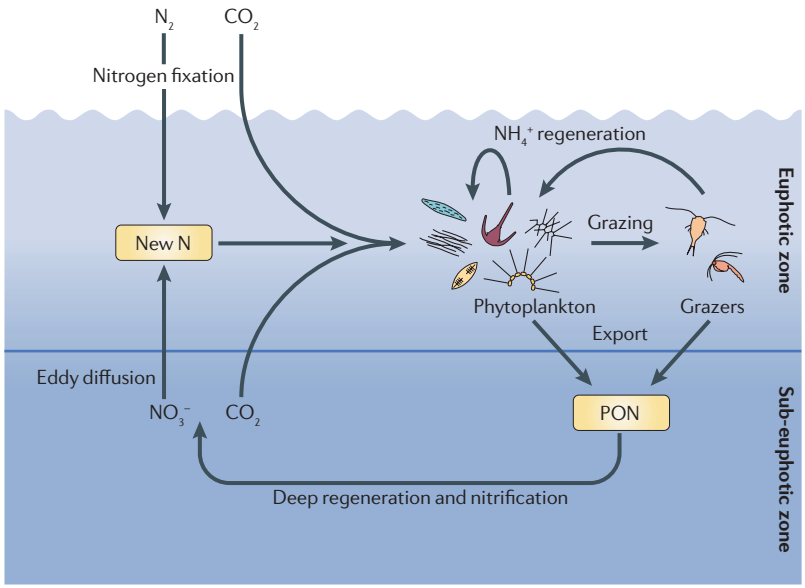

Figure 1 | **Overview of the importance of nitrogen fixation in the ocean.** The export of organic material from surface into deeper waters is dependent on the amount of CO_2 fixation (primary production) that is based on new nitrogen inputs (N_2 fixation or nitrate diffusion from deep waters). Alternatively, some primary productivity is based on the regeneration of organic material to ammonia in surface waters. PON, particulate organic nitrogen.

The marine diazotrophs

In the marine water column, substantial N_2 fixation is carried out by several different types of diazotrophs, including non-heterocystous filamentous cyanobacteria, unicellular cyanobacteria and heterocystous filamentous cyanobacteria (both free-living and symbiotic forms), all of which are phototrophs but not necessarily autotrophs. Heterotrophic bacteria containing the *nifH* gene have also been found in the open ocean[7], but their importance to global rates of N_2 fixation remains to be determined. The details of the physiology of marine diazotrophs have been presented in another recent review[8], so they will be only briefly described here. In oligotrophic tropical and subtropical waters the non-heterocystous, filamentous diazotrophs are most commonly represented by the colony-forming cyanobacteria *Trichodesmium* spp.[5]. This genus has a unique physiology that employs spatial and temporal segregation and increased oxygen consumption to allow it to simultaneously fix N_2 and CO_2 (and thus evolve O_2) without a heterocyst[9]. *Trichodesmium* spp. grow and fix N optimally at temperatures between ~24 and 30 °C[10] and are well known for forming large surface blooms when wind speeds are low and water temperature is above 25 °C[5]. The collapse of these blooms could contribute to organic matter export.

The occurrence of N_2 fixation and the diversity of diazotroph species in the <10 µm plankton fraction was only recognized about a decade ago[11]. These unicellular diazotrophs are divided into three groups: *Crocosphaera watsonii,* a cultured cyanobacterium that has many representative strains available; UCYN-A, an uncultured cyanobacterium; and UCYN-C, a distinct, uncultured clade that is related to *Cyanothece* sp. ATCC 51142 (REF. 8). They are commonly classified by group-specific quantitative PCR (qPCR) of the *nifH* gene in DNA samples, in which *nifH* copy number is assumed to equal the cell number of the specific diazotroph. These three groups of small diazotrophs have been found in diverse tropical and subtropical surface waters[12,13], and their N_2 fixation rates can equal or exceed that of *Trichodesmium* spp. when they are present at high densities[14,15].

C. watsonii carries out photosynthetic CO_2 fixation during the day and N_2 fixation at night[8]. Despite the fact that strains of this species are extremely similar phylogenetically[16,17], the isolates fall into two distinct phenotypic groups: large (>4 µm) and small (<4 µm) cells. The large isolates display greater rates of N_2 fixation than the small isolates[16] and also produce large amounts of extracellular polysaccharides (J.A.S., E.A.W., B. R. Edwards and B. G. Wilson, unpublished). The temperature range for growth of *C. watsonii* is ~22–36 °C[16]. In the field, DNA copy numbers of *nifH* from *C. watsonii* increase linearly with temperature, reaching a maximum of nearly 10^6 *nifH* copies per litre at temperatures of 30 °C[18], but are generally closer to 10^3–10^4 per litre[12,13]. Because UCYN-A remains uncultured, its physiology has been inferred from a recent analysis of an environmental metagenome. The UCYN-A genome contains photosystem I (PSI) genes but lacks genes for photosynthetic accessory pigments, the oxygen evolving photosystem II (PSII), the tricarboxylic acid cycle and metabolic pathways for some amino acids and purines, indicating that UCYN-A is photoheterotrophic and possibly lives in association with other phototrophs[19,20]. In the ocean, *nifH* DNA has been amplified from UCYN-A that are found at temperatures ranging from 15–30 °C[12,13,21], with the greatest copy number being found at ~24 °C[18], indicating that this group has a wider temperature range and lower minimum temperature than the other marine diazotrophs. UCYN-A bacteria are often the more abundant of the unicellular diazotrophs in oligotrophic waters, ranging from 10^3–10^5 *nifH* DNA copies per litre on average, and sometimes reaching abundances of 10^6 copies per litre[12,18].

