Trichodesmium – a widespread marine cyanobacterium with unusual nitrogen fixation properties

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Abstract

The last several decades have witnessed dramatic advances in unfolding the diversity and commonality of oceanic diazotrophs and their N₂-fixing potential. More recently, substantial progress in diazotrophic cell biology has provided a wealth of information on processes and mechanisms involved. The substantial contribution by the diazotrophic cyanobacterial genus Trichodesmium to the nitrogen influx of the global marine ecosystem is by now undisputable and of paramount ecological importance, while the underlying cellular and molecular regulatory physiology has only recently started to unfold. Here, we explore and summarize current knowledge, related to the optimization of its diazotrophic capacity, from genomics to ecophysiological processes, via, for example, cellular differentiation (diazocytes) and temporal regulations, and suggest cellular research avenues that now ought to be explored.

Introduction

Balancing of nitrogen (N) inputs and exports in global oceans requires substantial biogenic fixation of atmospheric nitrogen (N₂). In this context, planktonic colony-forming cyanobacteria of the genus Trichodesmium are recognized as major players. Representatives within the genus have consistently been shown to be stable components of tropical and subtropical segments of the Atlantic, Pacific, and Indian Oceans where they may form enormous surface accumulations (‘blooms’) visible to the naked eye (see Capone & Carpenter, 1982; Capone et al., 1998; Karl et al., 2002; Tyrrell et al., 2003; Davis & Mc Gillicuddy, 2006; Westberry & Siegel, 2006; Carpenter & Capone, 2008). Trichodesmium contributes to sustaining marine life via active release of key nutrients, for example carbon and nitrogen, and upon death and decay, hence making this fully photoautotrophic genus a vital player in the biogeochemical cycling of basic elements in contemporary oceans (Carpenter & Capone, 2008). The global input via N₂ fixation by Trichodesmium was initially estimated to amount to about 5 Tg N annually by Capone & Carpenter (1982), an estimate that by now has risen to about 60–80 Tg N annually (Capone et al., 1997; Mahaffey et al., 2005; Westberry & Siegel, 2006; Carpenter & Capone, 2008), which makes up a substantial part of the current estimate of global marine N₂ fixation, 100–200 Tg N annually (Karl et al., 2002).

As the N₂-fixing enzyme, nitrogenase, encoded by the nifHDK genes, is rapidly inactivated by O₂, diazotrophic cyanobacteria either fix N₂ at night (to avoid photosynthetically evolved oxygen) or differentiate a thick-walled, photosystem II-deficient heterocystous cell type to specifically sustain daytime N₂ fixation (Kumar et al., 2010). Members of the genus Trichodesmium fix N₂ exclusively in the light (Dugdale et al., 1961; Capone et al., 1997),
although the genus is affiliated to Section III filamentous cyanobacteria that are unable to form heterocysts and therefore expected to fix N\textsubscript{2} (primarily) during the dark phase (see Bergman et al., 1997). Knowledge has expanded dramatically in regard to the diazotrophic physiology and molecular biology of *Trichodesmium*, but there are still gaps related to its unique cell biology and overall behavior in an ecophysiological context. We here summarize our current knowledge by highlighting its diazotrophic peculiarities from various perspectives.

**Speciation**

*Trichodesmium erythraeum* was named by Ehrenberg in 1830 after observing blooms that discolored the water at the Bay of Tor in the Red Sea (Ehrenberg, 1830). Jules Verne (1869) in ‘20,000 leagues under the sea’ also mentions blooms in this Bay (Box 1). Two other species, *T. thiebautii* and *T. hildebrandtii*, were named by Gomont (1892), and Wille (1904) later described another three species, *T. contortum*, *T. tenue*, and *T. radians*. These species were re-examined in 1995 (Janson et al., 1995) using specimens from the Indian Ocean, Caribbean, and Sargasso Seas. Ultrastructural arrangement of gas vesicles and glycogen clusters (carbon storage) were used as primary markers and separated the species into two clades: (i) *T. tenue* and *T. erythraeum* and (ii) *T. thiebautii*, *T. hildebrandtii*, and *T. contortum*. In 2001, a close relationship between *Trichodesmium* spp. (Lundgren et al., 2001) and marine cyanobacterial members of the genus *Katagnymene* (*K. pelagica* and *K. spiralis*; Lemmermann, 1900) was discovered using phylogenetic analysis of *nifH* gene sequences. Using the more variable fragment of the *hetR* gene, and a few other genetic markers, as targets revealed that the two *Katagnymene* species in fact cluster within one of the two *Trichodesmium* clades (Orcutt et al., 2002; Lundgren et al., 2005). Despite the morphological differences, *K. pelagica* and *K. spiralis* were, in addition, found to be the same species (Lundgren et al., 2005). Examining 21 cultivated isolates of *Trichodesmium*Katagnymene, using genetic and morphological markers, Hynes et al. (2012) verified the existence of two *Trichodesmium* clades and suggested that these may inhabit different ecological niches, based on different pigment characteristics.

However, a full revision of the genera *Trichodesmium* and *Katagnymene* is now warranted, as the latter also includes several freshwater species (*T. iwanoフianum* Nygaard, *T. lacustre* Klebahn, *K. accurata* Geitler, *K. mucigera* Compère and *K. spirulinoidea* An; see Komárek & Anagnostidis, 2005), one of which, in addition, represents the ‘type strain’ of the genus *Katagnymene*. Sequencing of additional genomes within the *Trichodesmium* genus is also needed if we are to fully comprehend the taxonomy and phylogeny of the genus.

