Carbon acquisition by *Trichodesmium*: The effect of pCO$_2$ and diurnal changes

Sven A. Kranz  
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Dieter Sülttemeyer  
Fachbereich Biologie, Technische Universität Kaiserslautern, Postfach 3049, D-67653 Kaiserslautern, Germany

Klaus-Uwe Richter and Björn Rost  
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

**Abstract**

We investigated carbon acquisition by the N$_2$-fixing cyanobacterium *Trichodesmium* IMS101 in response to CO$_2$ levels of 15.1, 37.5, and 101.3 Pa (equivalent to 150, 370, and 1000 ppm). In these acclimations, growth rates as well as cellular C and N contents were measured. In vivo activities of carbonic anhydrase (CA), photosynthetic O$_2$ evolution, and CO$_2$ and HCO$_3^-$ fluxes were measured using membrane inlet mass spectrometry and the $^{13}$C disequilibrium technique. While no differences in growth rates were observed, elevated CO$_2$ levels caused higher C and N quotas and stimulated photosynthesis and N$_2$ fixation. Minimal extracellular CA (eCA) activity was observed, indicating a minor role in carbon acquisition. Rates of CO$_2$ uptake were small relative to total inorganic carbon (Ci) fixation, whereas HCO$_3^-$ contributed more than 90% and varied only slightly over the light period and between CO$_2$ treatments. The low eCA activity and preference for HCO$_3^-$ were verified by the $^{13}$C disequilibrium technique. Regarding apparent affinities, half-saturation concentrations ($K_{1/2}$) for photosynthetic O$_2$ evolution and HCO$_3^-$ uptake changed markedly over the day and with CO$_2$ concentration. Leakage (CO$_2$ efflux; Ci uptake) showed pronounced diurnal changes. Our findings do not support a direct CO$_2$ effect on the carboxylation efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) but point to a shift in resource allocation among photosynthesis, carbon acquisition, and N$_2$ fixation under elevated CO$_2$ levels. The observed increase in photosynthesis and N$_2$ fixation could have potential biogeochemical implications, as it may stimulate productivity in N-limited oligotrophic regions and thus provide a negative feedback on rising atmospheric CO$_2$ levels.

Marine phytoplankton contribute up to 50% of global primary production (Falkowski et al. 1998) and influence Earth’s climate by altering various biogeochemical cycles (Schlesinger 2005). In this respect, phytoplankton can be distinguished into so-called functional types, which affect these cycles differently. Next to diatoms (silicifiers) and coccolithophores (calcifiers), diazotrophic cyanobacteria (dinitrogen-fixers) contribute largely to overall marine primary production. The current increase in atmospheric CO$_2$ and rising sea-surface temperature are bound to affect phytoplankton communities in numerous ways (Boyd and Doney 2002). In view of potential ecological implications and feedbacks on climate, several studies have investigated CO$_2$ sensitivity in key phytoplankton species, mainly focusing on the groups of diatoms and coccolithophores (Nielsen 1995; Burkhardt and Riebesell 1997; Rost et al. 2003).

Diazotrophic cyanobacteria affect marine ecosystems by providing reactive nitrogen to otherwise nitrogen-limited regions. The filamentous nonheterocystous cyanobacterium *Trichodesmium* thrives in oligotrophic areas of tropical and subtropical seas. Forming large blooms, this species contributes about half of all marine N$_2$ fixation (Mahaffey et al. 2005). In contrast to other diazotrophs, *Trichodesmium* 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). This intriguing species has been investigated by several studies focusing on the effects of phosphorus and iron limitations as well as temperature, salinity, and irradiance (Berman-Frank et al. 2001a; Fu and Bell 2003; Breitbarth et al. 2007). The potential influence of CO$_2$-induced changes in seawater chemistry, however, has been ignored until very recently.

Barcelos e Ramos et al. (2007), Levitan et al. (2007), and Hutchins et al. (2007) observed a strong increase in photosynthesis and N$_2$ fixation in *Trichodesmium* under elevated CO$_2$ levels. This trend is predominantly attributed to changes in cell division (Hutchins et al. 2007; Levitan et al. 2007) but also altered elemental ratios of carbon to nitrogen (Levitan et al. 2007) or nitrogen to phosphorus (Barcelos e Ramos et al. 2007). Despite differences in their findings, e.g., in terms of absolute rates or elemental ratios,
the magnitudes of these CO₂ effects exceed those previously seen in other marine photoautotrophs. The underlying processes responsible for the strong CO₂ sensitivity in this important diazotroph are currently unknown.

Understanding CO₂ sensitivity in photosynthesis, which provides the energy for growth and any other downstream processes, requires information about modes of carbon uptake and fixation in phytoplankton. Most of the reductive power and energy generated in the light reactions of photosynthesis are allocated for assimilation of inorganic carbon (Ci) and subsequent reduction (Falkowski and Raven 2007). A large proportion of these costs is associated with the operation of so-called CO₂ concentrating mechanisms (CCMs), which function to increase the carboxylation reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This enzyme evolved during times of elevated CO₂ levels and is characterized by very low affinity for its substrate CO₂, a slow maximum turnover rate, as well as a susceptibility to a competing reaction with O₂. Since cyanobacterial RubisCO has one of the highest half-saturation constants ever measured (Kₘ of 105–185 μmol L⁻¹ CO₂; Badger et al. 1998), this group has to invest considerable resources into the CCM to avoid the risk of carbon limitation as well as the wasteful process of photorespiration. This CCM involves active uptake of CO₂ and/or HCO₃⁻ as well as carbonic anhydrase (CA), which catalyzes the otherwise slow conversion between HCO₃⁻ and CO₂. Processes that minimize the CO₂ efflux from the cell are also important components of an efficient CCM. To date, there are no physiological studies on these central processes in *Trichodesmium*.

