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REVIEW

Interactions between CCM and N_2 fixation in *Trichodesmium*

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Abstract In view of the current increase in atmospheric pCO_2 and concomitant changes in the marine environment, it is crucial to assess, understand, and predict future responses of ecologically relevant phytoplankton species. The diazotrophic cyanobacterium *Trichodesmium erythraeum* was found to respond strongly to elevated pCO_2 by increasing growth, production rates, and N_2 fixation. The magnitude of these CO_2 effects exceeds those previously seen in other phytoplankton, raising the question about the underlying mechanisms. Here, we review recent publications on metabolic pathways of *Trichodesmium* from a gene transcription level to the protein activities and energy fluxes. Diurnal patterns of nitrogenase activity change markedly with CO_2 availability, causing higher diel N_2 fixation rates under elevated pCO_2. The observed responses to elevated pCO_2 could not be attributed to enhanced energy generation via gross photosynthesis, although there are indications for CO_2-dependent changes in ATP/NADPH + H^+ production. The CO_2 concentrating mechanism (CCM) in *Trichodesmium* is primarily based on HCO_3^- uptake. Although only little CO_2 uptake was detected, the NDH complex seems to play a crucial role in internal cycling of inorganic carbon, especially under elevated pCO_2. Affinities for inorganic carbon change over the day, closely following the pattern in N_2 fixation, and generally decrease with increasing pCO_2. This down-regulation of CCM activity and the simultaneously enhanced N_2 fixation point to a shift in energy allocation from carbon acquisition to N_2 fixation under elevated pCO_2 levels. A strong light modulation of CO_2 effects further corroborates the role of energy fluxes as a key to understand the responses of *Trichodesmium*.

Keywords CO_2 concentrating mechanism · Diazotroph · Energy allocation · N acquisition · Ocean acidification · Photosynthesis

Introduction

Marine phytoplankton are responsible for almost half of all photosynthetic carbon fixation on Earth and play a vital role in altering the CO_2 exchange between ocean and atmosphere (Gruber 2004; Maier-Reimer et al. 1996). Some prokaryotic algae affect the primary productivity and thus CO_2 uptake capacity of the oceans by yet another process, the fixation of dinitrogen (N_2) into biomass. As nitrate is often limiting phytoplankton growth, so-called diazotrophs play a crucial role in many marine ecosystems by providing a new source of biologically available nitrogen.

One of these diazotrophs, *Trichodesmium*, is able to form massive blooms known as "sea-sawdust", covering large areas of the surface ocean in the tropical and subtropical regions (Capone et al. 2005; Mahaffey et al. 2005). The first mention of *Trichodesmium* was made in 1770 by Captain Cook in the Coral Sea near Australia. In 1839, Charles Darwin described a bloom by this species during his cruise with the Beagle as "The whole surface of the water, as it appeared under a weak lens, seemed as if covered by chopped bits of hay, with their ends jagged." In 1961, Dugdale and colleagues reported the "ability of *Trichodesmium* to fix atmospheric nitrogen." Recent estimates on its contribution to overall marine N_2 fixation range up to 50% (Mahaffey et al. 2005) and in oligotrophic

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areas of the oceans, *Trichodesmium* is responsible for a major part of the primary production (Falkowski 1997; Gruber and Sarmiento 1997). Henceforward, *Trichodesmium* was acknowledged to exert a significant influence on the global nitrogen and carbon cycle.

As a key species in the marine ecosystem, *Trichodesmium* was used in several studies on the regulation of N_2 fixation (e.g., Berman-Frank et al. 2001a, b, 2007; Capone et al. 2005; Kana 1993; Mulholland et al. 2004). Unlike other diazotrophs, this species has evolved special features allowing N_2 fixation to occur during the photoperiod. To protect the oxygen-sensitive enzyme nitrogenase, which catalyzes the reduction of N_2 to NH_3, from photosynthetic O_2 evolution, this species has developed distinct diurnal rhythms in photosynthesis and N_2 fixation (Berman-Frank et al. 2001b). By now, the genome of *Trichodesmium* IMS101 has been fully sequenced (US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/) and several aspects of its ecophysiology have been studied, e.g., effects of phosphorus, iron limitation, temperature, salinity, and irradiance (Berman-Frank et al. 2001a; Breitbarth et al. 2008; Fu and Bell 2003). The potential influence of CO_2-induced changes in seawater chemistry or combined effects of different environmental factors have, however, been ignored for a long time.

Four recent studies tested the effect of different CO_2 concentrations on growth, biomass production, and elemental composition of *Trichodesmium* (Barcelos é Ramos et al. 2007; Hutchins et al. 2007; Kranz et al. 2009; Levitan et al. 2007). They concordantly demonstrated higher growth and/or production rates under elevated pCO_2, with a magnitude exceeding those CO_2 effects previously seen in other marine phytoplankton. Nonetheless, the responses differed significantly in terms of absolute rates, cell quotas or C/N ratios (Table 1). For instance, the stimulation in N_2 fixation and/or PON production between present-day pCO_2 values (370–400 µatm) and those predicted for the year 2100 (750–1,000 µatm) ranged between 35 and 240%. Several of these laboratory studies also observed higher rates of growth or carbon fixation under elevated pCO_2, yet the degree of stimulation differed. Responses in cell quotas or elemental stoichiometry varied in degree as well as direction with changes in pCO_2.

These large differences in CO_2 sensitivity obtained by the different studies raise questions about experimental conditions other than the applied pCO_2 levels. It should be noted that all of the mentioned studies used the same *Trichodesmium* isolate (IMS101) from the Atlantic Ocean and even the same artificial seawater media (YBCII). They differed, however, in the light intensities applied during incubations, which may serve as an explanation for the differences in CO_2 sensitivity. A recent companion study assessed the combined effect of pCO_2 (150 vs. 900 µatm) and light (50 vs. 200 µmol photons m^{-2} s^{-1}) and could indeed show that light levels do modify the responses of *Trichodesmium* to pCO_2 (Kranz et al. 2010a; Levitan et al. 2010b). Notably, the relative stimulation in growth, POC, or PON production rates was highest in the low-light treatment and diminished under high light (Fig. 1). These observations not only corroborate the exceptionally high CO_2 sensitivity in the N_2 fixing *Trichodesmium*, but also reconcile previous findings.