**Box 1**

From Jules Verne, ‘20,000 leagues under the sea’ (translated by Lewis Mercier, (Verne, 1872)):

‘So, Captain Nemo, it is not the first time you have overrun the Red Sea on board the Nautilus? ’No, sir.’

**An expanding and flexible genome**

*Trichodesmium erythraeum* IMS101 (from now on *Trichodesmium* IMS101) was one of the first strains isolated into axenic cultures (Prufert-Bebout et al., 1993) and still represents the only sequenced genome within the genus (http://genome.jgi-psf.org/trier/trier.home.html). The genome, which comprises 7.75 Mbp, is among the larger cyanobacterial genomes sequenced to date (Fig. 1a). A recent phylogenetic survey of 58 sequenced cyanobacterial genomes, based on a concatenated alignment of 285 protein orthologs, verified that *Trichodesmium* IMS101 is affiliated to a lineage composed of other filamentous nonheterocystous species within Oscillatoriales (Fig. 1b; Larsson et al., 2011); the marine *Lyngbya* sp., PCC 8106 and two species within *Arthrospira* (*A. platensis* and *A. maxima*; previously denoted *Spirulina*). These are all ecologically successful and widespread inhabitants of marine waters and alkaline lakes, respectively. Recent analyses based on the 16S rRNA gene sequence give a similar clustering of *Trichodesmium* IMS101 (Schirmeister et al., 2011). Larsson et al. (2011) also showed that the capacity to fix N\textsubscript{2} within this *Trichodesmium* clade was lost in *A. platensis* and *A. maxima*, as also verified by Latysheva et al. (2012), while retained in the deeper-branching *Trichodesmium* and *Lyngbya* sp. PCC 8106. Interestingly, these four lineages all possess *hetR* (Larsson et al., 2011), a gene encoding a protease with a key function in cell differentiation (N\textsubscript{2}-fixing heterocysts and resting akinetes; see Kumar et al., 2010), although they all lack these
Fig. 1. Phylogeny and genome properties of Trichodesmium IMS101. (a) Genome sizes and proportions of coding and noncoding nucleotides in genomes of organisms included in (b) and (c). Genomes are sorted by total size. The genome of Trichodesmium IMS101 is indicated by an arrow. (b) Maximum-likelihood phylogenetic tree based on a concatenated alignment of 285 single-copy orthologs. The tree is a subtree of a larger phylogeny of 58 cyanobacteria (see Larsson et al., 2011). Specific phenotypes for cyanobacteria are shown by the colored boxes next to the tip labels. The clade containing Trichodesmium (order Oscillatoriales) is highlighted with blue branches. Thick and thin branches indicate bootstrap support values (200 replicates) of 100 and between 58 and 84, respectively. Bar, 0.4 expected substitutions per site. (c) Ancestral genome sizes (reconstructed by parsimony) in the phylogeny from b. Organism names are abbreviated (see below for full names). Contemporary genome sizes (Mb) are shown in the right margin and at specific nodes in the tree. Organism abbreviations are as follows: Acam = Acaryochloris marina MBIC11017, Anav = Anabaena variabilis ATCC 29413, Armt = Arthospira platensis str. Parao, Crow = Crocosphaera watsonii WH8501, Cya0110 = Cyanothece sp. CCY0110, Cya7425 = Cyanothece sp. PCC7425, Cyana7822 = Cyanothece sp. PCC7822, Cyana8801 = Cyanothece sp. PCC8801, Cyana8802 = Cyanothece sp. PCC8802, Cyl = Cylindrospermopsis raciborskii CS505, Lys = Lyngbya sp. PCC 8106, Mica = Microcystis aeruginosa NIES 843, Micc = Microcoleus chthonoplastes PCC7420, NoAz = 'Nostoc azollae' 0708, Nods = Nodularia spumigena CCY9414, Nosp = Nostoc punctiforme PCC73102, Noss = Nostoc sp. PCC7120, Raph = Raphidiopsis brookii D9, Sycys6803 = Synechocystis sp. PCC6803, Syn7002 = Synechococcus sp. PCC7002, Thee = Thermosynechococcus elongatus, Trie = Trichodesmium erythraeum IMS101, Ucyn = cyanobacterium UCYN-A. The figures are adapted from Larsson et al. (2011) with the author’s permission.
developmental capacities. Additionally, comparative genomic analyses show that several other gene orthologs involved in heterocyst differentiation are present in the Trichodesmium IMS101 genome (e.g. hetCF, patB) while others, not unexpectedly, are missing such as those involved in the deposition of the heterocyst outer envelope (e.g. hglCDE, hepB) (Table S1; El-Shehawy et al., 2003; Larsson et al., 2011). However, orthologous genes are not always functionally equivalent. For instance, the sepJ gene of Trichodesmium is missing a vital domain essential for filament integrity under nitrogen deprivation, although it fully complements a sepJ deletion mutant of Nostoc sp. PCC7120 when grown in the presence of combined nitrogen (Mariscal et al., 2011). The nif gene operon of Trichodesmium IMS101 is conserved in a manner typical of some heterocystous cyanobacteria, although Trichodesmium lacks the large DNA insertion element present in the structural nifD gene of several of the heterocystous cyanobacteria, as well as the intergenic region between nifB and nifVZT/cysE (Fig. S1). These findings strengthen an evolutionary relationship between the genus Trichodesmium and the heterocystous clade, whereas distinct differences are also apparent, relationships now worth examining in greater detail.