In the present study, we investigated the physiological responses of *Trichodesmium IMS101* to different CO₂ levels, comparing Last Glacial Maximum (15.1 Pa), present-day (37.5 Pa), and projected upper CO₂ values for the year 2100 (101.3 Pa; Raupach et al. 2007). To assess diurnal changes in these treatments, responses were generally measured at different time intervals over the photoperiod. In each CO₂ treatment, responses in growth rates, elemental ratios, and rates of photosynthesis and photorespiration were determined using an inverted microscope (Zeiss, Axiovert S50, Graticules). The average cell size for each pCO₂ treatment was estimated from total alkalinity (TA), pCO₂, temperature, and salinity using the program CO2Sys (Lewis and Wallace 1998). The carbonate chemistry for the respective pCO₂ treatments is given in Table 1.

### Methods

**Culture conditions**—Stock cultures of *Trichodesmium erythraeum* IMS101 (isolated by Prufert-Bebout et al. 1993) were grown at 25°C in a 12:12 h light:dark (LD) cycle at 150 μmol photons m⁻² s⁻¹ in 0.2-μm-filtered unbuffered YBCII media (Chen et al. 1996). For experiments, semicontinuous batch cultures were grown in 1-liter custom-made cylinder flasks (diameter 7 cm) at the same temperature and light regime. Air containing three different CO₂ partial pressures (pCO₂) of 15.1, 37.5, and 101.3 Pa (equivalent to 150, 370, and 1000 ppm) was sparged continuously through the cultures. CO₂ gas mixtures were generated with gas-mixing pumps (Digamix 5KA188-F and 5KA368-F, Woesthoff GmbH), using CO₂-free air (Nitrox CO₂ RP280; Domnick Hunter GmbH), pure CO₂ (Air Liquide Deutschland GmbH), or ambient air. Regular dilution with fresh, pre-acclimated media ensured that the carbonate chemistry remained constant and that the cells stayed in the midexponential growth phase. Cultures in which the pH had shifted in comparison to cell-free media at the respective pCO₂ (pH drift >0.06) were excluded from further analysis. Total alkalinity was measured in duplicate by potentiometric titration and calculated from linear Gran plots (Gran 1952). The carbonate system was calculated from total alkalinity (TA), pCO₂, phosphate, temperature, and salinity using the program CO2Sys (Lewis and Wallace 1998). Equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) were chosen.

### Table 1. Parameters of the seawater carbonate system calculated from pCO₂, alkalinity, pH, phosphate, temperature, and salinity using the CO2Sys program (Lewis and Wallace 1998) (n=3; ±SD).

<table>
<thead>
<tr>
<th>pCO₂ (Pa)</th>
<th>pH (NBS)</th>
<th>CO₂ (μmol L⁻¹)</th>
<th>DIC (μmol L⁻¹)</th>
<th>TA (μEq L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.1</td>
<td>8.56 ±0.03</td>
<td>3.9±0.3</td>
<td>1879±24</td>
<td>2535±12</td>
</tr>
<tr>
<td>37.5</td>
<td>8.26 ±0.03</td>
<td>9.9±0.7</td>
<td>2113±20</td>
<td>2535±12</td>
</tr>
<tr>
<td>101.3</td>
<td>7.89 ±0.03</td>
<td>27.2±1.9</td>
<td>2322±16</td>
<td>2535±12</td>
</tr>
</tbody>
</table>

**Growth, elemental composition, and fixation rates**— Cultures were acclimated to the respective pCO₂ for at least 14 d (>5 generations) before measuring. In general, samples were taken at the beginning of the photoperiod to account for diurnal changes. Cell densities were determined using an inverted microscope (Zeiss, Axiovert 200) by measuring the number of filaments, length, and cell size in a Sedgwick-Rafter Cell (S50, Graticules). The average cell size for each pCO₂ treatment was estimated based on the length of individual filaments and corresponding cell counts (>10,000 individual counts). Samples for chlorophyll a (Chl a) measurements were filtered onto cellulose nitrate filters (Sartorius) and stored at −80°C. Chl a was subsequently extracted in 5–10 mL acetone (overnight in darkness, at 4°C) and determined with a fluorometer (Turner Designs).

Samples for particulate organic carbon (POC) and nitrogen (PON) were filtered onto precombusted (500°C; 9 h) GFF filters and stored in precombusted (500°C; 9 h) petri dishes at −20°C. Prior to analysis, filters were treated with 200 μL HCl (0.1 μmol L⁻¹) to remove all inorganic carbon. POC and PON were subsequently measured in duplicate on an EA mass spectrometer (ANCA-SL 2020).
Growth rates were determined based on changes in cell density, Chl a concentration, as well as POC and PON, respectively, and are given as mean values. Growth rates (µ) were calculated as:

\[ \mu (d^{-1}) = \frac{\ln(N_1) - \ln(N_0)}{\Delta t} \]  

where \( N_0 \) and \( N_1 \) are concentrations of cells, Chl a, POC, or PON at time \( t_0 \) and \( t_1 \), respectively, and \( \Delta t \) is the time between sampling intervals. Production rates of PON (\( P_N \)) and POC (\( P_C \)) per day were calculated according to the following equations:

\[ P_N = \mu \times PON \times (Chl \, a)^{-1} \]  

\[ P_C = \mu \times POC \times (Chl \, a)^{-1} \]  

_Determination of CA activity—_After a minimum of 14 d acclimation to the respective pCO2, cells were concentrated by gentle filtration over a membrane filter (pore size 8 µm; Isopore, Millipore). The culture media was stepwise exchanged with the respective assay medium, and CA activities were determined using a membrane inlet mass spectrometer (MIMS). The system consisted of a temperature-controlled cuvette, a membrane-inlet (polytetrafluoroethylene membrane, 0.01 mm), and a sectorfield multi-collector mass spectrometer (Isoprime; GV Instruments). Gas molecules dissolved in solution permeated through the membrane and were ionized, and, depending on their mass:charge ratio (m/z), ions were then separated and detected.

