Trichodesmium spp. have proved to be enigmatic organisms, and their ecology and physiology are unusual among diazotrophs. Recent research shows that they can simultaneously fix N\textsubscript{2} and take up combined nitrogen. The co-occurrence of these two processes is thought to be incompatible, but they could be obligatory in Trichodesmium spp. if only a small fraction of cells within a colony or along a filament are capable of \(\text{N}_2\) fixation. Combined nitrogen is released from cells during periods of active growth and \(\text{N}_2\) fixation, and concomitantly taken up by Trichodesmium spp. or cells living in association with colonies. Although the nitrogenase of Trichodesmium spp. is affected by high concentrations of combined nitrogen, it might be relatively less sensitive to low concentrations of combined nitrogen typical of the oligotrophic ocean and culture conditions. Nitrogenase activity and synthesis exhibits an endogenous rhythm in Trichodesmium spp. cultures, which is affected by the addition of nitrogen.

In spite of the observation that nitrogen (N) is often the nutrient that limits primary productivity in many oceanic environments, marine \(\text{N}_2\) fixation has been largely overlooked as a source of fixed N in these systems. This is probably because reports in the early 1980s suggested that integrated rates of marine \(\text{N}_2\) fixation were small compared with overall phytoplankton N demand. However, a range of new evidence indicates that marine \(\text{N}_2\) fixation does provide a significant source of new N to tropical marine ecosystems. This evidence includes:

- Recognition of a more widespread occurrence and higher densities of the cyanobacteria *Trichodesmium* spp. than previously reported\textsuperscript{1,2}.
- Deficits and imbalances in the marine N budget indicating unquantified N sources\textsuperscript{3,4}.
- Particulate organic N and NO\textsubscript{3}\textsuperscript{−} could contribute substantially to the ocean’s fixed N budget\textsuperscript{8}.
- Integrations in plant science trends in the early 1980s suggested that integrated rates of marine \(\text{N}_2\) fixation were small compared with overall phytoplankton N demand. However, a range of new evidence indicates that marine \(\text{N}_2\) fixation does provide a significant source of new N to tropical marine ecosystems. This evidence includes:
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*Trichodesmium* spp. fix N\textsubscript{2} at about twice the rate of *T. erythraeum* at light intensities \(\gtrsim 500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}\) and fixed \(\text{N}_2\) at \(\sim 1.6\) times higher rates at 300 \(\mu\text{mol quanta m}^{-2} \text{s}^{-1}\textsuperscript{9} (\text{Ref. 20}). More rapid rates of \(\text{N}_2\) fixation under high light intensities might explain the greater abundance of *T. thiebautii* relative to *T. erythraeum* under such conditions. Low light intensities were used for cultures during the isolation of strains from natural populations, and might have selected for *T. erythraeum*.

Ecology of marine \(\text{N}_2\) fixation

Epiphytic, endosymbiotic and free-living unicellular and colonial cyanobacterial species, as well as bacteria, have been shown to fix N in pelagic marine systems\textsuperscript{1}. However, to date, *Trichodesmium* spp. appear to be the most quantitatively significant pelagic \(\text{N}_2\) fixers in marine systems. They are broadly distributed throughout the marine tropics and sub-tropics, and surface aggregations can occur over large expanses of ocean\textsuperscript{12}. Besides providing important N inputs, these species can dominate primary productivity and N cycling in the upper water column\textsuperscript{13,14}, particularly when they occur as episodic ‘blooms’\textsuperscript{15}.

In the ocean, multi-cellular trichomes of *Trichodesmium* spp. form spherical aggregates called ‘puffs’ and fusiform bundles called ‘tufts’, each containing up to several hundred trichomes\textsuperscript{16}. Free trichomes are also dispersed throughout the upper water column in tropical and subtropical seas\textsuperscript{1}. Cultures of *Trichodesmium* IMS101 and NIBB1067 occur largely as free filaments, forming colonies only during stationary-phase growth\textsuperscript{16,17,18}. The precise causes of bundle formation, aggregation and the formation of so-called ‘blooms’ are unknown, but many investigators have suggested that oxygen protection of oxygen-labile nitrogenase, Fe or other nutrient limitation might be important. Rates of \(\text{N}_2\) fixation by *Trichodesmium* spp. colonies vary widely between single sites\textsuperscript{19,20} and this might be attributed to environmental and physiological factors affecting natural populations. Highly variable rates of combined N uptake have also been measured\textsuperscript{21}.