Another notable feature of the Trichodesmium IMS101 genome is its comparatively low coding capacity (Larsson et al., 2011). With c. 40% noncoding DNA, it holds one of the lowest coding percentages among all to date sequenced genomes of cyanobacteria (Fig. 1a) and other bacteria (Hou & Lin, 2009). The intergenic sequences within the Trichodesmium IMS101 genome (459-bp median length) are also relatively large for cyanobacteria (14.5- to 231-bp median intergenic length in 39 other finished cyanobacterial genomes). The reason for these large and presumably noncoding intergenic spacers in the Trichodesmium IMS101 genome is unknown. It is, however, interesting to note that among the 58 genomes examined, the genome of Trichodesmium IMS101 is one of a few in which the genome is currently expanding in size, as is also the case for the genomes of the limnic Microcystis aeruginosa NIES 843 and the marine Acaryochloris marina MBIC11017 (Fig. 1c; Larsson et al., 2011). This suggests that the genome of Trichodesmium IMS101 is in an expanding dynamic state, in contrast to the shrinking genomes of the unicellular marine genera Synechococcus and Prochlorococcus (with genomes < 2 Mbp; Palenik et al., 2006; Kettler et al., 2007), genera which to a large extent share the same tropical/subtropical marine habitat as Trichodesmium. Based on these data, it is suggested that different strategies are used to cope with the various constraints enforced by these oligotrophic oceans (Larsson et al., 2011). Trichodesmium may use a strategy to flexibly adapt by incorporating functions and capacities when needed (e.g. via horizontal gene transfer, HGT) and maintain gene duplications (in-paralogs) affecting c. 10% of all genes in the Trichodesmium IMS101 genome, as a mechanism to promote genome expansion and organismal adaptations (Swingley et al., 2008; Treangen & Rocha, 2011). One example of HGT in Trichodesmium IMS101 is the acquisition of long eukaryotic triglyceride collagen protein fibers that may sustain Trichodesmium colony formation (Layton et al., 2008). Paralogs and horizontally gained genes that do not provide a fitness advantage will undergo inactivation (sequence divergence and loss of function) and eventually be lost. Indeed, 21% of in-paralogs in Trichodesmium IMS101 appear to have been subject to inactivation and are now present only as pseudogenes within the genome (Larsson et al., 2011), thereby contributing to the abundance of noncoding nucleotides (Fig. 1a). Considering that bacterial genomes are subject to a deletion bias (Mira et al., 2001), the large noncoding proportion of the Trichodesmium IMS101 genome which cannot be attributed to remnants of previously functional genes (pseudogenes) is enigmatic. However, it is possible that at least parts of these intergenic regions contain as yet non-annotated genes or small RNAs (Hewson et al., 2009; Shi et al., 2009). It appears that the unicellular marine cyanobacteria with reduced genomes (e.g. Prochlorococcus spp. in Clade II) use an opposite strategy to compete for life-space and survival, that is maintain a large surface-to-volume ratio and fewer genes, a strategy recently characterized as ‘cryptic escape’ (Yoooseph et al., 2010).

The diazocytes – separation in space

A reduced oxygen environment is a prerequisite for effective N2 fixation activity in Trichodesmium as in all other bacteria. Both transcription of nif genes and biosynthesis of the nitrogenase enzyme complex have been found to be sensitive to oxygen inactivation (Zehr et al., 1997; Staal et al., 2007). Ever since the pioneering studies by Dugdale and co-workers (Dugdale et al., 1961), a compelling research area has therefore been to elucidate how Trichodesmium reconciles oxygenic photosynthesis and oxygenophobic N2 fixation within its ‘heterocyst-free’ physiology. Initially, N2 fixation was proposed to be limited to low-oxygen or anaerobic regions in the center of Trichodesmium colonies (Paerl & Bebaut, 1988). However, later, it became apparent that colony formation was not a prerequisite as ‘free’ trichomes, the dominant form in Trichodesmium laboratory cultures, are also able to fix N2. Attention then switched to the structural differences along the Trichodesmium trichomes that early on were observed using light microscopy, recognized as ‘nongranulated’ or ‘lighter’ cell regions in parts of the trichomes (Carpenter & Price, 1976; Bryceson & Fay, 1981; Li & Lee, 1990), in both...
single trichomes, and colony-associated trichomes. This cellular arrangement occurs in both natural populations and cultures of *Trichodesmium* IMS101 (Lin et al., 1998; El-Shehawy et al., 2003). On average, ~15% of the total cell population of the trichomes may be described as less granulated or lighter (more transparent). These cells are arranged in strings or ‘zones’ composed of ~2–30 cells, but are not always obvious in LM (Fig. 2a). However, when stained (e.g. with Lugol’s solution; Fig. 2c), each trichome harbors typically 1–2 such zones per trichome, but up to four zones have been observed in longer trichomes (El-Shehawy et al., 2003). The nongranulated appearance is caused by a diminished number and/or size of subcellular structures such as cyanophycin granules, gas vacuoles, and polyphosphate granules (Fig. 2c and e), while additional membranes are synthesized (Fredriksson & Bergman, 1997).