CA activity was determined from the \(^{18}\text{O} \) depletion of doubly labelled \(^{13}\text{C}^{18}\text{O}_2 \) in water caused by several hydration and dehydration steps of CO2 and HCO\(_3^− \) (Silverman 1982). The reaction sequence of \(^{18}\text{O} \) loss from initial \(^{13}\text{C}^{18}\text{O}^{18}\text{O} \) (m/z = 49) via the intermediate \(^{13}\text{C}^{18}\text{O}^{16}\text{O} \) (m/z = 47) to the final isotopomer \(^{13}\text{C}^{18}\text{O}^{16}\text{O} \) (m/z = 45) was recorded simultaneously. The \(^{18}\text{O} \) enrichment was calculated as:

\[ \log \text{(enrichment)} = \log \frac{(13\text{C}^{18}\text{O}_2) \times 100}{(13\text{C}^{16}\text{O}_2 + 13\text{C}^{18}\text{O}^{18}\text{O} + 13\text{C}^{18}\text{O}_2)} \]  

\[ = \log \frac{m/z 49 \times 100}{m/z 45 + m/z 47 + m/z 49} \]  

CA measurements were performed in 8 mL of YBCII medium buffered with 2-(4-[2-hydroxyethyl]-1-piperazinyl)ethanesulfonic acid (HEPES, 50 mmol L\(^{-1} \), pH 8.00) at 25°C. If not stated otherwise, all measurements were carried out in the dark to avoid interference with light-dependent carbon uptake by the cells. Bicarbonate was added (1 mmol L\(^{-1} \) NaH\(^{13}\text{C}^{18}\text{O}_3 \)), and once the chemical equilibrium was reached, the uncatalyzed rate of \(^{18}\text{O} \) loss was recorded for at least 5 min. Subsequently, 100–200 µL of concentrated cell suspension were added to the media to yield a final Chl a concentration of 0.5–2.5 µg mL\(^{-1} \). For calculation of extracellular CA activities (eCA), the increasing rate of \(^{18}\text{O} \) depletion after addition of the cells (S\(_2\)) was compared to the uncatalyzed reaction (S\(_1\)) and normalized on a Chl a basis (Badger and Price 1989):

\[ U = \frac{(S_2 - S_1) \times 100}{S_1 \times \mu \text{g Chl a}} \]  

Consequently, 100 units (U) correspond to an enhancement in the interconversion between HCO\(_3^− \) and CO\(_2 \) relative to the spontaneous rate by 100% per µg Chl a. Following the eCA measurements, light was added (300 µmol photons m\(^{-2} \) s\(^{-1} \)) to monitor light-induced changes in the \(^{18}\text{O} \) exchange. This method is indicative of active transport of Ci, as there will be an enhanced influx of labelled Ci into the cell to the active site of internal CA, resulting in an increase of the \(^{18}\text{O} \) loss (Badger and Price 1989).

Intracellular CA (iCA) activity was determined in the presence of 50 µmol L\(^{-1} \) dextran-bound sulfonamide (DBS), a membrane-impermeable inhibitor of eCA. The activity of iCA was estimated from the rapid decline in log (enrichment) upon the injection of cells, defined as Δ, and calculated according to Palmqvist et al. (1994). Values of Δ are expressed per µg Chl a.

_Determination of photosynthesis and Ci fluxes—_The O\(_2 \) and Ci fluxes were determined during steady-state photosynthesis with the same MIMS as for the CA measurements. The method established by Badger et al. (1994) is based on simultaneous measurements of O\(_2 \) and CO\(_2 \) during consecutive light and dark intervals. Known amounts of inorganic carbon were added to measure photosynthesis and carbon uptake rates as a function of CO\(_2 \), HCO\(_3^− \), or dissolved inorganic carbon (DIC) concentrations. Photosynthesis, CO\(_2 \) uptake, and HCO\(_3^− \) uptake were calculated according to the equations of Badger et al. (1994). Cells were harvested in the same manner as for the CA measurements using CO\(_2\)-free YBCII medium (50 mmol L\(^{-1} \) HEPES, pH 8.00) and transferred into the cuvette before DBS was added (final concentration of 50 µmol L\(^{-1} \)). Light and dark intervals during the assay lasted 6 and 5 min, respectively. The incident photon flux density was 300 µmol photons m\(^{-2} \) s\(^{-1} \). Chl a concentrations in the assay ranged from about 0.5 to 4 µg mL\(^{-1} \). Further details on the method and calculations are given in Badger et al. (1994) and Rost et al. (2007).

_Determination of photosynthesis and Ci fluxes—_The O\(_2 \) and Ci fluxes were determined during steady-state photosynthesis with the same MIMS as for the CA measurements. The method established by Badger et al. (1994) is based on simultaneous measurements of O\(_2 \) and CO\(_2 \) during consecutive light and dark intervals. Known amounts of inorganic carbon were added to measure photosynthesis and carbon uptake rates as a function of CO\(_2 \), HCO\(_3^− \), or dissolved inorganic carbon (DIC) concentrations. Photosynthesis, CO\(_2 \) uptake, and HCO\(_3^− \) uptake were calculated according to the equations of Badger et al. (1994). Cells were harvested in the same manner as for the CA measurements using CO\(_2\)-free YBCII medium (50 mmol L\(^{-1} \) HEPES, pH 8.00) and transferred into the cuvette before DBS was added (final concentration of 50 µmol L\(^{-1} \)). Light and dark intervals during the assay lasted 6 and 5 min, respectively. The incident photon flux density was 300 µmol photons m\(^{-2} \) s\(^{-1} \). Chl a concentrations in the assay ranged from about 0.5 to 4 µg mL\(^{-1} \). Further details on the method and calculations are given in Badger et al. (1994) and Rost et al. (2007).
Carbon acquisition by Trichodesmium