The heterocystous diazotrophs are a filamentous group that includes both free-living and symbiotic cell types, which are typically restricted to more specialized marine environments. The cooler, brackish waters of the Baltic Sea host *Nodularia* spp., *Aphanizomenon* spp. and sometimes *Anabaena* spp. The temperature optima of *Aphanizomenon* spp. and *Nodularia* spp. isolates are 16–22 °C and 25–28 °C, respectively[22]; both are present at around 10^4 heterocysts per litre in their respective blooms[23]. In warmer waters of the tropics and subtropics, heterocystous diazotrophs (such as *Richelia intracellularis*) are only encountered as symbionts of diatoms like *Hemiaulus* spp., and *Rhizosolenia* spp. Filaments of *Calothrix* spp. can also be found in association with the diatom *Chaetoceros* spp.[24]. These diatom diazotroph associations (DDAs) are widely distributed through the warm oligotrophic ocean[25–27], averaging 10^3 heterocysts per litre in a bloom, but sometimes reaching 10^6 heterocysts per litre[26]. Because

Heterocyst
A specialized cell with a thick cell wall that lacks photosystem II (PSII) and is the site of N_2 fixation in some filamentous cyanobacteria.

Blooms
Areas of large growth or accumulation of a species.

Brackish
Describing water that has a salinity between fresh and marine water.

Box 1 | **The Redfield ratio**

More than half a century ago, Alfred C. Redfield[121] recognized that the dissolved nutrient ratios in the deep sea corresponded remarkably to the ratios of particulate organic matter in surface waters. He attributed this correlation to the fact that organic matter (phytoplankton biomass) is produced, on average, in a ratio that is determined by biochemical needs, and that this material is subsequently remineralized in the deep ocean, releasing inorganic nutrients in the same proportions. This ratio is now referred to as the Redfield ratio, which has been determined to be 106 C/16 N/1 P. In the marine environment, the ratio of N/P can vary substantially in dissolved forms in surface waters, and also in particulate matter, and the Redfield ratio is often cited as the line between N and P limitation, with higher ratios indicating P limitation and lower ratios indicating N limitation. It is important to acknowledge that many organisms have N/P ratios that differ from 16/1; however, the uniformity of the average deep water dissolved inorganic N/dissolved inorganic P (DIN/DIP) ratio around the globe strongly supports an average phytoplankton demand of 16/1.

of their heavy, silicon-containing cell walls, DDAs may have an important role in C export[28], and could therefore be an important part of the biological pump. Other planktonic marine diazotrophs that have been identified by *nifH* gene diversity assessments are members of the gammaproteobacteria and alphaproteobacteria, and members of the anaerobic cluster III diazotrophs[7,11,13]. The importance of these groups to open ocean marine N_2 fixation is currently unknown and represents an exciting area of future research.

Iron and phosphorus limitation

Marine phytoplankton are generally thought to be N limited through much of the tropical and subtropical oceans[29], whereas diazotrophs by definition are not. This suggests that other nutrients control diazotrophs in the ocean, with the most likely candidates being Fe and P. The importance of each of these nutrients is probably determined by the prevailing inputs and cycling of nutrients in the different ocean basins (see BOX 3 for background information).

Iron limitation of diazotrophs in culture. Fe limitation may be important for cyanobacterial N_2 fixation as Fe is a cofactor in PSI and nitrogenase (as well as in other redox enzymes), such that the Fe requirement for diazotrophy is ~5 times higher than that for ammonia assimilation[30,31]. The concentration of dissolved Fe (dFe) is also low in many of the environments where N_2 fixation occurs[32–35]. Early work established the importance of Fe to N_2 and CO_2 fixation and growth in natural populations and cultures[36,37], and recent research has probed the mechanisms behind this importance. Low free Fe concentrations decrease N_2 fixation rates in multiple strains of *Trichodesmium* spp.[31,38], causing the expression of Fe stress genes that are predicted to compensate for Fe deprivation, and downregulation of Fe-containing proteins that are involved in photosynthesis and N_2 fixation[38–40]. The expression of these genes is not necessarily specific to diazotrophs; however, group-specific expression of these genes can be used to detect Fe stress in the field. The observed decrease in N_2 fixation may be due to a decrease in nitrogenase protein levels under conditions of Fe limitation, while the cell maintains the abundance of the Fe-requiring PSI[39]. N_2 fixation and growth in *C. watsonii* are also reduced under conditions of Fe depletion[41]. However, to reduce its overall Fe needs, *C. watsonii* synthesizes Fe-containing proteins for N_2 fixation and CO_2 fixation only during the night or day, respectively, repurposing the Fe throughout the diel cycle and thus reducing the total cellular Fe requirement by 40%[42]. Fe deficiency can also reduce the growth and yield in *Nodularia spumigina*; however, *N. spumigina* may be able to overcome some of this effect by accessing ligand-bound Fe[43].

Phosphorus limitation of diazotrophs in culture. P is only supplied to the ocean by geological processes (that is, weathering) and is a major component of cells, having roles in information storage (DNA and RNA), protein synthesis (ribosomal RNA), energy generation (ATP) and as a component of cell membranes (phospholipids). Despite the fact that *Trichodesmium* spp. and other phytoplankton can substitute sulpholipids for phospholipids during P deficiency[44], they still are not released from a requirement for P for growth.