The ‘lighter’ cells were in 1991 (Bergman & Carpenter, 1991) proven to be the nitrogenase enzyme containing cells in *Trichodesmium* (Fig. 2d). The existence of a ‘spatial’ nitrogenase sequestration mechanism in a nonheterocystous cyanobacterium was thereby proven. The cells were subsequently termed diazocytes: *di* (two) *azo* (nitrogen) *cyte* (cell) (Fredriksson & Bergman, 1997). The exclusive localization of nitrogense in diazocytes was corroborated by immuno-TEM (sectioned trichomes) and immuno-LM (whole-mount intact trichomes; Fig. 2d) analyses of both cultured and natural populations from the Indian, Pacific, and Atlantic Oceans, using a battery of antibodies (Bergman & Carpenter, 1991; Bergman et al., 1993; Janson et al., 1994; Fredriksson & Bergman, 1997; Berman-Frank et al., 2001b), including also one monoclonal anti-*Trichodesmium* IMS101-NifH antibody (targeting the smaller Fe protein subunit; Zehr et al., 1990; Bergman et al., 1993). The frequency of the diazocytes is lower at dawn and increased toward noon and is negatively regulated by the presence of combined nitrogen (Fredriksson & Bergman, 1995; Lin et al., 1998; Sandh et al., 2009, 2011). Indeed, theoretical models suggest that a spatial separation of processes (such as nitrogen fixation and photosynthesis) favors biomass production compared to temporal separation (Rosetti & Bagheri, 2012) as, for instance, in unicellular cyanobacteria (Bergman et al., 1997).

As a few other studies have suggested that the nitrogenase enzyme is present in all cells within the trichomes (Paerl et al., 1989; Ohki, 2008; Orcutt et al., 2009), a variation in cellular localization may exist depending on different environmental conditions or species examined. Using $^{15}$N and Nano-SIMS, Finzi-Hart et al. (2009) observed that the fixed N is rapidly distributed into the majority of cells along *Trichodesmium* trichomes, although cells in the center showed a lower $^{15}$N label, a pattern that may suggest a zone of diazocytes. A lower $^{15}$N label is also typical for heterocysts analyzed by Nano-SIMS due to a most rapid transfer of fixed nitrogen out of these cells (Popa et al., 2007; Ploug et al., 2010).

It was recently shown that, as for the differentiation of heterocysts, removal of combined nitrogen from the growth medium is the sole and sufficient mean needed to elicit the development of the centrally located nitrogenase containing diazocytes (Fig. 2d) in *Trichodesmium* IMS101 (Sandh et al., 2012). The fact that the development of diazocytes takes between 8–27 h and that changes in cellular ultrastructure precede the expression of the nitrogenase enzyme (Sandh et al., 2012) strongly argues for a genetically based developmental background. Phylogenetically, the nonheterocystous *Trichodesmium* clade is a sister group to the heterocystous clade (Fig. 1b) and *Trichodesmium* shares several genomic and behavioral features with this clade (see above, below and Table S1). For instance, some heterocystous cyanobacteria (*Anabaena* sp.) first develop strings or subsets of adjacent proheterocysts upon nitrogen deprivation, while only the central cell develops into a mature heterocyst and the other regresses into vegetative cells (Wilcox et al., 1975). The similarity of the patterning of proheterocysts and diazocytes hints that several early regulatory elements may be shared in the nitrogen-regulated pathways of heterocystous genera and *Trichodesmium*. Diazocytes may during evolution have ‘frozen’ at this more minimalistic initial stage (Fig. 3), as a full differentiation is disadvantageous for the conditions offered in oceans (Staal et al., 2003; Stal, 2009). In contrast to heterocysts, the diazocytes are not terminally differentiated cells and retain their ability to divide (Fig. 2e; Fredriksson & Bergman, 1995), which may also contribute to their more flexible life style and allow the diazocytes (*Trichodesmium*) to more easily/rapidly adapt to prevailing conditions. To what extent *Trichodesmium* and heterocystous cyanobacteria share additional regulatory mechanisms that govern nitrogen deprivation signaling and pattern formation is now of great interest to be resolved.

**Diazotrophy – separation in time**

The timing of the $N_2$ fixation physiology of *Trichodesmium* is governed by the circadian clock (Chen et al., 1996, 1998; Dong & Golden, 2008). This regulates the transcription of the *nif* genes, the daily *de novo* synthesis of the subunits of the nitrogenase enzyme (NifHDK), a post-translational modification of NifH (Capone et al., 1990; Zehr et al., 1993; Chen et al., 1996), and the supply of appropriate levels of energy and reducing equivalents necessary for $N_2$ fixation activity (Staal et al., 2007).