Results

Growth, elemental ratios, and fixation rates—To assess the overall sensitivity of *Trichodesmium* to different CO₂ levels (15.1, 37.5, and 101.3 Pa), responses in growth rates, elemental ratios, rates of photosynthesis, and production rates of particulate organic nitrogen were measured. Growth rates were determined during mid-expontential growth phase based on cell counts, Chl *a*, POC, and PON. The mean growth rate was 0.31 ± 0.04 d⁻¹ (Fig. 1a) and did not differ significantly between pCO₂ treatments (*p* = 0.378; one way ANOVA). The C : N ratios (4.6 ± 0.1) and Chl *a*:cell ratios (1.0 ± 0.2) did not differ between the treatments. However, POC and PON increased from 4.1 ± 0.6 pmol C cell⁻¹ and 0.9 ± 0.1 pmol N cell⁻¹ at 37.5 Pa to 5.4 ± 0.6 pmol C cell⁻¹ and 1.2 ± 0.1 pmol N cell⁻¹ at 101.3 Pa CO₂ (Figs. 1b,c). The corresponding POC and PON production rates increased from 51.7 ± 8.0 to 67.6 ± 7.4 μmol C (mg Chl *a*)⁻¹ h⁻¹ and from 11.4 ± 2.2 to 14.9 ± 1.8 μmol N (mg Chl *a*)⁻¹ h⁻¹, representing a stimulation in carbon and nitrogen fixation by almost 40%.

In terms of diurnal changes, carbon and nitrogen contents per cell showed distinct patterns leading to strong changes in C : N ratios (Fig. 2a). During the course of the day, the C : N ratio increased from 4.76 ± 0.04 at the onset of the photoperiod to 4.91 ± 0.04 around midday (09:00 h–12:00 h). It decreased to 4.48 ± 0.08 during the afternoon (12:00 h–17:00 h) and subsequently increased to 4.95 ± 0.09 toward the scotoperiod (21:00 h). This diurnal variation in the C : N ratio indicates distinct differences in the patterns of carbon or nitrogen fixation over the day. Rates of photosynthesis and respiration (based on O₂ evolution) as determined by MIMS also showed pronounced diurnal changes in all acclimations (Fig. 2b). Rates of photosynthesis decreased by 48% during the first 3 h of the photoperiod, while dark respiration increased by 102%. After reaching lowest and highest rates around midday, respectively, this pattern reversed, and photosynthesis increased while dark respiration decreased toward the end of the photoperiod (Fig. 2b).

Carbonic anhydrase activities—External CA activity determined by MIMS directly reflects the acceleration in the conversion between CO₂ and HCO⁻₃ relative to the spontaneous rate. In *Trichodesmium*, eCA activities were about 50 ± 10 units (μg Chl *a*)⁻¹, and they neither varied between treatments nor over the photoperiod (data not shown). The activity of internal CA remained constant in all acclimations and was near the detection limit, i.e., Δ values were about 0.25 ± 0.08 (μg Chl *a*)⁻¹ following calculations of Palmqvist et al. (1994).

The ¹⁸O exchange technique can provide information about active Ci transport systems. As shown in Fig. 3, illumination resulted in an enhanced uptake of ¹⁸O-labelled ¹³CO₂ (m/z 49 and 47) and a large efflux of unlabelled ¹³CO₂ (m/z 45), leading to a light-dependent decrease in log (enrichment). Similar patterns were observed in all acclimations and throughout the photoperiod.

Photosynthetic O₂ evolution and carbon fluxes—Photosynthesis and Ci uptake are shown as functions of CO₂ and HCO⁻₃ concentration measured during steady-state conditions (Fig. 4) by MIMS. Maximum rates of photosynthesis (*V* max) and half-saturation concentrations (*K*₁/₂) were obtained from a Michaelis–Menten fit and are summarized for all pCO₂ treatments in Table 2. Kinetics for photosynthetic O₂ evolution were affected both by pCO₂ and photoperiod. While *V* max differed only slightly between pCO₂ treatments, diurnal variations were pronounced.

![Fig. 1](image-url) (a) Mean growth rates of *Trichodesmium* based on changes in cell density, Chl *a*, POC, PON (b) content of POC per cell, and (c) content of PON per cell in different acclimations of 15.1 Pa, 37.5 Pa, and 101.3 Pa pCO₂. Data present mean values (n ≥ 3; ±SD).
(Fig. 5a; Table 2). Minimum values were obtained about 3 h after illumination (125 to 170 µmol O₂ [mg Chl a]⁻¹ h⁻¹), and increased by nearly twofold toward the end of the photoperiod (303 to 330 µmol O₂ [mg Chl a]⁻¹ h⁻¹). As indicated by the K₁/₂ (DIC) values, affinities differed significantly between pCO₂ acclimations and also showed a strong diurnal pattern, with highest values around midday (Fig. 5b; Table 2). K₁/₂ (CO₂) values for photosynthesis ranged between 0.9 and 13.6 µmol L⁻¹ CO₂ (data not shown).

In terms of carbon fluxes, *Trichodesmium* showed a preference for HCO⁻₃ as a carbon source for photosynthesis (Fig. 4; Table 3). The high HCO⁻₃ contribution to net fixation was verified by the ¹⁴C disequilibrium method (see below). The K₁/₂ values for HCO⁻₃ uptake and diurnal variability therein strongly increased from low to the high pCO₂ acclimation (Table 2), ranging between 40 and 100 µmol DIC L⁻¹ in the low pCO₂ treatment and 85 and 520 µmol DIC L⁻¹ in the high pCO₂ treatment. This CO₂ effect on affinities persisted despite the large diurnal variations in K₁/₂, being most pronounced during midday and lowest at the beginning of the photoperiod. Rates for CO₂ uptake were very low in all acclimations and throughout the photoperiod (Table 2). In terms of gross CO₂ uptake, K₁/₂ and Vₘₐₓ remained unaffected by pCO₂ in the acclimation as well as over the photoperiod. K₁/₂ values ranged between 3.3 and 6.1 µmol L⁻¹ CO₂, and Vₘₐₓ ranged between 51 and 114 µmol CO₂ (mg Chl a)⁻¹ h⁻¹. Net CO₂ flux was often negative, showing lowest values between 12:00 h and 15:00 h, which made it impossible to calculate K₁/₂ values. These rates reflect the CO₂ efflux that occurs during steady-state photosynthesis. The proportion of Ci efflux compared to gross Ci uptake, i.e., cellular leakage, was estimated by MIMS from the CO₂ efflux observed directly upon darkening. Independent of the pCO₂ acclimation, *Trichodesmium* showed large variations in leakage during the photoperiod, and the highest ratio (~0.5) occurred at 12:00 h (Fig. 6).