But how is the process of N_2 fixation coupled to the availability of CO_2? Or why are CO_2 effects on growth and

Table 1 Comparison of rates and elemental composition for *Trichodesmium* IMS 101 grown at different pCO_2 levels and 25°C

Conditions		Rates and elemental composition				References
Light (µmol photons m^{-2} s^{-1})	pCO_2 (µatm)	Growth μ (d^{-1})	C/N ratios (mol:mol)	N_2 fixation PON production	C fixation POC production	
80–120				nmol N (mg Chl *a*)$^{-1}$ h^{-1}	mol C (mol Chl *a*)$^{-1}$ s^{-1}	Levitan et al. (2007)
	400	0.17	6.5	1.3	0.126	
	900	0.26	7.0	3.1	0.155	
~100				µmol N (mg Chl *a*)$^{-1}$ h^{-1}	mg C (mg Chl *a*)$^{-1}$ h^{-1}	Hutchins et al. (2007)
	380	0.35	5.1	14.8	1.25	
	750	0.39	5.1	20.0	1.75	
~150				fmol N_2 (fmol P)$^{-1}$ h^{-1}	n.d.	Barcelos é Ramos et al. (2007)
	380	0.44	5.3	0.06		
	800	0.48	4.8	0.11		
~150				µmol N (mg Chl *a*)$^{-1}$ h^{-1}	µmol C (mg Chl *a*)$^{-1}$ h^{-1}	
	370	0.27	4.6	11.4	51.7	Kranz et al. (2009)
	1,000	0.34	4.5	14.9	67.6	

The reported values relate to present day (370–400 µatm) and future scenarios for pCO_2 (750–1,000 µatm) used in the different studies. Please note the differences in normalization and light levels

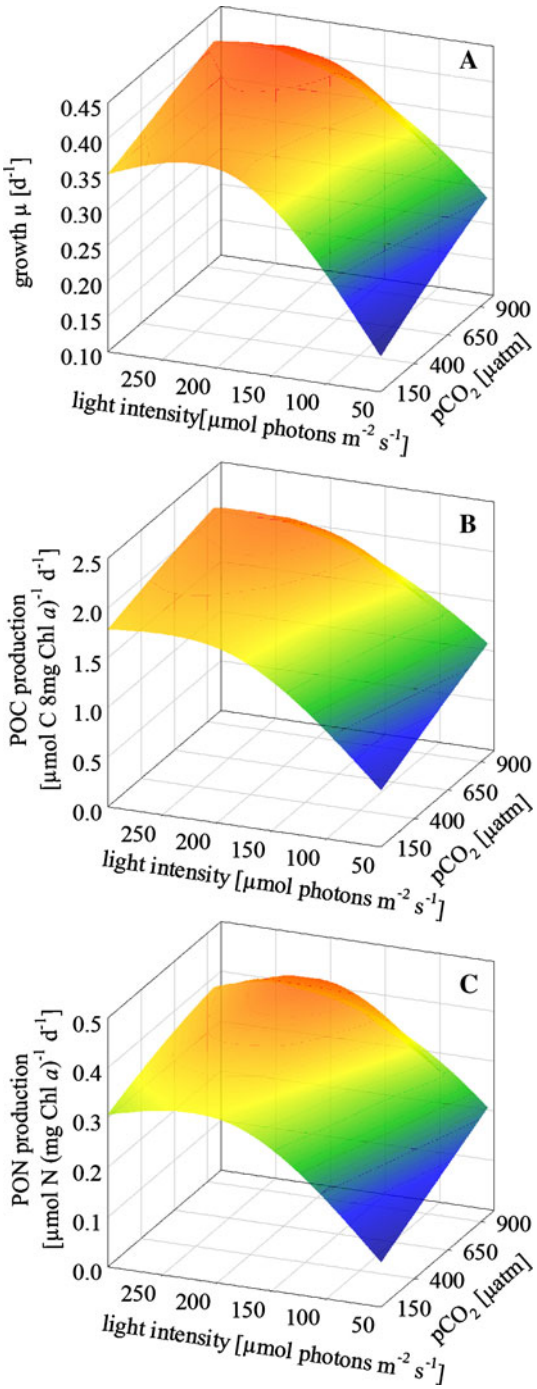

Fig. 1 Combined effects of pCO_2 and light. Plots are based on data taken from Kranz et al. (2010a) and Eichner et al. (unpublished; pCO_2 180 vs. 950 μatm at 300 μmol photons m^{-2} s^{-1}). **a** Growth rates, **b** production rates of particulate organic carbon (POC), and **c** production rates of particulate organic nitrogen (PON)

biomass production modulated by light availability? These and other questions regarding the underlying reasons for the strong CO_2 effects in *Trichodesmium* can only be answered if the responses of physiological key processes are understood. In the following, the processes of N_2 fixation, photosynthesis, and carbon acquisition in *Trichodesmium* will be described and discussed, focusing on the influence of light and CO_2 as well as possible interactions.

N_2 fixation

Thriving in the oligotrophic regions of the ocean, *Trichodesmium* mainly fuels its N demand by fixation of N_2 (Mulholland et al. 2004). The reduction of N_2 is conducted by the enzyme nitrogenase, which consists of two proteins, the iron protein (dinitrogenase reductase) and the iron–molybdenum protein (dinitrogenase). This ancient enzyme evolved under O_2-free conditions in the Archean (Falkowski 1997; Falkowski and Raven 2007) and its iron protein is especially sensitive to O_2 (Bergman et al. 1997). Thus, photosynthetic energy generation and N_2 fixation within the same cell appear to be mutually exclusive processes. To circumvent this inhibitory effect, diazotrophic organisms evolved biochemical as well as morphological adaptations to separate photosynthetic O_2 evolution and N_2 fixation in time and space (Berman-Frank et al. 2007). *Trichodesmium* differs from other diazotrophs in this respect, as it lacks the clear spatial (i.e., heterocysts) and temporal separation (day vs. night activity) of the two processes (Berman-Frank et al. 2007).