Nitrogenase synthesis and activity in natural\textsuperscript{3,20} and cultured\textsuperscript{22} populations of *Trichodesmium* spp. exhibits a daily cycle, and nitrogenase activity is confined to the light portion of the day. This daily cycle of \(\text{N}_2\) fixation corresponds with the pattern of photo-synthetic C fixation. In natural populations of *T. thiebautii*, new
nitrogenase is synthesized each morning\(^\text{12,16}\), the enzyme is modified and thereby inactivated during the afternoon and subsequently degraded through the night\(^\text{35}\). Peak N\(_2\) fixation occurs around midday\(^\text{22}\), suggesting that nitrogenase activity might be regulated by light. However, \(nifH\) (the gene encoding the nitrogenase protein) mRNA abundance in cells collected from natural populations is highest before dawn, indicating that light itself does not directly promote nitrogenase synthesis\(^\text{16}\). The metabolic signals causing the modification and inactivation of nitrogenase have not been identified\(^\text{16}\). An endogenous rhythm for N\(_2\) fixation and the transcription, translation, and activity of nitrogenase in \(Trichodesmium\) IMS101 has been confirmed\(^\text{22,27}\).

Recently, it has been observed that not all cells within a colony or along a filament of \(Trichodesmium\) spp. contain nitrogenase. Immunolocalization studies suggest that <10-15% of cells clustered along regions of filaments contain nitrogenase and are therefore capable of fixing N\(_2\) (Refs 21,28–31). Cells within these clusters have been named diazocytes. Although they have some ultrastructural similarities to heterocysts, they do not have thickened cell walls and resemble vegetative cells\(^\text{21,28–31}\). Cells within these clusters have been named diazocytes. Although they have some ultrastructural similarities to heterocysts, they do not have thickened cell walls and resemble vegetative cells\(^\text{21,28–31}\).

However, what is unclear is whether particular cells permanently differentiate to perform N\(_2\) fixation or if all cells retain the capacity but vary widely among and even within studies depending on whether they simultaneously fix N\(_2\) and take up combined N when it is available at low concentrations\(^\text{13,14}\). High rates of NH\(_4\)\(^+\), NO\(_3\)\(^-\), and urea-uptake have been reported in some studies using populations from the N Atlantic Ocean and from the Caribbean Sea, as well as cultured populations, all have a capacity to take up NH\(_4\)\(^+\) at high rates\(^\text{15}\). High rates of NH\(_4\)\(^+\) uptake have been correlated with NH\(_4\)\(^+\) concentrations in exponentially growing cultured populations of \(Trichodesmium\) NIBB1067 and IMS101 (Ref 13.14). A capacity for glutamate (Glu) and glutamine (Gln) uptake has also been demonstrated in natural and cultured populations of \(Trichodesmium\) spp.\(^\text{15,17,18}\).
changes over the course of the growth cycle as more N is fixed into the system and becomes available through recycling (Ref. M.R. Mulholland and D.G. Capone, unpublished). Because Trichodesmium spp. must first fix N\textsubscript{2} into the system to grow, rates of N regeneration might be lower during the early growth stages. If natural populations are encountered early in the growth cycle, when the proportion of N\textsubscript{2} fixation is a higher fraction of the total N uptake, then measurements of NH\textsubscript{4}\textsuperscript{+} uptake might be low. Alternatively, higher measured rates of Nuptake and lower rates of N\textsubscript{2} fixation might be expected in populations sampled during late stationary phase growth, when new biomass production is low (Fig. 1).