Trichodesmium spp. culture work has identified a number of mechanisms to deal with P limitation. Maximal dissolved inorganic P (DIP) uptake rates[45], alkaline phosphatase activity (APA)[46] and particulate N/P ratios[47,48], as well as the transcription of genes for DIP transporters and alkaline phosphatases[49], all increase under conditions of DIP-induced limitation of growth rate. This allows *Trichodesmium* spp. to deal with P limitation through a greater uptake of DIP and dissolved organic P (DOP), and through decreasing the requirement for cellular P. These physiological and gene expression measures can be used as markers for P stress in the field. Additionally, *Trichodesmium erythraeum* str. IMS101 possesses the full *phn* operon, which gives it the genetic capability to use phosphonate (C–P) bonds[50]. Other isolates of *Trichodesmium* spp. contain the *phnJ* and *phnD* genes, suggesting that the *phn* genes are common in the genus. In culture and field populations, expression of *phnJ*, which is thought to encode part of a phosphonate bond lyase complex, was detected when cells were DIP deficient[50]. Direct uptake of P from phosphonates has yet to be shown, but *Trichodesmium* spp. cultures seem to be able to grow with phosphonate bond-containing compounds as the sole P source[51]. Despite the fact that *Trichodesmium* spp. have many physiological adaptations for dealing with P limitation, N_2 fixation can still be reduced in cultures that are depleted of DIP[47]. Similarly, DIP deficiency in *C. watsonii* leads to the expression of a high-affinity phosphate transporter, but only the yield and not the growth rate of cultures is reduced[52]. *C. watsonii* isolates can also grow on a number of DOP compounds containing phosphomonoesters[52] (but not phosphonates). P deficiency can also affect the growth of Baltic Sea isolates of *Aphanizomenon* spp. and *Nodularia* spp. Both species decrease their requirement for P and growth rates under DIP deficiency; however, *Aphanizomenon* spp. seem to be adapted to take advantage of DIP pulses, whereas *Nodularia* spp. have adaptations for growing well at low DIP concentrations[53].

Dissolved Fe
Fe that can pass through a 0.4 μm filter.

Free Fe
A pool of Fe that is composed of Fe bound to inorganic ligands and a small amount of free ion (Fe^{3+}) and is presumed to be bioavailable (often denoted Fe′).

Ligand
In this article, an organic molecule that binds Fe (potentially a siderophore).

Sulpholipid
A membrane lipid containing a sulphur (as opposed to P)-based head.

Alkaline phosphatase
A hydrolytic enzyme that cleaves phosphomonoesters from P-containing dissolved organic matter.

Box 2 | **Emerging patterns in benthic marine nitrogen fixation**

In addition to occurring in the pelagic zone, N_2 fixation occurs in benthic habitats such as microbial mats and plant rhizospheres. Research in these areas was brisk in the 1980s and before[122,123], but few new discoveries were made in subsequent years. However, in the past 5 years interest in benthic marine N_2 fixation has been renewed and researchers are again turning to the sediments. Recent research has shown that N_2 fixation might be an important process in estuaries, with N_2 flux shifting into the sediment in Narragansentt Bay in 2006, indicating net N_2 fixation[124]. N_2 fixation has also been detected in areas that were not typically studied in the past, such as bare sediments (sediments free of visible microbial mats), depths >5 cm[125] and the deep sea[126,127]. In shallow marine sediments, N_2 fixation can be attributed almost entirely to sulphate-reducing bacteria[125], and this activity correlates with macrofauna behaviour. In the deep sea, a microbial consortium has been identified that contains an archaeon and bacterium that together carry out the anaerobic oxidation of methane. The archaeon also appears to have the ability to fix N_2 and share it with its associated bacterium[126]. Molecular methods are also driving research into diel patters of N_2 fixation by specific cyanobacteria and heterotrophic bacteria[128]. Still, there are many more questions left to be answered; for example, why do microorganisms fix N_2 in sediments when reduced nitrogen species are in abundance in porewaters?

Iron and phosphorus limitation in the Atlantic Ocean. Early work by Rueter[37] showed that the introduction of Fe increases CO_2 and N_2 fixation rates and cellular chlorophyll *a* content in natural samples of *Trichodesmium* spp. that were collected near Barbados. Aside from this study and a more recent study showing P and Fe co-limitation of bulk N_2 fixation at two stations in the eastern tropical North Atlantic Ocean[54], Fe does not seem to be an important factor in limiting the rate of N_2 fixation (in the sense of Blackman limitation) in the North Atlantic Ocean. However, it appears that the amount of Fe delivered in dust is important in determining the diazotrophic biomass (in the sense of Liebig limitation) in this basin[55], and that high rates of N_2 fixation (250 μM m^{-2} d^{-1} from *Trichodesmium* spp. alone (BOX 3)) in turn drive the North Atlantic Ocean towards P limitation, with DIP concentrations as low as 1 nM[56,57] (FIG. 2). P stress in *Trichodesmium* spp. is suggested by the maximal DIP uptake rates, levels of APA and the particulate N/P ratios from the North Atlantic Ocean and the South Atlantic Ocean offshore of Brazil[47,58,59]. Furthermore, N_2 fixation by *Trichodesmium* spp. is proportional to P requirements in colonies in the western North Atlantic Ocean[60]. These data are consistent with recent research showing a higher degree of P stress in *Trichodesmium* spp. in the western versus the eastern North Atlantic Ocean[61]. Across the South Atlantic Ocean gyre, surface concentrations of DIP are one to two orders of magnitude greater than in the North Atlantic Ocean, whereas dFe is ~3 times lower[55], indicating that Fe may be limiting to N_2 fixation in the South Atlantic Ocean. Indeed, dFe may be so low as to preclude N_2 fixation, as rates are ≤ 20 μM m^{-2} d^{-1} (REF. 55) (BOX 3).