In *Trichodesmium*, nitrogenase is localized in subsets of neighboring cells, so-called diazocytes, which comprise about 15–20% of cells within a trichome (Berman-Frank et al. 2003; Durner et al. 1996; Fredriksson and Bergman 1995). In contrast to heterocysts, diazocytes contain both, photosystem I (PSI) and photosystem II (PSII) (Bergman et al. 1997) and can, hence, conduct oxygenic photosynthesis (Fig. 2). To protect nitrogenase from photosynthetically produced O_2, *Trichodesmium* has developed a distinct diurnal rhythm in PSII activity and N_2 fixation (Berman-Frank et al. 2001b; Lin et al. 1999). In combination with O_2-reducing mechanisms like the Mehler reaction (Berman-Frank et al. 2001b; Küpper et al. 2004; Milligan et al. 2007), N_2 fixation in *Trichodesmium* reaches rates similar or even higher than those reported for heterocystous or other non-heterocystous cyanobacteria (Bergman et al. 1997). As only the diazocytes are capable of fixing N_2, the residual cells of a filament depend strongly on the supply of bioavailable N from the N_2 fixing cells. These N sources are either the direct outcome of the N_2 reduction, i.e., ammonia (NH_3) or ammonium (NH_4^+), or the amino acid glutamine (Mulholland et al. 2004; Mulholland and Capone 2000; Wannicke et al. 2009), which is the product of the glutamine synthetase (GS) reaction. The inorganic or organic N sources released by the diazocytes

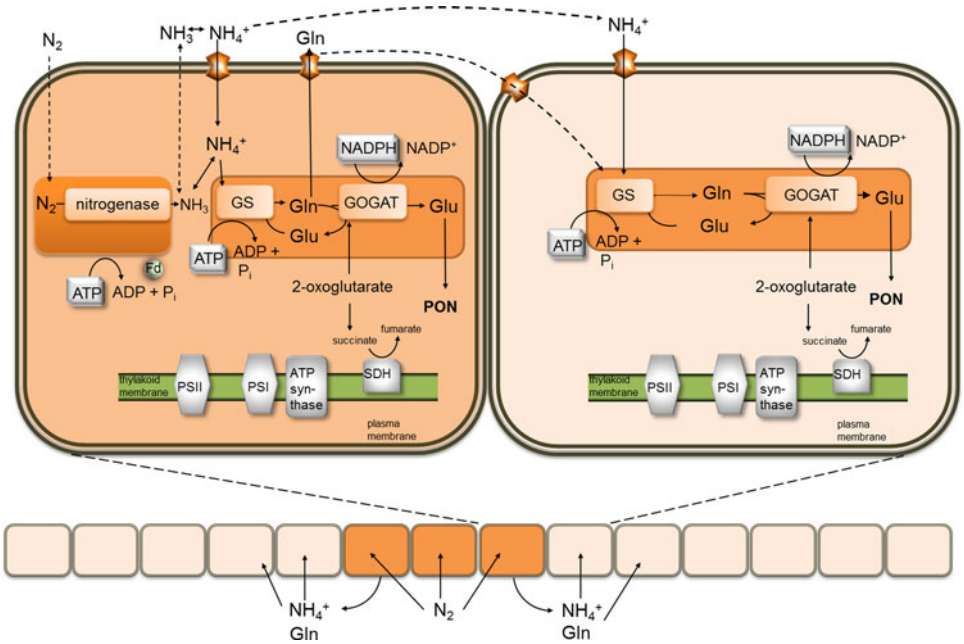

Fig. 2 A conceptual model of N_2 fixation and N cycling within for *Trichodesmium*. One filament is shown consisting of several single cells, with *dark orange* representing the N_2 fixing cells (diazocytes) while the *bright orange cells* represent the non-N_2-fixing vegetative cells. Processes of N acquisition in those types of cells are depicted. N_2 diffuses into the diazocytes, where it is reduced to NH_4^+. The latter is then converted into Glu by the GS/GOGAT reaction. The inorganic and organic N sources can be released/transported into the surrounding media and subsequently taken up by the non-N_2-fixing cells of *Trichodesmium*. Oxygenic photosynthesis as well as respiration is conducted in both types of cells, generating the energy and reductants as well as carbon skeletons required for N assimilation. *ADP* adenosine-5′-diphosphate, *ATP* adenosine-5′-triphosphate, *ATP synthase* adenosine-5′-triphosphate synthase, *Glu* glutamate, *GOGAT* glutamine oxoglutarate aminotransferase, *Gln* glutamine, *GS* glutamine synthetase, P_i inorganic phosphorus, *PON* particulate organic nitrogen, *PSI* photosystem 1, *PSII* photosystem 2, *SDH* succinate dehydrogenase

can subsequently be taken up by the vegetative cells to fuel their N demand (Fig. 2, Mulholland et al. 2004).

In terms of the diurnal regulation, N_2 fixation was shown to be controlled on different levels. Diazocytes develop primarily during the dark period at the same time when cell division takes place in *Trichodesmium* (Sandh et al. 2009). In these diazocytes, the nitrogenase is synthesized de novo each morning and for the most part degraded during the afternoon and night (Capone et al. 1990; Levitan et al. 2010a; Sandh et al. 2009). On the post-translational level, part of the iron protein pool is modified to an inactive form in the afternoon, which can persist throughout the night, being activated again in the morning (Zehr et al. 1993). The switch between active and inactive state of the iron protein is regulated by ADP-ribosylation via the activating enzyme dinitrogenase reductase-activating glycohydrolase (DRAG; Halbleib and Ludden 2000), which requires ATP and $MnCl_2$ in a specific ratio (Saari et al. 1986). Both, the transcription of nitrogenase and the post-translational modification of its iron protein were shown to be controlled by an endogenous rhythm (Chen et al. 1998). Rates of N_2 fixation followed the diurnal pattern in nitrogenase abundance and its post-translational modification, with highest activities during midday (Berman-Frank et al. 2001b; Levitan et al. 2010a, 2007; Milligan et al. 2007). The timing of both, demodification of the iron protein and nitrogenase activity was shown to be affected by light intensities (Kranz et al. 2010a; Zehr et al. 1996). The described diurnal pattern in cell division, protein build-up and degradation, as well as physiological activity may represent a mean for *Trichodesmium* to optimize its energy budget.