Another possible explanation for the variability in reported NH\textsubscript{4}\textsuperscript{+} uptake rates is that NH\textsubscript{4}\textsuperscript{+} concentrations in the water in interfilamental spaces of colonies can be higher relative to concentrations measured in surrounding waters. In field studies, although ambient water column NH\textsubscript{4}\textsuperscript{+} concentrations have always been at or near the limits of analytical detection, Glu, Gln, NH\textsubscript{4}\textsuperscript{+} and urea concentrations are enriched in the interfilamental spaces of colonies (Ref. 14,36). Consequently, cells within or adjacent to these microenvironments are exposed to higher ambient nutrient concentrations. Calculations of NH\textsubscript{4}\textsuperscript{+} uptake using stable isotopes are sensitive to the ambient concentrations of nutrients used in the calculations. If, in actuality, cells are exposed to higher concentrations of N substrates because of elevated N within the interfilamental spaces, NH\textsubscript{4}\textsuperscript{+}-labelled substrates diffusing in would be diluted relative to the enrichment factor used in the calculation based on ambient seawater concentrations. Using the extracolonal or ‘ambient’ concentrations in making uptake-rate calculations might thereby result in an underestimation of the true rate of uptake.

With respect to the natural abundance data, although low δ\textsuperscript{15}N sig- natures in aquatic communities typically indicate a N\textsubscript{2} N source, this does not preclude the utilization of other isotopically light N sources. For instance, the NH\textsubscript{4}\textsuperscript{+} and DON pools derived directly from the release of recently fixed N\textsubscript{2} would be isotopically lighter than NH\textsubscript{4}\textsuperscript{+} or DON derived from recycled material derived from non-atmospheric N sources. Rapid reassimilation of this isotopically light pool of regenerated N would allow Trichodesmium spp. to retain their light isotopic signature while using regenerated N sources. If only a small fraction of Trichodesmium spp. cells contain nitrogenase, a high capacity for NH\textsubscript{4}\textsuperscript{+} uptake and an ability to concur- rently take up nitrogenous substrates and fix N\textsubscript{2} during the light period might be an important adaptation for the extracellular distribu- tion of N among cells and filaments (Ref. 13). Examination of Trichodesmium spp. filaments has not produced evidence of structural mechanisms for along-filament transport of fixed N (Ref. 30). Consequently, it is unlikely that fixed N is transferred between cells along filaments. An extracolonal mechanism for the transfer of fixed N among Trichodesmium spp. cells that are fixing N\textsubscript{2} and among those that are not has been proposed. It is suggested that fixed N, primarily amino acids, are released by cells fixing N\textsubscript{2} and these are available for uptake by cells that are not fixing N\textsubscript{2} (Ref. 35). High rates of amino acid (Ref. 37) and NH\textsubscript{4}\textsuperscript{+} (Ref. 13) release, and a simultaneous uptake capacity for amino acids and NH\textsubscript{4}\textsuperscript{+} by Trichodesmium spp. colonies supports this contention (Fig. 2).

In cultures of Trichodesmium NIBB1067 and IMS101 growing exponentially on a defined seawater medium without added N sources, N\textsubscript{2} fixation accounts for ~15% whereas the sum of NH\textsubscript{4}\textsuperscript{+} and glu uptake accounts for ~85% of the total-measured daily N uptake (Ref. 13). This is also consistent with the idea that 85% of Trichodesmium spp. cells along a filament or within a colony do not fix N\textsubscript{2} but instead must rely on the uptake of NH\textsubscript{4}\textsuperscript{+} and NH\textsubscript{4}\textsuperscript{+} released from fixed N\textsubscript{2} by other Trichodesmium spp. colonies near Japan, can grow on NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{-} and urea-enriched media in the laboratory (Ref. 36). However, nitrogenase synthesis and activity are
affected by the N source. Cultures grown on NO₃⁻, NH₄⁺, and urea do not exhibit nitrogenase activity or reduce nitrogenase activity during light periods. In a study using *Trichodesmium* NIBB1067, the modified, inactive form of the nitrogenase Fe-protein was found in cells grown on NO₃⁻ or NH₄⁺, but nitrogenase was absent entirely from cells grown on urea. In *Trichodesmium* IMS101, the Fe-protein was absent from cells treated with 20 μM of either NH₄⁺ or urea. In another study of *Trichodesmium* NIBB1067, cells cultured on medium containing excess NO₃⁻ or urea retained nitrogenase and a capacity to fix N₂ during at least a portion of the light period. However, they did so at rates lower than those measured in cultures maintained on medium without added N substrates. Cultures growing in medium with excess urea turned over their cell N much more rapidly than cultures growing on NO₃⁻, enriched medium or on medium without added N sources. However, growth rates were similar among cultures grown with or without combined N sources. The variation in N turnover rates between cultures growing on different N sources could occur because N₂ fixation is confined to the light period regardless of the N regime in natural and cultured populations of *Trichodesmium* spp. By contrast urea and NH₄⁺ are taken up at comparable rates during both the dark and the light periods. Uptake of NO₃⁻ occurs primarily during the light period, and probably competes with N₂ fixation for photosynthetic energy and reductant. The absolute growth rate (e.g. doubling of biomass) might be limited intrinsically.