**¹⁴C disequilibrium method**—Figure 7 shows an example of the ¹⁴C incorporation of a culture acclimated to 101.3 Pa CO₂. Monitoring the ¹⁴C incorporation for more than 12 min, i.e., well into equilibrium, yielded a high level of precision for determining the carbon sources. In measurements of the same culture without DBS (control), similar rates of ¹⁴C incorporation were obtained, indicating a lack of significant eCA activity. The ratio of HCO⁻₃ to net fixation did not significantly differ between the acclimations or throughout the photoperiod; values ranged between 0.86 and 0.95 (Table 3).

**Discussion**

This study assessed the sensitivity of *Trichodesmium erythraeum* (IMS101) to changes in CO₂ concentration by measuring responses to the different acclimations (e.g., growth, elemental ratios, fixation rates) and by describing the modes of carbon acquisition (e.g., CA activities, O₂ evolution, carbon fluxes). Cells were acclimated in unbuffered artificial seawater and maintained at low cell densities to match the natural environment in nonbloom situations as closely as possible. *Trichodesmium* showed no responses in growth rate, but particulate organic carbon and nitrogen production rates increased strongly at elevated pCO₂ (Fig. 1) The apparent K₁/₂ values for photosynthetic O₂ evolution were significantly lower than values known for RubisCO (Badger et al. 1998), demonstrating the operation of a CCM in this species. *Trichodesmium* showed a strong
preference for HCO$_3^-$ as a carbon source, which did not change with CO$_2$ availability or over the diurnal cycle. In terms of CO$_2$ and HCO$_3^-$ affinities, however, cells showed strong responses to CO$_2$ treatments and photoperiod.

**Growth, elemental ratios, and production rates**—Previous studies have observed CO$_2$ effects on growth (Riebesell et al. 1993), photosynthesis (Nielsen 1995; Rost et al. 2003), and elemental ratios (Burkhardt and Riebesell 1997) in diatoms and coccolithophores. Large responses in growth, photosynthesis, and elemental ratios with respect to changes in pCO$_2$ have recently been found in the diazotrophic species *Trichodesmium* (Barcelos e Ramos et al. 2007; Hutchins et al. 2007; Levitan et al. 2007). It should be noted that all these studies, including the present one, have used the same *Trichodesmium* isolate from the Atlantic Ocean (IMS101).

Our findings indicate no sensitivity in growth rates over the tested CO$_2$ range (15.1 to 101.3 Pa CO$_2$), but they do show CO$_2$-dependent changes in the elemental composition of *Trichodesmium* (Fig. 1a). In comparison to the previously published data, growth rates ($\mu = 0.32$) are slightly higher than those obtained by Levitan et al. (2007; $\mu = 0.27$) and lower than those from Barcelos e Ramos et al. (2007; $\mu = 0.45$) under high CO$_2$. For low CO$_2$ levels, our data do not agree with the diminished rates observed by Levitan et al. (2007; $\mu = 0.12$) and Barcelos e Ramos et al. (2007; $\mu = 0.15$ to 0.3) or the absence of growth observed by Hutchins et al. (2007). According to the latter study, *Trichodesmium* cannot thrive under the CO$_2$ levels that

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Chl $\alpha$–specific rates of (a) photosynthesis and HCO$_3^-$ uptake and (b) photosynthesis and gross as well as net CO$_2$ uptake and as a function of HCO$_3^-$ and CO$_2$ concentration in the assay media. The given examples show results from cells acclimated to 101.3 Pa CO$_2$ and were measured at 07:00 h. Curves were obtained from a Michaelis–Menten fit.

Table 2. $K_{1/2}$ and $V_{max}$ of photosynthesis, HCO$_3^-$ uptake, and gross CO$_2$ uptake over a diurnal cycle and acclimated to 15.1, 37.5, and 101.3 Pa pCO$_2$. The photoperiod started at 09:00 h and ended at 21:00 h. Kinetic parameters were calculated from a Michaelis–Menten fit to the combined data of several ($n=3$, SD) independent measurements. Values for $K_{1/2}$ and $V_{max}$ are given in $\mu$mol L$^{-1}$ and $\mu$mol (mg Chl $a$)$^{-1}$ h$^{-1}$, respectively.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>pCO$_2$ (Pa)</th>
<th>Photosynthesis</th>
<th>HCO$_3^-$ uptake</th>
<th>Gross CO$_2$ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pCO$_2$ (Pa)</td>
<td>$K_{1/2}$ (DIC)</td>
<td>$V_{max}$ (DIC)</td>
<td>$K_{1/2}$ (HCO$_3^-$)</td>
</tr>
<tr>
<td>07:00</td>
<td>15.1</td>
<td>61±24</td>
<td>233±14</td>
<td>63±25</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>126±13</td>
<td>219±7</td>
<td>111±9</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>214±18</td>
<td>274±8</td>
<td>190±16</td>
</tr>
<tr>
<td>09:30</td>
<td>15.1</td>
<td>40±11</td>
<td>187±9</td>
<td>41±11</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>72±12</td>
<td>207±6</td>
<td>27±8</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>85±33</td>
<td>233±20</td>
<td>52±23</td>
</tr>
<tr>
<td>12:00</td>
<td>15.1</td>
<td>102±33</td>
<td>147±9</td>
<td>30±6</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>286±96</td>
<td>171±16</td>
<td>112±29</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>519±54</td>
<td>125±4</td>
<td>111±23</td>
</tr>
<tr>
<td>15:00</td>
<td>15.1</td>
<td>54±12</td>
<td>298±12</td>
<td>25±6</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>94±29</td>
<td>270±20</td>
<td>51±11</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>443±105</td>
<td>192±15</td>
<td>188±38</td>
</tr>
<tr>
<td>18:00</td>
<td>15.1</td>
<td>25±35</td>
<td>319±21</td>
<td>30±8</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>112±25</td>
<td>330±16</td>
<td>61±15</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>257±70</td>
<td>303±22</td>
<td>181±56</td>
</tr>
</tbody>
</table>
prevailed during glacial times and commonly occur under bloom conditions. Some of the strong CO$_2$ dependence observed by Barcelos e Ramos et al. (2007) is also caused by reduced growth rates in the low pCO$_2$ range, which furthermore shows significant variability.