Regarding energy requirements for N_2 fixation, the splitting of the triple-bond of N_2 to form NH_3 requires at least 16 ATP as well as eight electrons:

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

The subsequent production of glutamine and glutamate within the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) reaction further demands energy in form of 1 ATP and 1 NADPH + H^+ per glutamate produced. Thus, N acquisition in *Trichodesmium* is strongly dependent on availability of energy. Although respiratory processes may contribute some of the energy, most of the ATP and reductants are provided through photosynthesis (Bergman et al. 1997; Breitbarth et al. 2008). Light appears to play a crucial role both as the

source of energy for N_2 fixation (Breitbarth et al. 2008; Kranz et al. 2010a) and as a cue for synthesis and/or demodification of nitrogenase (Zehr et al. 1993). N_2 fixation rates, obtained from acetylene reduction assays, have been shown to increase up to light intensities of about 300 µmol photons m^{-2} s^{-1} (Bell and Fu 2005; Breitbarth et al. 2008). As cell densities of *Trichodesmium* are often highest at depths of 20–40 m (Capone et al. 1997), prevailing light intensities may commonly limit the energy supply to nitrogenase in natural populations (Sanudo-Wilhelmy et al. 2001).

The increase in N_2 fixation or PON production under elevated pCO_2 (Barcelos é Ramos et al. 2007; Hutchins et al. 2007; Kranz et al. 2009; Levitan et al. 2007) was found to be caused by a prolongation of the N_2 fixation period (Kranz et al. 2010a). As these enhanced N_2 fixation rates were not accompanied by larger protein pools of nitrogenase (Levitan et al. 2010b), they may have been achieved by post-translational modification and/or higher energy availability for nitrogenase activity. Higher rates of N_2 fixation also requires more C skeletons in the form of 2-oxoglutarate, which are provided via the tri-carboxylic acid pathway, for instance, during high rates of respiration in the early hours of the photoperiod (Berman-Frank et al. 2001b; Kranz et al. 2009). Enhanced respiration rates at elevated pCO_2, which could be expected from the stimulation in PON production, have, however, not been reported.

As a direct effect of CO_2 on nitrogenase seems unlikely, a higher share of energy to nitrogen fixation was suggested to be the cause for the observed stimulation in N_2 fixation and PON production at high pCO_2 (Kranz et al. 2010a). This explanation is further substantiated by the observation that the CO_2 effects in *Trichodesmium* were strongly modulated by light (Fig. 1). Yet, what is the source of the additional energy and resources supporting the observed stimulation in N_2 fixation and PON production under elevated pCO_2? To answer this question, one must first look at photosynthetic energy production and how it responds to changes in CO_2 availability.

Photosynthesis and electron transport

The energy for metabolic processes is provided by the phosphorylation of ADP as well as the reduction of $NADP^+$ due to photosynthetic and respiratory electron transport. The electron transport chain (ETC) in the thylakoid membrane of cyanobacteria differs in several important aspects from that of eukaryotes. As cyanobacteria lack chloroplasts, both photosynthesis and respiration are conducted on the thylakoid membrane, sharing specific protein complexes (Schmetterer 1994), including the plastoquinone (PQ) pool, the cytochrome b_6f (cyt b_6f) complex, and plastocyanin (Fig. 3). A concerted regulation of photosynthetic and respiratory electron flow is, hence, required to avoid detrimental feedbacks and/or photodamage.

During photosynthesis, electrons are introduced into the ETC via PSII, while respiration feeds electrons into the PQ

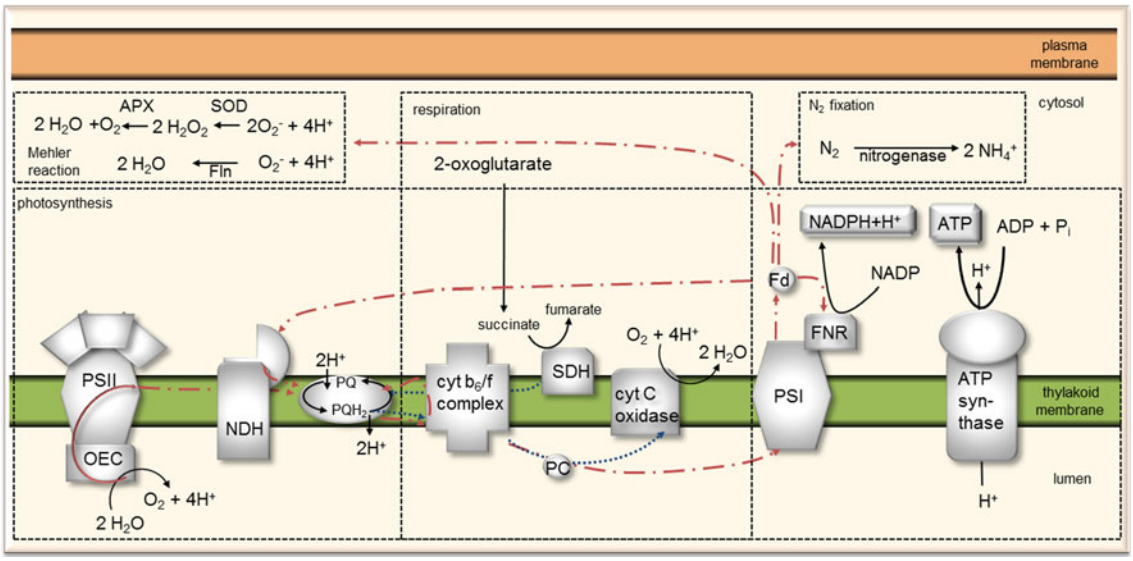

Fig. 3 Schematic representation of major cellular complexes involved in energy transport (photosynthesis, respiration, N_2 fixation, and Mehler reaction). *Red lines* represent photosynthetic electron flux, while *blue lines* represent respiratory electron flux. *ADP* adenosine-5′-diphosphate, *APX* ascorbate peroxidase, *ATP* adenosine-5′-triphosphate, *ATP synthase* adenosine-5′-triphosphate synthase, *Cyt b_6/f complex* cytochrome b_6/f protein complex, *Cyt C oxidase* cytochrome *C* oxidase, *Fd* ferredoxin, *Fln* flavoprotein, *FNR* ferredoxin NADP reductase, H^+ proton, *NADP* nicotinamide-adenine-dinucleotide-phosphate, *NDH* NADPH dehydrogenase, *OEC* oxygen evolving complex, *PC* plastocyanin, *PQ* plastoquinone, *PSI* photosystem 1, *PSII* photosystem 2, *SDH* succinate dehydrogenase, *SOD* superoxide dismutase