Iron and phosphorus limitation in the Pacific Ocean. Based on observational data of dust inputs and nutrient concentrations, many researchers have suggested that Fe limits N_2 fixation in the Pacific[35,57,62,63]. Subsequent field work has shown that addition of extra Fe can substantially increase N_2 fixation rates in the North Pacific at some times of the year[64], and that *Trichodesmium* spp. express an Fe stress gene over much of the South Pacific Ocean (P. D. Chappell, J. W. Moffett, A. M. Hynes and E.A.W., unpublished). Additionally, dust inputs seem to drive N_2 fixation in waters near Japan[65]. A decrease observed over a 10 year period in DIP stocks at station ALOHA (an autonomous ocean observatory in the North Pacific Ocean near Hawaii) is thought to be linked to the rise of N_2 fixing cyanobacteria and suggests a shift in this system to P limitation[66]. More recent data suggest that although P is becoming scarcer in the North Pacific Ocean, diazotrophs living there are not P limited[58,67,68] (FIG. 2). In the South Pacific Ocean gyre, dFe concentrations are some of the lowest that have been measured in the ocean: 0.1–0.2 nM in open ocean areas[33]. The extremely low DIN (<0.02 μM)[33] and DIP concentrations that are around an order of magnitude greater than in the North Atlantic Ocean[69] (and similar to the South Atlantic Ocean) would seem to favour N_2 fixation. However, N_2 fixation rates observed in the region are ~50 μM m^{-2} d^{-1} (REF. 70) (BOX 3), leading to the hypothesis that the low dFe concentrations limit N_2 fixation in the South Pacific Ocean.

Iron and phosphorus limitation in the Baltic Sea. The Baltic Sea is a brackish inland sea that is known to host large blooms of *Nodularia* spp., *Aphanizomenon* spp. and *Anabaena* spp. during the summertime. In coastal zones, Fe can stimulate diazotroph biomass[71], but in the open sea of the Baltic, research has converged on the importance of P to diazotrophic activity[72–74]. A number of studies have linked the strength of diazotroph blooms to the amount of excess DIP left over after the spring bloom[72,75], although another study suggested that an unidentified P source must also be important, probably DOP[76]. Nevertheless, the cyanobacterial blooms perform luxury uptake of DIP in spring, exhibiting particulate N/P ratios of ~5. As the bloom grows, N/P ratios increase to ~80 before bloom collapse[77]. These blooms induce a transient P limitation in the Baltic Sea microbial community during the summer[74,78] and also the limitation of N_2 fixation itself[73,78]. Interestingly, the *Aphanizomenon* spp. bloom generally initiates a few weeks before the numbers of *Nodularia* spp. increase, and the intensity of the *Aphanizomenon* spp. bloom can be linked to the amount of excess DIP[75]. *Nodularia* spp. seem to respond more to temperature and may be able to use regenerated P[75], a strategy that explains the delay in blooming of this species compared to *Aphanizomenon* spp.

Iron and phosphorus limitation in other areas. Relatively limited information is available for other bodies of water. The Mediterranean Sea is thought to be severely P limited[79] owing to high DIN/DIP ratios[80] that also point to the Mediterranean Sea as a site of N_2 fixation. Conversely, high N/P ratios of nutrient inputs to the Mediterranean Sea may account for the high DIN/DIP ratios. Despite this suggestion, there have been few N_2 fixation studies in the region. Recent work has identified putative diazotrophs from the cyanobacteria, proteobacteria, cluster III and methanogenic archaea groups[81]. Microscopy methods have been used to show that diazotrophic cyanobacteria are generally found free-living in

Blackman limitation
The limitation of the growth rate of an organism.

Liebig limitation
The limitation of the yield of an organism (as in a crop yield).

Gyre
A giant circular surface current that is present in the ocean.

Luxury uptake
The uptake of nutrients that are in excess of demand, and that can be made into storage products.

Box 3 | **Setting the stage: the biogeochemistry of the ocean**

N_2 fixation is generally thought to occur in warm, oligotrophic waters stretching roughly from 30°N to 30°S (REF. 5). Fe in the open ocean derives from the deposition of dust from adjacent desert areas. In general, the oceans in the northern hemisphere receive greater dust inputs than the southern oceans owing to the greater land mass in the northern hemisphere[63]. This is particularly true in the North Atlantic Ocean, where the Sahel and Sahara provide a large dust source causing dissolved Fe (dFe) concentrations of around 1 nM, compared to ~0.3 nM in the South Atlantic Ocean[32,55] (see the table). dFe levels are consistent across the entire Pacific at 0.1–0.2 nM[33,62]. However, dFe is elevated near islands such as the Hawaiian archipelago[62]. In the Arabian, Baltic and Mediterranean Seas dFe concentrations are relatively high, ≥ 1 nM[129–131], with concentrations in the Baltic Sea probably being affected by land runoff[71,131].