Hence, it appears that *Trichodesmium* spp. not only separates $N_2$ fixation physically from net oxygen evolution via
the development of a special cell type (diazocytes), although this may be the major protective mechanism, but also separates these two incompatible processes temporarily (Berman-Frank et al., 2001b), although in a more subtle way than in other nonheterocystous cyanobacteria (Bergman et al., 1997). For instance, in contrast to in the latter and in concert with heterocystous cyanobacteria, nitrogenase activity in *Trichodesmium* operates within the day/light phase of the diel cycle, however, at a period around mid-day when the oxygen production is lowered and oxygen-scavenging mechanisms enhanced (respiration/Mehler reaction). This ‘mid-day depression’ in photosynthetic oxygen evolution is manifested as a lower quantum yield (~ 50%) and a low or negative net O₂ evolution (Berman-Frank et al., 2001b). Chlorophyll fluorescence kinetic microscopy at the single-cell level has also revealed flexible temporal and spatial switching between high fluorescence states (within a row of cells) and recovery states during subsequent non-N₂-fixing periods in *Trichodesmium IMS101* (Küpper et al., 2004). This rapid sequential switching within type I cells (functional PSII activity and enhanced Mehler reaction; potentially being the diazocytes) would allow diazotrophy even in cells lacking thick cell walls, may be orchestrated by rearrangements of the phycobilisomes between PSI and PSII (Küpper et al., 2009; Andresen et al., 2010), and may constitute the part of *Trichodesmium*’s nitrogenase-protecting mechanism.

The increase in Mehler reaction (Kana, 1993; Milligan et al., 2007) and dark respiratory activity during the
enzymes involved in central carbon metabolism (Sandh... under diazotrophy, that is, a down-regulation of enzymes verifies a shift toward a catabolic carbon metabolism (Sandh... et al., 2003; Sandh... 2011), oxygen levels at mid-day.

Trichodesmium IMS101 also practices a light/dark (day/night) separation of other basic cellular processes. While the highly energy-demanding processes, such as CO₂ fixation and diazotrophy, take place in light/day phase, cell division and diazocyte development are more pronounced in the dark/night phase (Chen et al., 1999; Sandh... 2009). Temporal separation of similar processes on a diel basis has previously been observed in marine unicellular cyanobacteria (Holtzendorff et al., 2001, 2002; Stockel et al., 2008; Shi et al., 2010). The circadian clock governs many of these processes and is entrained by the cellular ATP/ADP ratio, which in turn is governed by photosynthesis (Rust et al., 2011). Taken together, current data suggest that besides the development of diazocytes, some more subtle physiological mechanisms may act in concert to optimize diazotrophy in Trichodesmium.

Adaptation to nutrient stress

The nitrogen fixed in Trichodesmium is, as in other cyanobacteria, assimilated via the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway, and as in heterocysts, the GS protein levels are higher in the diazocytes (Carpenter et al., 1992) to prevent feedback inhibition of the nitrogenase activity by the accumulation of the ammonia produced. Likewise, externally administrated sources of nitrogen negatively affect the expression of the nif genes, the synthesis of the nitrogenase enzyme, the nitrogenase activity, and diazocyte abundance in Trichodesmium (Ohki et al., 1991; Lin et al., 1998; Mulholland et al., 2001; El-Shehawy et al., 2003; Holl & Montoya, 2005; Sandh... et al., 2011). As in other cyanobacteria (Herrero et al., 2004), when subject to N deprivation, there is a significant upshift in the cellular C : N ratio in Trichodesmium (Kranz et al., 2009), which may be a signal for enhanced transcription by the transcription factor NtcA (Table S1) of N-regulated genes. However, Trichodesmium appears to be flexible in this context, being able to fix N₂ in the presence of low concentrations of dissolved inorganic and organic nitrogen (Holl & Montoya, 2005), and the transcript of ntcA is not exclusively regulated by the availability of, for example, ammonium (Post et al., 2012), which suggests that our knowledge in this area is still limited.

Phosphorus and iron are critical nutrients restricting growth and N₂ fixation in today’s oceans (Sanudo-Wilhelmy et al., 2001; Mills et al., 2004; Sohm et al.,...
To overcome P limitations in oligotrophic waters, the *Trichodesmium* colonies migrate vertically in the water column to scavenge P and other nutrients using a buoyancy-regulating mechanism. This is provided by the pronounced gas vacuoles of *Trichodesmium*, which can withstand pressures down to depth of about 100–200 m (the highest known; Kromkamp & Walsby, 1992). In the upper euphotic zone, the colonies capture and store carbon and nitrogen (as glycogen and cyanophycin granules; Romans et al., 1994), and with this ‘ballast’, the colonies sink into deeper waters where P species may be acquired (Romans et al., 1994; Villareal & Carpenter, 2003; White et al., 2006b; Hewson et al., 2009). As the cellular ballast is metabolized in the deeper darker waters, the subsequently lighter colonies return to the euphotic zone to again capture light energy.