pool via a succinate dehydrogenase (SDH) (Schmetterer 1994). Thus, photosynthetic as well as respiratory electron transport generate the proton motive force needed for ATP production (Mitchell 1961). Electrons from plastocyanin can be allocated to cytochrome *C* oxidase (cyt *C* oxidase), the terminal electron acceptor of the respiratory ETC, leading to the reduction of O_2 to H_2O. During the dark, all electrons from respiration are donated to the cyt *C* oxidase, while in the light, cyt *C* oxidase competes with PSI for electrons (Milligan et al. 2007). The flux of electrons to O_2, producing H_2O at the cyt *C* oxidase, is likely to be influenced by the ratio of PSI:PSII and the location of the phycobilisomes (Küpper et al. 2004), but may also be affected by kinetic constraints of the terminal oxidase and PSI (Milligan et al. 2007). In contrast to eukaryotes, the ratio of PSI:PSII in cyanobacteria is usually above one and highly variable (Fujita et al. 1994). For *Trichodesmium*, reported values cover a large range from 1.3 (Berman-Frank et al. 2001b, 2007) to values between 2 and 4 (Brown et al. 2008; Levitan et al. 2010b, 2007) and up to values as high as 24 (Subramaniam et al. 1999). Thus, the high ratio of PSI:PSII allows for efficient electron flow through PSI, preventing an overly high-redox state of the components of the ETC.

From PSI, electrons can be transferred to ferredoxin and allocated to a ferredoxin NADP reductase (FNR), producing the reductant $NADPH + H^+$ in the so-called linear electron transport (Vermaas 2001). In addition to linear electron transport, several alternative electron transport routes have been found to operate. In the so-called cyclic electron transport around PSI, electrons transferred from PSI to ferredoxin can be fed back to the cyt b_6f complex, thereby increasing proton translocation and thus ATP synthesis (Stroebel et al. 2003). Electrons needed in N_2 fixation are allocated from the reduced ferredoxin onto nitrogenase (Flores and Herrero 1994). Therefore, N_2 fixation represents a strong quenching mechanism in *Trichodesmium*, yielding an oxidized PQ pool and improved electron transport. Alternatively, electrons can be transferred from ferredoxin onto O_2 in the pseudo-cyclic electron transport called the Mehler reaction. Such an electron transfer does not necessarily lead to H_2O_2 production by superoxide dismutase (SOD) as superoxide can directly be reduced to H_2O by a flavoprotein in cyanobacteria (Helman et al. 2003). Nonetheless, H_2O_2 was shown to be present in *Trichodesmium* cells during the time of N_2 fixation, which could only be explained by SOD activity (Berman-Frank et al. 2001b). High rates of Mehler reaction have been found in *Trichodesmium* both in laboratory experiments (Kranz et al. 2010a; Levitan et al. 2007; Milligan et al. 2007) and in the field (Berman-Frank et al. 2001b; Kana 1993). The Mehler reaction has been suggested to serve as a nitrogenase protection mechanism as it can strongly decrease the O_2 concentrations in the vicinity of nitrogenase (Berman-Frank et al. 2001b; Kana 1993; Milligan et al. 2007). However, light-dependent $^{18}O_2$ uptake was also observed at times of low nitrogenase activity (Kranz et al. 2010a) or under conditions when N_2 fixation was repressed (Milligan et al. 2007; Eichner et al. unpublished). In addition, measurements of $^{18}O_2$ uptake showed that Mehler reaction can function as a photoprotection mechanism in *Trichodesmium* at high light intensities (Kranz et al. 2010a). In any case, the transfer of electrons to either N_2 fixation or Mehler reaction needs to be balanced in order to prevent inhibition of nitrogenase by O_2 and electron shortage for nitrogenase.

The energy produced in photosynthesis and respiration is subsequently allocated according to the needs of the different metabolic pathways. The largest share of ATP and reductants is used for the fixation of CO_2 in the Calvin Cycle (Table 2). Other major energy sinks in *Trichodesmium* are the fixation of N_2 and the active uptake of inorganic carbon. All these pathways compete for energy but differ in their proportional demands for ATP and reductants (Table 2). CO_2 fixation needs a ratio of ATP:NADPH + H^+ of 3:2, while N_2 fixation requires ATP and electrons in a ratio of 2:1. Inorganic carbon uptake also requires energy in the form of ATP and reductants, however, not much is known about respective requirements for this process in *Trichodesmium*. To cover the energetic demand of these processes, which vary strongly over the photoperiod, a concerted regulation in the production of ATP and reductants is necessary. Linear electron transport yields ATP and NADPH + H^+ in a ratio close to one, while non-linear electron flow raises this ratio significantly. In *Trichodesmium*, the high PSI:PSII ratios and alternative electron flow indicate generally higher generation of ATP than of NADPH + H^+, which may serve to satisfy the need for a high ATP:NADPH + H^+ ratio imposed by N_2 fixation.