Dissolved inorganic P (DIP) concentrations in much of the open ocean range from 100–200 nM[55,69,132] except in the North Atlantic, where they can be below 1nM in the western basin[56,57]. Dissolved inorganic nitrogen (DIN) concentrations, conversely, are universally low in oligotrophic surface waters[33,55,132], causing nutrient ratios in the North and South Pacific Oceans, and the South Atlantic Ocean to be much lower than the Redfield ratio, and much higher than 16/1 in the North Atlantic. DIP concentrations in the Arabian and Baltic Seas are seasonally variable and can be >1 μM during mixing periods[72,86], but undetectable in the warm stratified period, with DIN/DIP ratios of less than 16/1 (REFS 78,86). DIP concentrations are also extremely low in the Mediterranean Sea owing to the anti-estuarine circulation of water, and DIN/DIP ratios are consequently high at ~30 (REF. 80).

Because of differences in sampling effort, N_2 fixation rates in some areas are better understood than others. In the North Atlantic Ocean, N_2 fixation by *Trichodesmium* spp. alone can be 250 μmol N m^{-2} d^{-1} in the season in which N_2 fixation occurs[4], whereas bulk water rates are only about 20 μmol N m^{-2} d^{-1} in the South Atlantic Ocean[55]. In the North Pacific Ocean, rates are about 100 μmol N m^{-2} d^{-1} (REFS 15,133) but can be much higher, and rates in the South Pacific Ocean are 50 μmol N m^{-2} d^{-1} (REF. 70). Biological estimates of N_2 fixation in the Arabian Sea range from 16–122 μmol N m^{-2} d^{-1} (REF. 85). N_2 fixation in the Baltic Sea is highly seasonal, ranging from as low as 20 μmol N m^{-2} d^{-1} in the winter up to 2,500 μmol N m^{-2} d^{-1} during summertime cyanobacterial blooms[134], whereas rates seem extremely low in the Mediterranean Sea at ~3 μmol N m^{-2} d^{-1} (REF. 83).

Location	Dust deposition	dFe (nM)	DIP (nM)	DIN/DIP	N_2 fixation (μmol N m^{-2} d^{-1})
North Atlantic Ocean	very high (REF. 63)	0.2–1.2 (REFS 32,55)	0.2–5 (REFS 56,57)	~30 (REF. 57)	250 (annual average) (REF. 4)
South Atlantic Ocean	very low (REF. 63)	0.1–0.4 (REF. 55)	~200 (REF. 55)	~0.1 (REF. 55)	~20 (spring) (REF. 55)
North Pacific Ocean	low (REF. 63)	0.1–0.3 (REF. 62)	10–100 (REF. 132)	~1 (REF. 57)	30–120 (annual range) (REF. 133)
South Pacific Ocean	very low (REF. 63)	0.1–0.2 (REF. 33)	110–240 (REF. 69)	~0.1 (REF. 33)	30–70 (spring) (REF. 70)
Arabian Sea	high (REF. 63)	0.5–1 (REF. 129)	BD–1,000 (REF. 86)	>13 (REF. 86)	16–122 (annual estimate) (REF. 85)
Mediterranean Sea	high (REF. 63)	0.2–1.2 (REF. 130)	BD–70 (REF. 135)	~28 (REF. 80)	~3 (spring and summer) (REF. 83)
Baltic Sea	low (REF. 63)	3–7 (REF. 131)	BD–800 (REF. 72)	5–40	~2500 (summer peak) (REF. 134)

BD, below detectable limits.

low abundance and ranging in size from 0.2–3 μm[82], and DDAs are also found in low abundance[25]. Measured N_2 fixation rates are also very low (~3 μM m^{-2} d^{-1})[83] (BOX 3), and it was recently concluded that extreme P limitation precludes substantial N_2 fixation in the eastern basin of the Mediterranean Sea[84].

The Arabian Sea has long been known to host large blooms of *Trichodesmium* spp.[85]; however, the constraints on N_2 fixation rates in this region are not well understood. The presence of large amounts of excess DIP in surface waters[86] and the apparent link between *Trichodesmium* spp. abundances and dust deposition[87] suggest that Fe may be the limiting nutrient. This area is also strongly affected by physical factors, and blooms may be triggered by the calm, inter-monsoon periods[87]. In the nearby Red Sea, *Trichodesmium* spp. and gammaproteobacteria have been detected, and N_2 fixation rates are low and possibly P limited[88].

Diazotrophic distribution in the open ocean

The distribution of physiologically and morphologically different diazotrophic species has ramifications for the potential for N_2 fixation in a given area and the fate of recently fixed N in surface waters. It is apparent from qPCR quantification and microscopy techniques that *Trichodesmium* spp. are the most abundant and perhaps the dominant diazotrophs in parts of the North Atlantic Ocean[12–14,89], whereas the Pacific can be dominated — at least numerically — by the unicellular diazotrophs, particularly UCYN-A[12,18] (FIG. 2). Recent work indicates that dFe concentrations could be controlling the distribution of diazotrophic species. *Trichodesmium* spp. are abundant in the North Atlantic Ocean, where dFe concentrations are relatively high, but not in the South Atlantic Ocean, where dFe concentrations are extremely low[55]. Additionally, *Trichodesmium* spp. may have a selective advantage in low P areas (that is, the North Atlantic