The carbon and nitrogen contents per cell increased at high pCO$_2$ compared to the lower pCO$_2$ acclimations (Figs. 1b,c) while the C : N ratios remained constant at 4.6 (obtained at the beginning of the photoperiod).

Measured carbon quotas and elemental ratios are comparable with those obtained by Hutchins et al. (2007) and similar to C : N ratios reported for the low pCO$_2$ acclimation by Barcelos e Ramos et al. (2007). However, in their study, the cell quotas for C and N decreased with increasing pCO$_2$, which is the opposite to the trend.

**Table 3.** Contribution of HCO$_3^-$ uptake relative to net C fixation. Values of MIMS measurement were obtained at 2 mmol L$^{-1}$ DIC. Values of $^{14}$C were obtained by fitting the $^{14}$C incorporation pattern. Values represent the mean of three independent measurements ($n$=3; ±SD).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>pCO$_2$ (Pa)</th>
<th>HCO$_3^-$ uptake : C fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIMS</td>
</tr>
<tr>
<td>07:00</td>
<td>15.1</td>
<td>1.02±0.20</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>1.01±0.08</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>09:30</td>
<td>15.1</td>
<td>1.08±0.28</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>1.02±0.19</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>1.02±0.22</td>
</tr>
<tr>
<td>12:00</td>
<td>15.1</td>
<td>1.11±0.35</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>1.09±0.31</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>1.13±0.38</td>
</tr>
<tr>
<td>15:00</td>
<td>15.1</td>
<td>0.99±0.19</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>0.98±0.15</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>1.06±0.27</td>
</tr>
<tr>
<td>18:00</td>
<td>15.1</td>
<td>0.99±0.17</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>1.01±0.24</td>
</tr>
<tr>
<td></td>
<td>101.3</td>
<td>1.02±0.35</td>
</tr>
</tbody>
</table>

**Fig. 5.** Maximum rates ($V_{max}$) and half-saturation concentrations ($K_{1/2}$) of photosynthesis over a diurnal cycle acclimated to different pCO$_2$ conditions. Kinetic parameters were calculated from a Michaelis–Menten fit to the combined data of several independent measurements. Error bars denote ±SD.

**Fig. 6.** Ratio of CO$_2$ efflux : gross Ci uptake in Trichodesmium at different acclimations over a day. Values indicate leakage measured with MIMS method calculated for the CO$_2$ concentrations in the acclimation. Data present mean values ($n$=3; ±SD).

**Fig. 7.** Examples of disintegrations per minute (DPM) of $^{14}$C during a short-term incubation of cells acclimated to 101.3 Pa CO$_2$ and measured at 15:00 h. Values of $f$ in DBS-treated cells (closed symbols) and the control (open symbols) represent the proportion of HCO$_3^-$ uptake relative to net C fixation.
observed by Levitan et al. (2007), who found an increase in C and N quota as well as the respective ratio under elevated pCO₂. In all studies, the observed magnitude in CO₂ sensitivity to carbon and N₂ fixation differed strongly. Using the acetylene reduction assay, Barcelos e Ramos et al. (2007) and Levitan et al. (2007) observed stimulation in N₂ fixation by approximately 40% and even up to 400%, while Hutchins et al. (2007) obtained stimulation by up to 35% over the respective CO₂ range. In our study, we assessed the process of N₂ fixation by measuring the production of particulate organic nitrogen. The results show a 40% increase in the production rates under high pCO₂ (Fig. 1c) as well as elevated C fixation (Fig. 1b). Both processes were equally stimulated, which is reflected by the constant C:N ratios in Trichodesmium. Responses consistent within all mentioned studies on Trichodesmium show that elevated CO₂ stimulates both C and N₂ fixation rates.

The discrepancy between studies may be attributed to differences in methodology (e.g., midexponential growth versus late stationary phase) or growth conditions (light intensities). Preliminary data (S. Kranz and O. Levitan unpubl. data) showed that light levels strongly influenced CO₂ dependency of growth as well as C and N quotas in Trichodesmium. Responses consistent within all mentioned studies on Trichodesmium show that elevated CO₂ stimulates both C and N₂ fixation rates.

\textbf{Diurnal variations in photosynthesis and N₂ fixation—} Diazotrophic organisms have developed numerous strategies to fix N₂ efficiently (Berman-Frank et al. 2007). Nitrogenase, the enzyme that catalyzes the reduction of atmospheric N₂ to ammonia, is inhibited by O₂, and thus N₂ fixation has to be separated from photosynthesis. In the nonheterocystous \textit{Trichodesmium}, a distinct diurnal pattern of N₂ fixation and O₂ evolution has been observed (Berman-Frank et al. 2001b; Milligan et al. 2007). Our study verifies these diurnal rhythms, finding a pronounced decrease in photosynthesis and increased dark respiration during midday (Fig. 2b). The concomitant decrease in C:N ratio during that time also reflects increasing rates of N₂ fixation (Fig. 2a). Additionally, the Mehler reaction appears to be involved in light-dependent O₂ uptake during N₂ fixation (Kana 1993; Milligan et al. 2007). The inverse correlation between photosynthesis and respiration observed in the present and previous studies is caused by the fact that both processes share the same protein complex in the electron transport chain. Consequently, the increase in dark respiration results in a down-regulation of the water splitting in Photosystem II (PSII) due to a negative feedback reaction in the electron transport chain (Milligan et al. 2007).