*Trichodesmium* is also known to adjust to periods of low P bioavailability by adopting high cellular N : P ratios (White et al., 2006a) in part by a substitution of its phospholipids by non-P membrane lipids (Van Mooy et al., 2009). Phosphorus uptake is also maximized via the uptake of both inorganic and organic phosphorous species (Stihl et al., 2001; Fu et al., 2005; Dyhrman et al., 2006; Orchard et al., 2009; Beversdorf et al., 2010; White et al., 2010). Enzymes hydrolyzing phosphoesters (alkaline phosphatase; Stihl et al., 2001; Orchard et al., 2009) and phosphonates (e.g. phosphonate hydrolase; Dyhrman et al., 2006) to yield phosphate have been identified in *Trichodesmium*. However, the globally widespread *T. thiebautii* lacks one of the alkaline phosphatase-encoding genes, *phoA* (Orchard et al., 2003). It has been shown that colonial alkaline phosphatase activities in *Trichodesmium* may rather be further enhanced by quorum-sensing signals (acylated homoserine lactones) released from colony-associated microorganisms (Van Mooy et al., 2012). The various phosphate pools available can be utilized either individually or in combination to sustain growth and N₂ fixation (White et al., 2010). Any ‘luxury’ uptake of phosphate is stored in subcellular structures (polyphosphate granules), which are common in natural *Trichodesmium* populations (Romans et al., 1994). As increased polyphosphate storages have been found in phosphate-starved cells (Orchard et al., 2010), the regulation of these subcellular structures in *Trichodesmium* is enigmatic. The variability among *Trichodesmium* species in relation to P-uptake genes (Orchard et al., 2003) also raises the question of niche differentiation and calls for further investigation.

Iron is a pivotal cofactor in a number of cellular processes, such as photosynthesis, N₂ fixation (in nitrogenase), and oxygen scavenging (Kustka et al., 2003; Shi et al., 2007). The high iron content of *Trichodesmium* cells suggests an efficient uptake and detainment capacity of iron and may even make *Trichodesmium* colonies ecologically valuable sources of this otherwise poorly soluble element in oligotrophic oceans (Kustka et al., 2003; Whittaker et al., 2011). No obvious genes in the *Trichodesmium* genome seem to encode for siderophores (Chappell & Webb, 2010), while the genes for transporters of siderophore-bound Fe³⁺ and Fe²⁺ and for enzymes related to cellular storage of iron are present (Castruita et al., 2006; Chappell & Webb, 2010). However, the involvement of siderophores in enhancing the uptake of low iron concentrations in *Trichodesmium* colonies has been reported (Achilles et al., 2003). The current hypothesis is that these siderophores are synthesized by associated microorganisms that indirectly facilitate the uptake of iron by *Trichodesmium* (Achilles et al., 2003). On the other hand, a recent study showed that such siderophore-bound iron is rather consumed by the bacteria than by the *Trichodesmium* cells (Roe et al., 2011). Another option is that the *Trichodesmium* colony formation *per se* facilitates the capture of enough particulate iron from, for example, eolian dust depositions to feed the colonies (Rubin et al., 2011).

Iron depletion is known to lead to a decrease in the frequency of diazocytes (Berman-Frank et al., 2001a; Küpper et al., 2008) and to a down-regulation of N₂ fixation, while photosynthetic capacities are maintained (Shi et al., 2007; Brown et al., 2008; Küpper et al., 2008). Iron limitation may also elicit a switch in the phycobiliproteins being used (Küpper et al., 2008). Other cyanobacteria have also been shown to replace ferredoxin with the iron-free flavodoxin (Sandmann et al., 1990). Monitoring isiB transcription, encoding a flavodoxin, has suggested that *Trichodesmium* populations in the Atlantic Ocean are rarely, or not at all, iron limited, while those in the Pacific Ocean are (Chappell et al., 2012). However, the expression of the two *fld* genes, encoding flavodoxins, in *Trichodesmium* IMS101 is also regulated by N availability and growth stage (Lin et al., 2009; Chappell & Webb, 2010; Sandh et al., 2011), and their proposed use as iron limitation ‘markers’ may be questioned. Rather, the enhanced *idiA* transcription and IdiA levels noted under iron limitation may be a more suitable marker for iron stress in *Trichodesmium* (Webb et al., 2001; Chappell & Webb, 2010). As a strong up-regulation of Dps, yet another protein related to iron acquisition, was observed on the transfer of *Trichodesmium* to diazotrophic conditions (N stress), the role of Dps and other proteins related to iron acquisition and metabolism now also needs attention.

**Impact on the ecosystem**

The *Trichodesmium* abundance is roughly limited to waters warmer than 20 °C, and temperature tolerance for growth...
and N2 fixation in cultured strains of Trichodesmium (T. erythraeum IMS101, T. erythraeum GBRTL1101 and T. tenue H94) ranges from 20 to 34 °C, with optimal temperatures being 24–30 °C depending on species and other growth conditions (Breithbarth et al., 2007; Chappell & Webb, 2010). However, Trichodesmium-like cyanobacteria (nifH and hetR phyllogenies) were recently reported in Arctic waters suggesting wider temperature limits (Diez et al., 2012). Indeed, the very spotty nature of surface blooms of Trichodesmium does not represent the entire population in the ecosystem monitored and illustrates the difficulty in estimating the full global distribution of Trichodesmium via bloom registrations. In spite of this limitation, monitoring such blooms via remote sensing (via the SeaWiFS satellite; Subramaniam et al., 2002) verified that Trichodesmium blooms occur roughly between 20°N and 20°S in the eastern Pacific Ocean and that patches may occur even toward 40°N and 40°S in the Atlantic and the western Pacific and Indian Oceans (1998 and 2003; Westberry & Siegel, 2006).