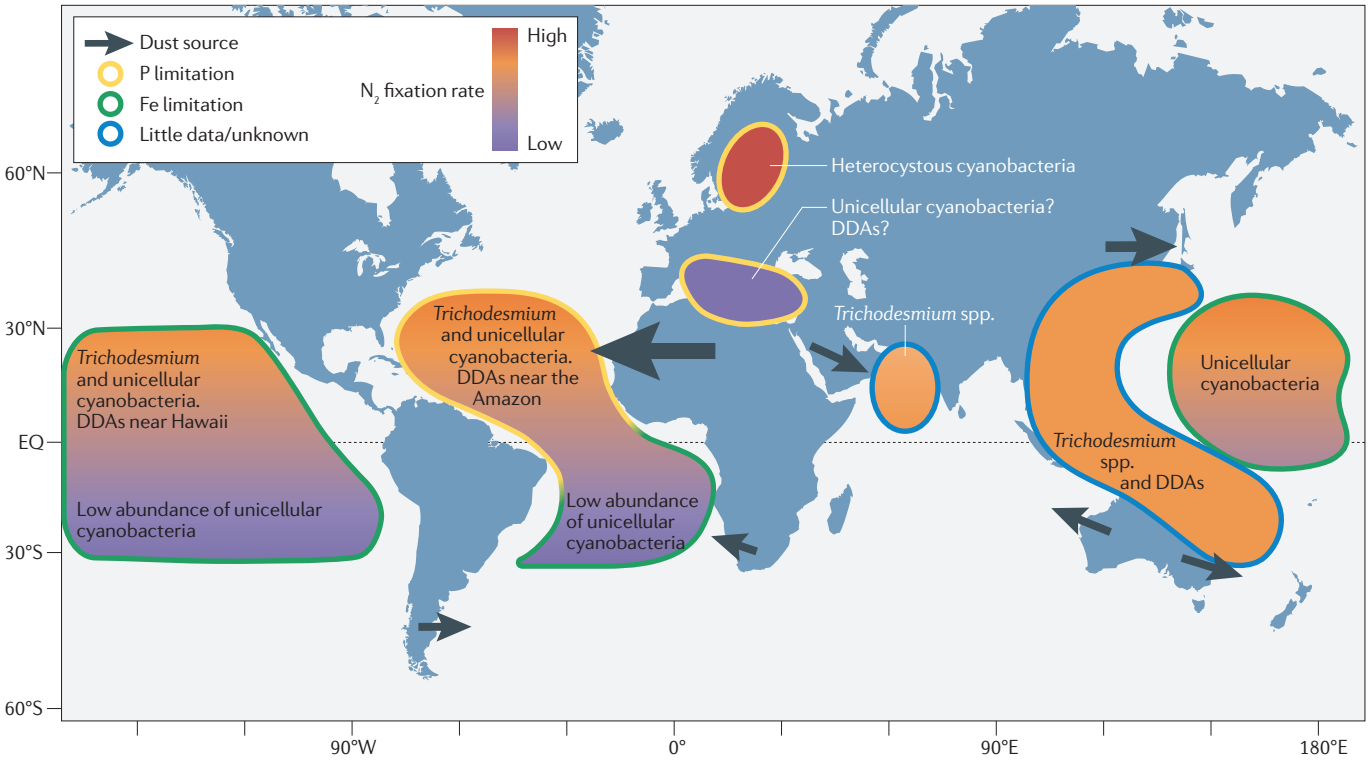

Figure 2 | **Summary of the distribution of nitrogen fixation rates, diazotrophic species and nutrient limitation in the ocean.** Relative N_2 fixation and information on the prevailing limiting nutrient are shown in each area by the fill colour and outline, respectively, and the source and relative strength of dust sources are shown by the size of the grey arrows. The diazotrophic species that dominate in each basin are indicated, with other types noted if they are found at high abundance in specific environments. Much of the abundance data available come from late spring–early autumn in each hemisphere, when higher overall abundances are expected. Robust seasonal data sets of diazotroph biomass are only available for limited locations, so it is less clear how the global patterns may change temporally. DDA, diatom diazotroph association; EQ, equator.

Ocean), as they have the genetic capability to use phosphonates. Within each basin, there is also evidence for smaller-scale variation in the populations. In one study in the tropical North Atlantic Ocean, *Trichodesmium* spp. were shown to dominate across much of the basin, except near the Cape Verde Islands where UCYN-A was most abundant[14]. In the Pacific, *Trichodesmium* spp. can be seen in relatively high abundances near the Hawaiian Islands[90], where dFe concentrations are elevated[34,62]. In the central gyre, however, dFe concentrations are very low (~0.1 nM)[62], *Trichodesmium* spp. are not common, and N_2 fixation seems to be carried out by small unicellular cyanobacteria[12,90]. The apparent dominance of the North Pacific Ocean by UCYN-A[12] supports the hypothesis that the extremely low Fe concentrations inhibit *Trichodesmium* spp. growth in the North Pacific Ocean, but are high enough for picodiazotrophs and nanodiazotrophs. A similar distribution of N_2 fixation is seen in the equatorial Pacific, with fixation being carried out by *Trichodesmium* spp. near Papua New Guinea but by small diazotrophs in open waters[91]. In the understudied central southern gyres of the Atlantic and Pacific Oceans, there is evidence of the occurrence of unicellular cyanobacteria[18], but more work needs to be done to confirm the importance of unicellular diazotrophs in this region.

Heterocystous symbionts have a much patchier distribution throughout the world's oceans, probably because the diatom hosts require silicon to build their cell walls. Hence, research to date has identified the largest densities of DDAs in the Amazon River plume[14,26,28,92], which delivers high concentrations of nutrients — including silicate — to a large area of the tropical North Atlantic Ocean[28]. DDAs have also been found in the Gulf of Guinea near the Congo River plume[93], the Mediterranean Sea[25] and across the Pacific Ocean[27]. In these regions, abundance is low compared to the Amazon River plume, and it is not clear what promotes the growth of these organisms in the open ocean. Although their distribution and abundance appear patchy, evidence suggests that DDAs are highly efficient at exporting C (REF. 28), and thus these species could be very important where they occur.