As energy supply to the different metabolic pathways is first and foremost driven by light availability, efficient photosynthesis is crucial for an organism like *Trichodesmium* with its high energy demands. In its natural habitat, *Trichodesmium* is subject to a highly variable light regime and so it is not surprising that *Trichodesmium* shows high plasticity in light acclimation (Andresen et al. 2010). These acclimation mechanisms include modifications in pigment composition, enhanced protein turnover (Andresen et al. 2010) and state transition, i.e., reversible uncoupling of phycobilisomes or individual pycobiliproteins from PSI and PSII (Küpper et al. 2004). This high flexibility in light capture efficiency enables effective energy generation by *Trichodesmium*, thereby avoiding drawbacks like D1 protein degradation or superoxide production at PSII.

The effects of different pCO_2 levels on photosynthetic electron generation and its subsequent utilization in

Table 2 Relative energy demand for carbon and nitrogen fixation for *Trichodesmium*

Process	Energy demand			References
	ATP	Electrons from $NADPH + H^+$ or Fd_{red}	ATP:$NADPH + H^+$	
C fixation	18	24	1.5	Allen (2002)
N acquisition	9	6	3	Flores and Herrero (1994)

Estimated values relate to the proportional demand relative to units of N, assuming a C:N ratio of 6. N acquisition includes assimilation steps from N_2 to glutamate. For calculation of the ATP:$NADPH + H^+$ ratios, the quantity of electrons needed in N_2 fixation was converted to a corresponding amount of $NADPH + H^+$ produced in linear electron transport. Please note that the active C uptake would add to the energetic cost for C assimilation, while N_2 can diffuse into the cell. The energetic demand of the different C acquisition systems is, however, still under debate

Trichodesmium have been examined using MIMS measurements (Kranz et al. 2009, 2010a; Levitan et al. 2007), chlorophyll fluorescence measurements (Levitan et al. 2007, 2010a), and protein analysis (Levitan et al. 2010b). These studies showed that gross O_2 production and PSII chlorophyll fluorescence were not affected by the availability of CO_2, although changes were found in the composition of the ETC, with an increased ratio of PSI to PSII at elevated pCO_2 (Levitan et al. 2010b). This finding indicates a higher capacity for cyclic electron transport around PSI, allowing for an increase in ATP production at the expense of NADP reduction. Considering the high ratio of ATP:reductant required in N_2 fixation (Table 2), this may in turn contribute to the observed stimulation in nitrogenase activity under elevated pCO_2. Nonetheless, as the applied pCO_2 levels did not alter the overall energy generation it cannot explain the observed stimulation in growth and biomass production. In consequence, one must search for other causes of the apparent surplus of energy under elevated pCO_2. As inorganic carbon (C_i) uptake and its subsequent fixation represent the largest energy sink (Table 2), these processes certainly need to be considered to understand CO_2-dependent changes in the energy budget of *Trichodesmium*.

Inorganic carbon acquisition

As autotrophs, cyanobacteria use energy to produce organic carbon compounds from inorganic CO_2 in the Calvin Cycle, the first step being catalyzed by the enzyme Ribulose bisphosphate carboxylase/oxygenase (RubisCO). This ancient enzyme evolved, similarly to the nitrogenase, during times of elevated CO_2 levels and low O_2 concentrations and is characterized by a very low-affinity for its substrate CO_2, a slow maximum turnover rate, as well as a susceptibility to a competing reaction with O_2 (Badger and Andrews 1987; Tortell 2000). Cyanobacterial RubisCO has one of the highest half-saturation concentrations for CO_2 of all autotrophic organisms (K_M of 105–185 μmol l^{-1} CO_2; Badger et al. 1998). Consequently, RubisCO represents a potential bottle neck for primary production by cyanobacteria in today's oceans. All cyanobacterial species investigated to date, however, possess mechanisms to enhance the carboxylation efficiency of their RubisCO, reducing the risk of carbon limitation. These processes are commonly referred to as CO_2 concentrating mechanisms (CCMs; Badger et al. 2006; Giordano et al. 2005; Kaplan and Reinhold 1999). Although CCM functioning should significantly repress the oxygenase function of RubisCO (Colman 1989), photorespiration has recently been found to occur under certain conditions in *Synechocystis* PCC6803 (Eisenhut et al. 2008). If operative in *Trichodesmium*, this pathway would allow decreasing the O_2 concentration in the vicinity of nitrogenase.

In cyanobacteria, the operation of a CCM generally involves a suite of CO_2 and HCO_3^- acquisition systems, as well as a distinct assembly of RubisCO and carbonic anhydrase (CA) within so-called carboxysomes (Badger and Price 2003; Cannon et al. 2001; Price et al. 2008; Raven 2003). Characterization of cyanobacterial C_i uptake has thus far revealed five different systems, primarily based on studies with cyanobacterial strains such as *Synechococcus* spp. and *Synechocystis* spp. These five C_i acquisition systems comprise two inducible high-affinity HCO_3^- transporters (BCT1, Omata et al. 1999; SbtA, Shibata et al. 2002), a constitutive low-affinity HCO_3^- transporter (BicA, Price et al. 2004), a constitutive low-affinity system to convert CO_2 into HCO_3^- (CO_2 uptake facilitator) at the NDH-1 complex located at the thylakoid membrane (NDH-1_4, Maeda et al. 2002; Price et al. 2002; Shibata et al. 2001), as well as an inducible high-affinity CO_2 uptake facilitator based on a modified NDH-1 complex (NDH-1_3, Maeda et al. 2002; Shibata et al. 2001). All of these measures function to increase the HCO_3^- concentration in the cytosol of the cell. The accumulated HCO_3^- passes the protein shell of the carboxysome and, catalyzed by a CA, gets converted to CO_2 for subsequent fixation by RubisCO. The differences in the composition of the carboxysomes and the details of their genetic assembly allow

differentiating the genera of cyanobacteria into two classes, the α- and β-cyanobacteria. Further information on the role of carboxysomes in the cyanobacterial CCM can be found in several reviews (Badger et al. 2006; Cannon et al. 2001; Kaplan and Reinhold 1999; Price et al. 2002). All in all, a large diversity in CCMs has evolved that allows cyanobacteria to grow over a wide range of C_i concentrations. Differences in CCMs may, however, explain why certain species like *Trichodesmium* respond strongly to changes in C_i availability.