Clearly, additional studies investigating the effect of combined N on growth and the competitive advantage of *Trichodesmium* spp. need to be pursued. Although *Trichodesmium* NIBB1067 and IMS101 appear to be able to take up combined N and fix N₂ simultaneously or alternately in cultures, little is known about the growth characteristics of *Trichodesmium* spp. under different N regimes in nature. *Trichodesmium* spp. can alleviate N limitation and therefore out-compete other species in N-limiting environments in nature, although blooms persist even after N concentrations become elevated. It is unclear how increasing availability of combined N species affects the growth of *Trichodesmium* spp. and their competitive success in natural systems.

The modification and expression of nitrogenase might be at least partially regulated by the N regime. NH₄⁺, Gln and Gln additions have been shown to reduce N₂ fixation over time, probably because of feedback inhibition of nitrogenase by metabolite pools. From studies using metabolic inhibitors, it appears that metabolites that accumulate during N assimilation, are important in regulating nitrogenase activity and synthesis in natural and cultured populations of *Trichodesmium* spp. However, the presence of low concentrations of NH₄⁺ and amino acids in the colony medium during growth do not inhibit N₂ fixation. The biosynthetic capacity of GS is sufficient to allow *Tri-

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**Nitrogen metabolism**

Nitrogen assimilation in natural populations of *Trichodesmium* spp. proceeds via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. GS is necessary for NH₄⁺ assimilation regardless of the primary form of N being used. High rates of GS transferase activity (a reverse reaction assay that provides an approximate total GS active sites) relative to rates of total N uptake have been observed in natural and cultured populations of *Trichodesmium* spp. This high ratio of GS transferase:biogenic activity during the period of maximum N₂ fixation, indicating that the proportion of the GS pool that is biogenic synthetically active increases during the day.

The biosynthetic capacity of GS is sufficient to allow *Trichodesmium* spp. colonies to turnover their cell N at least three times per day, suggesting that N assimilation does not limit the rate of N utilization by cells, even during midday when N₂ fixation rates are high. Cells appear to have sufficient capacity to assimilate all three of the intracellular N substrates derived from N₂ fixation and N uptake in cultures growing on media with or without added N. Excess GS activity is characteristic of cells limited by N or using N₂ as their N source. A positive correlation between GS and nitrogenase enzyme abundance and distribution has been observed in a variety of heterocystous and non-heterocystous *cyanobacteria* including *Trichodesmium* spp. The GS enzyme is twice as abundant in *Trichodesmium* spp. cells that contain nitrogenase.
than in those that do not. Although different types of GS might be present in cells, different promoters for transcribing the gene encoding GS during growth on fixed N and molecular N, have also been observed. Thus, there might be both a constitutive pool of GS, regulated for the general assimilation of N derived from various N sources, and a nitrogenase-linked pool co-regulated specifically with nitrogenase under low N conditions.

In support of two separately regulated GS pools in *Trichodesmium* spp., glnA transcript abundance is elevated both during the pre-dawn period and again in the late afternoon. The early morning peak in glnA transcript abundance corresponds with peak nifH (a gene encoding the Fe-protein of nitrogenase) transcript abundance, whereas the afternoon peak does not. In *Trichodesmium* NIBB1067 grown on medium without added N, biosynthetic GS activity increased before the onset of the light period and then again in the late afternoon (M.R. Mulholland et al., unpublished), consistent with the pattern of glnA mRNA abundance in natural populations. The absence of a strong diel cycle of GS activity might reflect the observation that *Trichodesmium* spp. colonies and filaments take up nitrogenase activity in a diel cycle might be significant in terms of the capacity for N assimilation in cells fixing N. The ratio of GS trans- ferase-biosynthetic activity in natural and cultured populations of *Trichodesmium* spp. varies by +20% 30% over a diel cycle.