Given the differential dominance of different groups of diazotrophs in the oceans, it is likely that the fate of recently fixed N will also differ geographically. The modes of transfer of newly fixed N into the food web or of export from surface waters are not well known, but probably differ based on morphology, chemical composition and size differences between diazotrophic species[6]. *Trichodesmium* spp. have only one well

characterized grazer, *Macrosetella gracilis*, which lives its life attached to *Trichodesmium* spp. colonies and is probably only responsible for a small amount of mortality[94]. However, *Trichodesmium* spp. also appear to release newly fixed N[95]. To date, nothing is known about the grazing, recycling or export of *C. watsonii* and UCYN-A. Grazing, sinking and nutrient release in diazotrophs control the fate of newly fixed N and thus its impact on the global C cycle. These factors are important to consider in the future.

Future study of marine nitrogen fixation

Nitrogen fixation in lower temperature waters. Although marine N_2 fixation is generally associated with warmer tropical and subtropical surface waters, the process of N_2 fixation is not intrinsically inhibited by temperature and can occur at temperatures near 0 °C[96]. However, individual species have optimal temperatures, thus temperature may affect species distribution; for example, it has been proposed that warm waters allow for the high respiration rates that preclude the need for a heterocyst and thus select against free-living heterocystous forms[97]. Colder surface waters at higher latitudes are often associated with upwelling of nutrients (including fixed N), presumably selecting against N_2 fixation. Despite this, N_2 fixation by small diazotrophs has been reported in two Leuwin Current eddies at temperatures of 18 °C and 15 °C[98]. It has also been found at 18–20 °C in the English Channel (which was linked through an mRNA *nifH* library to the presence of UCYN-A, proteobacteria and the anaerobic cluster III group[99]) and at 19 °C off the coast of California (which was attributed to UCYN-A[21]). Thus, N_2 fixation by small diazotrophs can occur at temperatures that are lower than previously thought, but deeper investigation of the physiological basis of these occurrences is warranted.

Control of nitrogen fixation by carbon dioxide. In light of increasing atmospheric CO_2 concentrations and acidification of surface waters, it is important to understand how these related factors will affect N_2 fixation in the ocean in the future. Experiments by multiple investigators with *T. erythraeum* str. IMS101 show higher growth and N_2 fixation rates under increased CO_2 concentrations[100–102], suggesting that N_2 fixation by open ocean diazotrophs could provide negative feedback on the increase in global CO_2 concentrations through an increase in new N to drive the biological pump. However, the positive effect of increased CO_2 at current ocean temperatures is not as pronounced when temperatures are increased[103]. *C. watsonii* str. WH8501 shows a similar response to *T. erythraeum* str. IMS101 when faced with increased CO_2[41]; however, CO_2 had no effect during Fe depletion, indicating that low Fe could temper the effect of CO_2 on *C. watsonii* in future climate scenarios. Higher CO_2 concentrations may allow greater efficiency in the C concentrating mechanism, freeing up energy to be shunted towards N_2 fixation[100–102,104]. In *N. spumegina*, increased CO_2 may have the opposite effect, substantially decreasing growth and N_2 fixation rates[105]. The authors speculate that more acidic conditions impede the transfer of compounds between heterocystous and vegetative cells[105]. However, more research on this topic is needed before general conclusions can be drawn.

Research on the interaction of factors that are likely to change in concert and the long-term effects of exposure to higher CO_2 are the next step to truly understanding how N_2 fixation will change in the future in a human-influenced ocean.

Fixed nitrogen as an inhibitor of nitrogen fixation. Fixed N has long been recognized as an inhibitor of N_2 fixation, as it is an energetically costly process to carry out in comparison to ammonia assimilation. N_2 fixation in most diazotrophs examined is inhibited by mM concentrations of ammonia[106], whereas mM concentrations of nitrate shut down N_2 fixation in some strains[107] but have little to no effect in others[108]. The increased energy requirement for the reduction of nitrate after uptake compared to 'direct' assimilation of ammonia might explain these differences.A recent modelling exercise predicted that some of the largest areas of N_2 fixation in the ocean occur in or near waters that contain measurable amounts of nitrate[109], thus some recent studies have begun to question the strength of feedback inhibition of fixed N species on marine N_2 fixation. Addition of ammonia to natural samples of *Trichodesmium* spp. can shut down nitrogenase activity[110]; however, culture studies have shown mixed results, with some indicating that short-term additions of fixed N sources do not reduce activity[111,112], whereas others show a reduction in activity under these conditions[113]. Finally, N_2 fixation has been shown in the nitrate-rich upwelling areas off Chile[70], demonstrating that N_2 fixation may not be as strongly regulated by fixed N as previously presumed.

Physical forcing of N_2 fixation. It has long been known that biological processes in the ocean can be driven by physical processes, but only recently have researchers specifically begun to look at physical forcing of N_2 fixation. Davis and McGillicuddy[114] found that *Trichodesmium* spp. abundance in the North Atlantic Ocean correlated with anticyclonic eddies, suggesting that N_2 fixation is enhanced inside these mesoscale features. This phenomenon has since been studied in the North Pacific Ocean, where N_2 fixation in an anticyclonic eddy was found to be higher than the rates that are typically found at nearby Station ALOHA[115]. Over a 3-year timecourse, spikes in N_2 fixation at Station ALOHA can also be linked to sea surface height anomalies that suggest the presence of anticyclonic eddies[116]. Finally, depth-integrated N_2 fixation was found to be 2.5 times greater in a warm core eddy versus a cold core eddy off the west coast of Australia[98]. These studies provide intriguing insights, but more research is needed to fully understand the mechanisms behind physical forcing of N_2 fixation in the ocean.