Genomic analysis for the CCM in *Trichodesmium* revealed that it belongs to the β-cyanobacteria and its CCM comprises only few of the known CCM components (Badger et al. 2006; Price et al. 2008). Two C_i acquisition systems have been identified in *Trichodesmium*, the HCO_3^- transporter BicA and the CO_2 uptake facilitator NDH-1_4 (Fig. 4). In contrast to other marine β-cyanobacteria (e.g., *Anabaena*, *Crocosphaera*, *Lyngbya*, *Nodularia*, *Synechococcus PCC7002*), *Trichodesmium* lacks CO_2-responsive genes (*CcmR*/*CmpR*) as well as genes associated with high-affinity C_i acquisition systems (NDH-1_3, BCT1, SbtA; Price et al. 2008). In view of the strong CO_2 dependence in growth and biomass production (Barcelos é Ramos et al. 2007; Hutchins et al. 2007; Kranz et al. 2009, 2010a; Levitan et al. 2007), one could assume that the CCM of *Trichodesmium* functions only insufficiently to saturate the carboxylation reaction of RubisCO (Raven et al. 2005). Physiological studies have, however, revealed

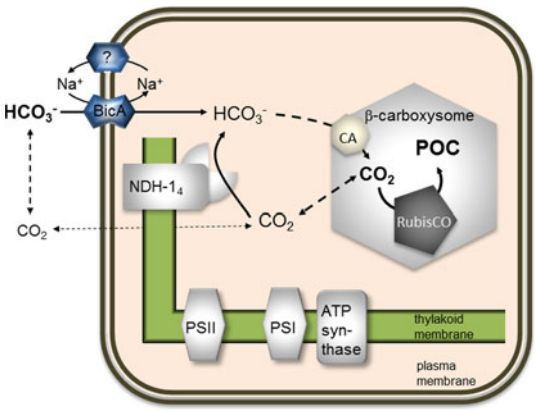

Fig. 4 A schematic model for the CO_2 concentrating mechanism in *Trichodesmium*, based on genetic homologies (Price et al. 2008). BicA represents the low-affinity, high-flux rate HCO_3^- transporter, which is driven by a Na^+ gradient. The buildup of the Na^+ gradient needed to drive the BicA transporter is not fully understood yet. NDH-1_4 represents a low-affinity CO_2 uptake facilitator located at the thylakoid membrane. The NDH-1_4 is thought to be involved in cyclic electron transport of photosynthesis. *ATP synthase* adenosine-5′-triphosphate synthase, *CA* carbonic anhydrase, *POC* particulate organic carbon, *PSI* photosystem 1, *PSII* photosystem 2, *RubisCO* ribulose bisphosphate carboxylase/oxygenase

C_i affinities high enough to saturate rates of photosynthesis under CO_2 concentrations typical for the present-day ocean (Kranz et al. 2009).

In *Trichodesmium*, inorganic carbon was found to be supplied mainly by the uptake of HCO_3^- via the BicA transporter (Kranz et al. 2009, 2010a). This transporter was first described in the marine cyanobacterium *Synechococcus* PCC7002 (Price et al. 2004) as being a low-affinity, high-flux transporter for HCO_3^-. Further characterization revealed that BicA is dependent on a sodium (Na^+) gradient across the plasma membrane, which energize a Na^+/HCO_3^- symporter (Espie and Kandasamy 1994). The Na^+ extrusion is thought to be operated via a plasma membrane located, so-called Mnh complex (Woodger et al. 2007). Alternatively, the Na^+ gradient might be generated by a H^+/Na^+ antiport via PxcA (Price et al. 2008). Despite uncertainties about the actual functioning, genes encoding both Mnh and PxcA are present in *Trichodesmium*. Provided that these complexes exchange H^+ and Na^+, the overall process might mitigate the cytosolic alkalinization resulting from HCO_3^- uptake.

The gene encoding BicA appears to be constitutively expressed (Price et al. 2004), at least in the freshwater cyanobacteria *Synechocystis* PCC6803. Only recently, also in *Trichodesmium* the regulation of CCM genes was investigated over a range of CO_2 concentrations, different temperatures and over the photoperiod (Levitan et al. 2010c), showing constitutive gene expression for *bicA1*, *ndhF4* (encoding subunits of BicA and NDH-1_4) as well as carboxysomal-related genes. Despite constitutive gene expression, a high plasticity in response to different pCO_2 (150–1,000 µatm) was found in the affinities of the HCO_3^- transport (Kranz et al. 2009). The apparent discrepancy between CCM gene expression and CCM activities points to a post-translational modification of BicA or alterations in the Na^+ gradient driving this transporter. Next to the influence of pCO_2 on HCO_3^- uptake affinities, the CCM activity of *Trichodesmium* was greatly affected by the diurnal rhythm in N_2 fixation and photosynthesis (Kranz et al. 2009).

Even though CO_2 uptake via NDH-1_4 plays only a minor role in C_i uptake of *Trichodesmium*, this component of the CCM might be important to avoid efflux of CO_2 from the cytosol. NDH-1_4 is a protein complex located at the thylakoid membrane and its subunit, the chpX, converts CO_2 into HCO_3^-, by using electrons from reduced ferredoxin or NADPH + H^+ (Fig. 4). Due to its ability to use electrons from the ETC and to drive H^+ translocation through the thylakoid membrane, this protein may be involved in cyclic electron flow around PSI and thus contribute to ATP production (Price et al. 2002). In Kranz et al. (2010a), it was shown that CO_2 utilization by NDH-1_4 increases with elevated pCO_2 as well as light intensities, which may

consequently enhance the production of ATP and fuel the respective needs of processes such as N_2 fixation. Based on isotopic fractionation data as well as MIMS-based carbon fluxes, it was further demonstrated that the CO_2 efflux in *Trichodesmium* is moderate (20–50% of total C_i uptake) and internal C_i cycling via the NDH-1_4 is highly efficient, especially under high CO_2 concentrations (Kranz et al. 2010a). This C_i cycling might yield an energetic surplus, both by preventing the efflux of previously taken up carbon as well as by the buildup of the H^+ gradient over the thylakoid membrane.