Due to high adenosine triphosphate (ATP) and electron requirements, N₂ fixation is among the most costly processes for the cell next to carbon assimilation (Falkowski and Raven 2007). However, this process occurs during midday when photosynthesis is down-regulated and hence the ATP and nicotinamide adenine dinucleotide phosphate (NADPH) supply is low. The way in which \textit{Trichodesmium} copes with this shortage in energy supply is not yet fully understood. Another enigma relates to CO₂ sensitivity in photosynthetic carbon assimilation and N₂ fixation, as both processes compete for ATP and reductants provided by the light reaction of photosynthesis. While external CO₂ levels could affect the C fixation directly by controlling the carboxylation efficiency of RubisCO or indirectly by modifying the energy costs of their CCM, there are currently no CO₂-related processes known to directly influence nitrogenase activities. The strong effects of CO₂ as well as diurnal changes in C and N fixation observed in this and previous studies must be reflected in the modes of carbon acquisition of \textit{Trichodesmium}. In the following, we will characterize the CCM of \textit{Trichodesmium}, the diurnal changes, and regulation with respect to CO₂ availability.

\textbf{Carbonic anhydrase activities—} External CA (eCA), which accelerates the interconversion between HCO₃⁻ and CO₂ at the cell surface, has been found to increase in response to decreasing CO₂ supply in various microalgal species (Berman-Frank et al. 1995; Rost et al. 2003). It is a common notion that eCA is involved in indirect HCO₃⁻ utilization by converting HCO₃⁻ to CO₂, which could then diffuse into the cell or be actively transported through the plasma membrane and subsequently used for photosynthesis (Sütlemeyer et al. 1998; Elzenga et al. 2000). External CA activity in \textit{Trichodesmium} was low—values of about 50 units per µg Chl a—and did not change with CO₂ supply (data not shown). In species that express significant quantities of eCA, activity is usually an order of magnitude higher (Rost et al. 2003; Trimborn et al. 2008). Moreover, there was no stimulation of photosynthesis by the addition of bovine CA (data not shown). The lack of significant eCA activity was further verified by the \textsuperscript{14}C disequilibrium method (Elzenga et al. 2000), which yielded similar \textsuperscript{14}C incorporation patterns in the presence and absence of DBS (Fig. 7). Consequently, the low activity and the lack of induction under low CO₂ supply indicate that eCA does not play an important, if any, role in the carbon acquisition by \textit{Trichodesmium}.

Internal carbonic anhydrase (iCA) in cyanobacteria is required for the rapid conversion from HCO₃⁻ to CO₂ prior to the fixation by RubisCO. When interpreting iCA activity, as defined according to Palmqvist et al. (1994), one has to bear in mind that Δ values are in vivo estimates, which depend not only on the rate of intracellular \textsuperscript{18}O depletion (i.e., CA activity) but also on the diffusive influx of doubly labelled CO₂ and, thus, on the diffusive properties of cyanobacterial membranes and cell shape. Therefore, despite being semiquantitative estimates, Δ values are still appropriate for direct comparison between treatments within the same species. Internal CA in \textit{Trichodesmium} is presumably located in the carboxysome to operate near RubisCO (Price et al. 2008). These data show that \textit{Trichodesmium} possesses low iCA activity, which is constitutively expressed. The iCA activity observed, despite being low, possibly reflects the CA activity inside the carboxysome catalyzing the interconversion between HCO₃⁻ and CO₂ (Price et al. 2008). In addition, the CO₂ uptake system in \textit{Trichodesmium}, located
at the thylakoid membrane, functions like CA by accelerating the conversion of CO\textsubscript{2} into HCO\textsubscript{3}\textsuperscript{-} (Price et al. 2008). However, this function may only play a role under illumination when electrons and NAD(P)H are available to drive this process.

Using the \textsuperscript{18}O exchange technique, we examined the presence of light-dependent Ci transport systems. In the case of active Ci uptake, a decline in log (enrichment) during illumination would be expected as a result of an enhanced uptake of \textsuperscript{18}O-labelled CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{-} into the cells, increased \textsuperscript{18}O exchange catalyzed by internal CA, and subsequent efflux of \textsuperscript{18}O-unlabelled CO\textsubscript{2} (Badger and Price 1989; Palmqvist et al. 1994). Such a net CO\textsubscript{2} efflux from photosynthetically active cells can only be explained by an active accumulation of Ci and the presence of iCA within the cells. As shown in Fig. 3, illumination resulted in a decrease of \textsuperscript{13}C\textsuperscript{18}O\textsubscript{2} (m/z = 49) due to uptake of labelled carbon species and an increase in \textsuperscript{13}C\textsuperscript{16}O\textsubscript{2} (m/z = 45) as a result of efflux of the latter. The consequent decrease in log (enrichment) was accompanied by an increase in oxygen production (data not shown). These patterns were measured in all acclimations throughout the photoperiod and are indicative of the presence of a CCM.

**Photosynthetic O\textsubscript{2} evolution**—Early studies demonstrated a CO\textsubscript{2}-dependent regulation of the CCM of cyanobacteria (Kaplan et al. 1980). More recent studies have shown that the apparent affinity for Ci increases strongly with decreasing Ci availability in the culture medium (Woodger et al. 2003; Price et al. 2004). These studies typically compared present-day (i.e., \( \sim 37.5 \) Pa) with unnaturally high CO\textsubscript{2} levels (i.e., 2000–5000 Pa), and it is therefore not yet fully understood to what extent this regulation occurs under environmentally relevant CO\textsubscript{2} concentrations. In the present study, photosynthetic O\textsubscript{2} evolution as a function of CO\textsubscript{2} concentration was monitored to gain information about the overall efficiency and regulation of carbon acquisition in *Trichodesmium*. Half-saturation constants were generally lower (0.9–13.6 \( \mu \)mol L\textsuperscript{-1} CO\textsubscript{2}) than those reported for cyanobacterial Rubisco (105–185 \( \mu \)mol L\textsuperscript{-1} CO\textsubscript{2}; Badger et al. 1998). Moreover, we observed a gradual regulation by pCO\textsubscript{2} in the acclimations. While we obtained lowest apparent affinities for Ci at high pCO\textsubscript{2}, maximum photosynthetic O\textsubscript{2} evolution rates were not affected in the bioassays (Fig. 5a; Table 2). The high apparent affinities, as well as the CO\textsubscript{2}-dependent changes therein, demonstrate the operation of a CCM for *Trichodesmium*, and these findings are consistent with kinetics observed in other cyanobacteria (Sültemeyer 1998; Price et al. 2004).