Heterotrophic nitrogen fixation. Heterotrophic N_2 fixation is a potentially new paradigm in marine N_2 fixation, as heterotrophic organisms are secondary producers that use dissolved organic C for energy generation. This

Eddies
Small-scale circular currents in which the water inside is typically different from the water outside.

C concentrating mechanism
The mechanism that increases the concentration of CO_2 around ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), in order to increase the rate of photosynthesis.

Anticyclonic eddies
Eddies with warm interior water and a centre that is slightly higher than the surrounding sea surface. They rotate clockwise in the northern hemisphere and anticlockwise in the southern hemisphere.

is an important difference from the more commonly recognized primary producing diazotrophs discussed above (not including UCYN-A, which is photoheterotrophic), as they will not concomitantly fix CO_2, but will instead produce it. Throughout the world's oceans, non-cyanobacterial *nifH* genes have been identified including those of alphaproteobacteria, gammaproteobacteria and phylotypes from *nifH* of cluster III (see REF. 7); however, it is unclear whether this genetic potential translates into nitrogenase activity. Evidence of expression of these *nifH* genes has been found in cDNA libraries from the Mediterranean Sea[81], the eastern North Atlantic Ocean and the English Channel[99], and qPCR has been used to quantify expression of *nifH* in gammaproteobacteria[117,118]. However, direct uptake of N_2 by heterotrophic bacteria in the water column has yet to be shown, thus the potential contribution of these organisms to marine N_2 fixation is undetermined. Additionally, how do organic C availability and concentration control the process of N_2 fixation, and what mechanism(s) do diazotrophs use to protect nitrogenase from O_2 inactivation? These questions highlight only a few reasons why research on heterotrophic N_2 fixation is likely to gain momentum in the coming years. We speculate that the attachment of bacteria to particles could be a mechanism to deal with some of the challenges heterotrophic diazotrophs might face.

The emerging global picture

The accumulating data suggest a range of factors that may constrain N_2 fixation *in situ*. On the larger scale, temperature seems to set the limits of where different diazotrophs can exist, although it is important to note that temperature may be correlated to other factors that control geographical ranges. Large dust inputs into the tropical North Atlantic Ocean from the Sahel and Sahara appear to foster higher availability of Fe in the tropical and subtropical North Atlantic Ocean, thereby stimulating N_2 fixation, which causes drawdown of DIP and makes DIP availability a more important constraint on N_2 fixation than Fe. By contrast, excess DIP in surface waters of the tropical and subtropical Pacific Ocean (both North and South) and the South Atlantic Ocean coupled with low deposition rates of Fe from the atmosphere seems to drive diazotrophy to Fe limitation[55]. Recent modelling efforts have integrated physiological parameters of diazotrophs and generated global maps of diazotroph limitation that are roughly consistent with this picture[119], suggesting that the underlying controls on diazotrophy are becoming better understood. However, it is important to note that high or low dust deposition does not necessarily predict the presence of N_2 fixation. For example, relatively high dust deposition to the Mediterranean Sea should support N_2 fixation there except DIP levels are too low, whereas N_2 fixation occurs at extremely high rates in the landlocked Baltic Sea despite the relatively low dust deposition (Fe and other nutrients are probably supplied by river and terrestrial runoff). The presence of fixed N probably also has an effect on global diazotroph distributions by selecting against N_2 fixation; however, the concentration at which inhibition occurs may be higher than previously thought and dependent on the type of diazotrophs present. Finally, at current atmospheric concentrations, CO_2 may also have a role in determining the total amount of N_2 fixation in the ocean and might increase N_2 fixation rates in the future as humans continue to discharge CO_2 into the atmosphere.

In parallel, recent molecular evidence indicates that the relative dominance of different diazotrophic groups also varies among ocean basins. Microdiazotrophs such as *Trichodesmium* spp. seem to have a more predominant role in the North Atlantic Ocean compared to nanodiazotrophs such as *C. watsonii* and other small coccoid cyanobacteria[13,92], whereas nanodiazotrophs are seemingly more dominant though much of the North Pacific Ocean[12,91]. We posit that the cause of these emergent patterns is the differences in nutrient inputs and cycling in distinct parts of the ocean (FIG. 2). Understanding the distribution of these diazotrophic species is important because the fate of the N (and potentially C) fixed by these different and contrasting groups may follow distinct pathways into marine food webs, thus differentially influencing the biological pump.

With the prospect of upper ocean warming and acidification as a result of global climate change, as well as the increasing anthropogenic flux of reactive N to the sea[120], the magnitude, distribution and importance of marine N_2 fixation is likely to change, although research into how is just beginning. Despite decades of advances, the marine N cycle remains poorly understood, and with climate- and human-induced changes on the horizon, the continued study of oceanic N_2 fixation is important for our broader understanding of ocean biogeochemistry now and in the future.

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FURTHER INFORMATION
Jill A. Sohm's homepage:
http://www-scf.usc.edu/~sohm/
Eric A. Webb's homepage:
http://dornsife.usc.edu/labs/webb/home/index.cfm
Douglas G. Capone's homepage:
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