Besides Trichodesmium, numerous unicellular cyanobacteria share the same marine aquatic environment, notably the small-celled non-N2-fixing genera Prochlorococcus and Synechococcus (cell diameter ~ 1 μm, genome sizes of ~ 2 Mbp; Partensky et al., 1999; Scanlan et al., 2009), but also several N2-fixing unicellular cyanobacteria (Zehr et al., 2001; Montoya et al., 2004; Moisander et al., 2010). Among the latter are representatives of the marine genera Cyaanotheca, Crocosphaera, and N2-fixing cyanobacteria of the ‘group A’ nifH phylotype (e.g. UCYN-A). These diazotrophic unicellular cyanobacteria may show a broader temperature tolerance (15–30 °C) than Trichodesmium, and some have been recovered from waters with detectable nitrate concentrations (Langlois et al., 2005). Observed community shifts from filamentous cyanobacteria in surface waters to unicellular cyanobacteria and/or heterotrophic bacteria in deeper waters (Langlois et al., 2005) may also suggest different ecological niche occupancies. Although estimates of the relative contribution to the total biogenic N2 fixation in oceans by unicellular cyanobacteria (and heterotrophic bacteria) are increasing (Halm et al., 2012; Sohm et al., 2011a,b; Turk et al., 2011; Zehr & Kudela, 2011), the role of Trichodesmium as a C and N source in the world’s oceans is still profound. For instance, Trichodesmium may account for up to 50% of the nifH genes in the North Atlantic Ocean (0°N – 42°N and 67°W – 13°W; Langlois et al., 2008), and Trichodesmium/Katagymnene represent up to 106 nifH genes per liter, while the unicellular cyanobacteria were represented by 105 nifH genes per liter and proteobacteria by 104 nifH genes per liter (Rijkenberg et al., 2011). Tyrrell et al. (2003) reported an even higher colony abundance of Trichodesmium in the tropical Atlantic Ocean (0–15°N and 20°W), and a Trichodesmium surface bloom covered about 100 000 km2 in the Arabian Sea (Capone et al., 1998). For still unknown reasons, large segments of the Trichodesmium population are suddenly trapped at the surface forming easily observed pigmented layers of dying and decomposing cells (‘blooms’) (Capone et al., 1998). Such decomposing blooms function as gigantic ‘fertilizer heaps’ releasing large quantities of carbon, nitrogen, and other nutrients for the benefit of nondiazotrophic and heterotrophic biota in the surrounding water bodies. The cause of this destructive ‘bloom’ phenomenon is unknown, while the involvement of viral infections (Hewson et al., 2004) and/or autocatalyzed cell death processes (Berman-Frank et al., 2004) have been proposed, but the question is still open. The nitrogen fixed by Trichodesmium may, in addition, enter marine food webs via grazing by tunicates, copepods, and fish (Roman, 1978; Bryceson, 1980; Carpenter, 1983; Oneil & Roman, 1994; Eberl & Carpenter, 2007). Nitrogen isotope ratios in zooplankton in the North Atlantic Ocean strongly indicate that N2 fixation is a major source of nitrogen for the marine zooplankton community (Montoya et al., 2002). This is at the same time unexpected, as the Trichodesmium toxin production has been inferred as a predator-deterring function (Layton et al., 2008) as well as a cause of death of several eukaryotic organisms, notably the copepod Acartia tonsa (Guo & Tester, 1994), several species of fish (Endean et al., 1993), and pearl oysters (Negri et al., 2004). Trichodesmium is now known to release different secondary metabolites, such as toxins, including the lipophilic chlorinated trichotoxin (Schock et al., 2011), the palytoxin causing clupeotoxism in humans via fish ( Kerbrat et al., 2011), and the neurotoxin β-N-methylamino-L-alanine (Cox et al., 2005), the latter also found in diazotrophic bloom-forming cyanobacteria in the Baltic Sea (Jonasson et al., 2010). The ecological function of these toxins is still unknown.

Yet another factor that may play a role besides the mere cell number of an organism is their cell size/cell volume. As illustrated in Fig. 4, the size of Trichodesmium cells (approximate sizes given) makes the cellular volumes of this organism ‘gigantic’ compared to, for instance, cells of the unicellular genus Prochlorococcus and the diazotrophic genus Crocosphaera. As about 10–20 cells in each Trichodesmium filament are diazocytes (filled with NifH; Fig. 2b) and a large fraction of the nitrogen fixed may be released (as dissolved organic nitrogen or ammonium) from actively growing Trichodesmium populations (Capone et al., 1994; Gilbert & Bronk, 1994; Mulholland & Capone, 2001; Mulholland et al., 2004; Mulholland, 2007), each Trichodesmium cell and filament may be viewed as a highly important source of new nitrogen for all nondiazotrophic small cells (unicellular cyanobacteria...
and bacteria) when sharing similar N-depleted aquatic marine environments.