The magnitude of this change is consistent with the suggestion that nitrogenase is confined to <10–15% of cells along a filament or within a colony may contain nitrogenase and are, therefore, capable of N fixation. If two pools of GS are present in *Trichodesmium* spp., the small changes in total filament or colony GS activity over a diel cycle might be significant in terms of the capacity for N assimilation in cells fixing N. The ratio of GS transferred to N fixation, it has been suggested that GS plays an important regulatory role in N fixation, either directly or indirectly, by preventing feedback inhibition from accumulated metabolites. Glutamate and glutamine are primary products of N metabolism via the GS/GOGAT metabolic pathway. Intracellular Glu and Gln concentrations parallel the daily cycle of GS fixation and might be factors in subcellular regulation of nitrogenase. However, in cultures of *Trichodesmium* NIBB1067 growing with and without combined N, the Gln/Glu ratio reflects the pattern of total N uptake (positive relationship). In natural populations, the magnitude and timing of the daily peak in Gln/Glu ratios varies among sites and populations. However, the highest Gln/Glu ratios are observed during the period when rates of N fixation are highest.

Light might be an important determinant for N fixation and the N status of *Trichodesmium* spp. cells. Higher Gln/Glu ratios (0.7) were observed in *Trichodesmium* spp. collected in the surface waters compared with colonies collected from deep waters. The decrease in the intracellular Gln/Glu ratios in colonies collected from deeper in the euphotic zone might be due to light or energy limitation of N fixation rates. Higher rates of photosynthesis by populations growing in surface waters might result in increases in the supply of energy to support higher rates of N fixation. In cultures of *Trichodesmium* IMS101 and NIBB1067, grown on medium without added N substrates, the intracellular ratios of Gln/Glu are lower (a maximum of ~0.3) and this might reflect the low light levels at which these isolates are maintained.

### Nitrogen regulation

Investigators have suggested that the nitrogenase of *Trichodesmium* NIBB1067 is regulated at different levels. An endogenous rhythm has been identified for nitrogenase transcription, translation and activity. Alternatively, it has been suggested that the modification and expression of nitrogenase and glutamine synthetase is the result of the activity of a regulatory gene, ntrA, which is co-regulated with the nitrogenase genes. It is unclear what the mechanism is.

### Community nitrogen dynamics and future directions

Although we are beginning to understand the physiology of N fixation by *Trichodesmium* spp., there remain many uncertainties regarding the mechanisms that support the growth of *Trichodesmium* spp. populations in nature and the growth of organisms living in association with *Trichodesmium* spp. colonies. For example, *Trichodesmium* spp. might have a larger role in the marine N cycle than previously suspected. Although net growth of populations might depend on inputs of new N from N fixation, *Trichodesmium* spp. also contribute to overall N turnover in oceanic systems. They contribute both directly through the release of amino acids, DON and NH4+, and indirectly through the regeneration of dissolved organic and organic N by bacteria and grazers living in association with *Trichodesmium* spp. colonies. Pathways of N cycling among *Trichodesmium* spp. filaments and colonies and associated organisms remain to be directly quantified.

Although *Trichodesmium* spp. alleviate N limitation of growth by fixing N, the effects of deficits of other nutrients on *Trichodesmium* spp. growth and N fixation are poorly understood. In particular, Fe, an essential component of the nitrogenase enzyme complex, might limit N fixation in oceanic regions with low Fe concentrations. Phosphorus is also in short supply in oceanic regions where *Trichodesmium* spp. grow and form blooms. It is unclear what the nutrient demands are for *Trichodesmium* spp. growth and how they acquire sufficient P to support observed growth rates. High N:P ratios have been suggested to be important for growth. Although we are gaining in our understanding of how these species affect the marine N cycle when they occur, much needs to be done to integrate N fixation into global C, N and P budgets.

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