In conclusion, the CCM of *Trichodesmium* appears to be strongly affected by light intensities, competing processes like N_2 fixation, as well as by changes in CO_2 availability. This complex interplay of factors can be explained by the high energetic costs of the CCM. An effect of light levels on C_i affinities has been observed in several species from different taxa (Beardall and Giordano 2002), which was ascribed to changes in the degree of energy limitation on active C_i uptake. Energy limitation on the CCM can, however, also be imposed by N_2 fixation, simply by competing for energy at times of high nitrogenase activities. This would explain the diurnal changes in C_i affinities following N_2 fixation (Fig. 5). Last but not least, similarly to many other species (Giordano et al. 2005), the availability of CO_2 was shown to strongly alter the CCM in *Trichodesmium* (Kranz et al. 2009, 2010a). Such high plasticity in CCM regulation may serve as a reason for the observed CO_2-dependent stimulation in growth and biomass production. Considering energy and its allocation as a key to the observed phenomena also explains why the benefit from down-regulation of the CCM is highest under low light intensities (Figs. 1, 5).

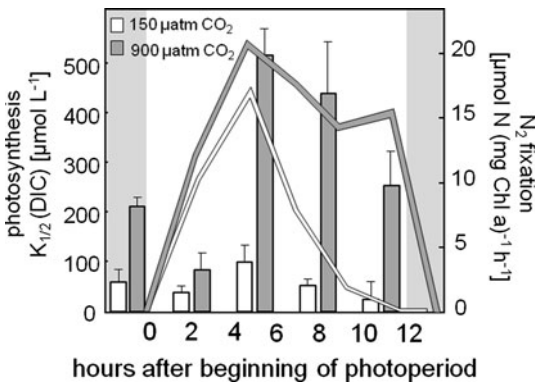

Fig. 5 Half-saturation concentrations ($K_{1/2}$ DIC) of photosynthesis (*bars*) and rates of N_2 fixation (*lines*) in *Trichodesmium* over a diurnal cycle. $K_{1/2}$ values were taken from Kranz et al. (2009), while rates of N_2 fixation were taken from Kranz et al. (2010a). Please note the differences in light intensities between studies (150 vs. 200 µmol photons m^{-2} s^{-1})

Ecological implications

Owing to the poor catalytic properties of RubisCO (Badger et al. 1998), the energetic costs of the CCM are high but its operation is nonetheless crucial for *Trichodesmium*. This is especially true during bloom conditions, when pH levels rise and dissolved inorganic carbon (DIC) concentrations can be significantly lowered in the ambient seawater (Kranz et al. 2010b). In addition, the aggregation of several hundreds of filaments to so-called puffs or tufts, a typical phenomenon for *Trichodesmium* under bloom conditions, can lead to a distinct boundary layer effect with lowered DIC concentration in the close vicinity of the cells (Kranz et al. 2010b). The reduced C_i availability during the progression of a bloom coincides with decreasing levels of other nutrients, which may in turn also influence the CCM.

Regulation of the CCM in response to changes in N availability has been reported for different phytoplankton species (Beardall and Giordano 2002). In *Chlamydomonas reinhardtii*, for instance, the CCM was down-regulated under N depletion (Giordano et al. 2003), which may reflect lower carbon demand and serves to maintain the C:N ratio relatively constant. Controlling elemental stoichiometry is of fundamental importance for ensuring optimal functioning of the enzymatic machinery (Beardall and Giordano 2002). In *Dunaliella tertiolecta* and *Chlorella emersonii*, however, affinities for CO_2 were enhanced under N depletion (Beardall et al. 1991; Young and Beardall 2005). This type of CCM regulation may serve to improve the N use efficiency by reducing the N requirement for synthesis of RubisCO (Beardall et al. 1991; Raven 1991). By suppressing photorespiration, up-regulation of CCMs under N-limitation may also prevent the exudation of downstream products such as glycolate and thus the loss of N (Giordano et al. 2005).

For diazotrophs like *Trichodesmium*, a regulation of the CCM would intuitively seem to be uncoupled from N availability as N_2 fixers are not dependent on scarce nitrogen sources such as NO_3^- or NH_4^+ or dissolved organic nitrogen. The acquisition of C and N are, however, coupled via their demand for energy (cf. Fig. 5). In *Dunaliella salina*, an increase in CCM activity was measured in cells grown on NH_4^+ instead of NO_3^-, supposedly due to increased energy availability to the CCM in the case of the more reduced nitrogen source (Giordano 1997; Giordano and Bowes 1997). Similarly, changes in the availability of dissolved organic nitrogen compounds or NH_4^+ (released by the diazocytes or provided by other sources) might influence CCM activities in *Trichodesmium* by altering the energy demand of N assimilation. As C and N assimilation compete for energy, the interaction will be strongly influenced by the overall energy supply.

For *Trichodesmium*, effects of CO_2 on growth and production were shown to be light dependent (Fig. 1). In its natural environment, *Trichodesmium* is subject to a wide range of light intensities, mostly because of the vertical motion of the cells in the euphotic zone (Villareal and Carpenter 1990). The CO_2 effect on *Trichodesmium* will therefore differ depending on its vertical distribution in the water column. As CO_2 effects on *Trichodesmium* proved to be diminished at higher light intensities, which prevail close to the ocean's surface, the current rise in pCO_2 levels will mainly affect cells residing in deeper water layers. The predicted shoaling of the upper mixed layer will yield an increase of average light intensities, while nutrient availability will diminish (Doney 2006). As NO_3^- depleted areas will expand, the importance of diazotrophs like *Trichodesmium* may increase. Data on CO_2 dependency of N_2 fixation rates from recent publications suggest that N_2 fixation by *Trichodesmium* spp. might increase by more than 20 Tg N a^{-1} to about 100 Tg N a^{-1} until the end of this century (Hutchins et al. 2009). These predictions, however, do not consider the light modulation of CO_2 effects nor the possible changes in the distribution pattern of *Trichodesmium*. Future predictions on the overall N_2 fixation should thus include interactive effects on physiological and ecological aspects, i.e., changes in the rates of processes and shifts in the dominance of diazotrophs.

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