**Trichodesmium in future scenarios**

The global importance of *Trichodesmium* in oceanic biogeochemistry has triggered numerous studies mimicking future global warming scenarios. As mentioned above, the optimum temperature range for growth and nitrogen fixation of *Trichodesmium* is 24–30 °C (Breithbarth et al., 2007; Chappell & Webb, 2010), but *Trichodesmium* also survives lower temperatures and darkness (White et al., 2006b; Breithbarth et al., 2007). The ability to live at lower temperatures and in darkness is essential for their vertical migrations (White et al., 2006b) and may explain the occurrence of *Trichodesmium* in temperate (see LaRoche & Breithbarth, 2005) and potentially in cooler (Diez et al., 2012) waters. Global warming will lead to increased stratification resulting in shallower mixed layers and increased irradiance. High light intensity (up to 1000 μE) stimulates growth, diazocyte abundance, and N₂ fixation in *Trichodesmium* (Andresen et al., 2010; Kranz et al., 2010; Levitan et al., 2010) and provokes changes in pigment composition (Andresen et al., 2010), and correlates with increased O₂ evolution and CO₂ fixation (Kranz et al., 2010). Higher light intensities also cause faster protein turnover (Andresen et al., 2010) and an increase in RuBisC/O:PSII ratio (Brown et al., 2008). The down-regulation in O₂ production via PSII correlates with the earlier noted peaks in N₂ fixation at noon (Berman-Frank et al., 2001b). Increased pCO₂ levels will not only stimulate CO₂ fixation, but also stimulate N₂ fixation (dependent on carbon skeletons for sequestration of the ammonium produced) and growth in *Trichodesmium* (Hutchins et al., 2007; Levitan et al., 2007, 2010; Ramos et al., 2007; Kranz et al., 2009, 2010). One mechanism may be energy relocation from the costly carbon-concentrating mechanism (CCM; Badger et al., 2006; Kranz et al., 2011) toward CO₂ and N₂ fixation (Levitan et al., 2007; Kranz et al., 2011). This may in turn increase the release of the newly fixed N into the surrounding water body, thereby enhancing primary production of other organisms (Hutchins et al., 2007). Hence, in a scenario of increased temperatures and CO₂ concentrations in the world’s oceans, the abundance of surface blooms of *Trichodesmium* is expected to increase (Breithbarth et al., 2007; Hutchins et al., 2007; Levitan et al., 2007), unless other unforeseen natural factors provoke the opposite reaction. For instance, element colimitations, rather than single-element limitations, may regulate the growth and N₂ fixation of natural *Trichodesmium* populations (Mills et al., 2004; Hutchins et al., 2007). Also, Garcia et al. (2011) have shown that the positive effect of higher CO₂ concentrations (on, e.g., N₂ fixation) is primarily seen at lower light intensities, and Rijkenberg et al. (2011) stress that the stimulations expected may be counteracted by decreased supply of nutrients from deeper waters as a consequence of enhanced stratification.

**Conclusions and future outlooks**

In 1968, Fay et al. (1968) proposed that the reducing conditions in the cyanobacterial cell type recognized as ‘heterocysts’ could be the site for N₂ fixation. In 1973, Fleming & Haselkorn (1973) were the first to isolate nitrogenase from such heterocysts. Hence, heterocysts have during four decades acted as the ‘consensus model’ for successful light-driven N₂ fixation in cyanobacteria. In 1991, Bergman and Carpenter were the first to show that nitrogenase is localized in subsets or short strings of cells (diazocyte) in *Trichodesmium*. These are now recognized as a prerequisite for the light-driven N₂ fixation in *Trichodesmium*. This developmental mechanism is combined

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*Fig. 4.* Schematic illustration of approximate cell size differences of *Trichodesmium* species and some unicellular cyanobacteria. The approximate cell sizes and volumes of different species of *Trichodesmium* are compared to the cell volumes of representatives of the nondiazotrophic but ubiquitous unicellular cyanobacterial genus *Prochlorococcus* and the unicellular diazotrophic genus *Crocosphaera*. Note the many-fold larger volume of the *Trichodesmium* cells. Hatched line shows maximum cell sizes. *Trichodesmium* cell sizes are according to Janson et al. (1995).
with mechanisms temporarily lowering oxygen evolution and orchestrating energy-competing processes in a multifaceted fashion. Hence, *Trichodesmium* is ‘second-to-none’ among potent daytime N₂ fixers, as is in particular evidenced by its great global ecological impact. However, many questions still remain. These include identification of genes/proteins that underpin the development of the diazocytes and their regulation, including mechanisms involved in the protection of the oxygen-sensitive nitrogenase. Because freshwater species exist within the (former) genus *Katagyneme* (see e.g. Komárek & Anagnostidis, 2005), an intriguing question is whether these are capable of developing diazocytes. Sequencing additional genomes within this globally important genus will allow comparative genomic analyses and potentially shed light on, for example, its uniquely low DNA coding proportion and the significance of its apparently expanding genome. Besides genomic analyses, other ‘omics’ and ‘meta-omics’ approaches (transcriptomics, proteomics, and metabolomics) need to be introduced if we are to comprehend the unique N₂-fixing physiology of *Trichodesmium* at all organization levels. ‘Metomics’ may provide more accurate information pertaining to the genetic diversity and the role of *Trichodesmium* and associated microorganisms than cultures, which often represents a minor part of the total species radiation. Such data may also reveal mutual and potentially life-sustaining interplays between *Trichodesmium* and the numerous associated microorganisms and open exciting research avenues into microbial evolution and marine microbial interphylum interactions, some potentially of a symbiotic nature. Focus should also be given to *Trichodesmium*-affiliated cyanobacteria with unusual diazotrophic behavior are yet other compelling research areas to explore.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:
Fig. S1. The Trichodesmium nif region.
Table S1. Gene orthologs related to heterocyst differentiation.

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