The strongest variation in the CCM was, however, observed over the diurnal cycle. As an example, \( K_{\text{ci}} \) for photosynthetic O\textsubscript{2} evolution varied between 85 and 520 \( \mu \)mol L\textsuperscript{-1} DIC in the 101.3 Pa CO\textsubscript{2} treatment (Fig. 6b). This up- and down-regulation of the CCM is most likely associated with the diurnal pattern of N\textsubscript{2} fixation. During midday, when N\textsubscript{2} fixation is greatest, the apparent affinities as well as maximum rates for photosynthetic O\textsubscript{2} evolution are down-regulated. The down-regulation of the CCM and the up-regulation of dark- and light-dependent respiration (see previous discussion) result in lower net O\textsubscript{2} evolution, which is a prerequisite for efficient N\textsubscript{2} fixation, as shown previously (Berman-Frank et al. 2001b; Milligan et al. 2007). The trigger for this diurnal CCM regulation may be changes in the redox state of the photosynthetic electron chain, which could result from lower PSII activity in line with higher respiration in *Trichodesmium* and/or the concentration of photoresporatory metabolites (Kaplan et al. 2001).

Although the CO\textsubscript{2} dependence of O\textsubscript{2} evolution provides information about the efficiency and regulation of carbon acquisition, it cannot provide any details about the underlying mechanisms. To get a process-based understanding, we therefore have to look at the carbon source(s) and respective uptake kinetics.

**Carbon source and uptake kinetics**—An essential component of a CCM is the active uptake of inorganic carbon and its accumulation within the cell. Several methods have been employed to distinguish between CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{-} uptake in microalgae and cyanobacteria. In this study, estimates of CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{-} uptake were obtained by means of mass spectrometry (Badger et al. 1994) and the \textsuperscript{14}C disequilibrium technique (Espie and Colman 1986; Elzenga et al. 2000). This is the first time such techniques have been applied to *Trichodesmium*.

Rates of CO\textsubscript{2} uptake determined by MIMS were very low in *Trichodesmium*, representing generally less than 10% relative to net carbon fixation (Table 2). Net CO\textsubscript{2} fluxes were low, even negative under some conditions, reflecting higher CO\textsubscript{2} efflux than uptake. Since the CO\textsubscript{2} uptake could not support the observed rates of photosynthesis, most of the inorganic carbon was taken up as HCO\textsubscript{3}\textsuperscript{-} (Table 3). In the instances when net fluxes of CO\textsubscript{2} were negative, HCO\textsubscript{3}\textsuperscript{-} uptake exceeded net fixation (Badger et al. 1994). The strong preference for HCO\textsubscript{3}\textsuperscript{-} in *Trichodesmium* did not change with CO\textsubscript{2} treatments or photoperiod. These findings were confirmed by the \textsuperscript{14}C disequilibrium method (Espie and Colman 1986; Elzenga et al. 2000), which showed on average 92% contribution of HCO\textsubscript{3}\textsuperscript{-} uptake relative to net carbon fixation. Please note that the contribution of HCO\textsubscript{3}\textsuperscript{-}, as determined by \textsuperscript{14}C disequilibrium approach, can never exceed net fixation (Elzenga et al. 2000). These results are consistent with previous studies showing that CCMs in cyanobacteria are generally based on active HCO\textsubscript{3}\textsuperscript{-} uptake (Price et al. 2008). With respect to the high accumulation of Ci necessary to compensate for their low-affinity Rubisco, cyanobacteria may prefer HCO\textsubscript{3}\textsuperscript{-} over CO\textsubscript{2} because of the higher equilibrium concentration of HCO\textsubscript{3}\textsuperscript{-} in marine systems. Moreover, as a charged molecule, HCO\textsubscript{3}\textsuperscript{-} can be accumulated more efficiently in the cytoplasm than CO\textsubscript{2} (Price and Badger 1989).

The apparent affinities of the HCO\textsubscript{3}\textsuperscript{-} uptake systems differed among CO\textsubscript{2} treatments and over the photoperiod. With decreasing CO\textsubscript{2} availability, apparent affinity for HCO\textsubscript{3}\textsuperscript{-} uptake increased (Table 2), and this trend generally persisted throughout the photoperiod. Various studies have shown that changes in apparent affinity can be accomplished by expression of high versus low affinity transporters (Omata et al. 1999; Price et al. 2004) or by post-translational modifications of existing transport proteins,
e.g., by phosphorylation (Sültemeyer et al. 1998). For *Trichodesmium*, deoxyribonucleic acid (DNA) sequence analysis indicates the presence of one medium and/or low affinity HCO$_3^-$ transporter (BicA) and a low-affinity NDH-I$_4$ CO$_2$ uptake system (Price et al. 2004, 2008). The observed $K_{i/2}$ values for HCO$_3^-$ uptake and the low contribution of CO$_2$ to the overall uptake observed in our study support the findings from these molecular studies.

The uptake kinetics for HCO$_3^-$ varied strongly over the photoperiod, although they were less pronounced than variations in photosynthetic O$_2$ evolution (Table 2). Apparent affinities for HCO$_3^-$ were highest at the beginning of the photoperiod, with $K_{i/2}$ values between ~25 and 50 $\mu$mol L$^{-1}$ HCO$_3^-$, and lowest during and following N$_2$ fixation, with $K_{i/2}$ values up to 190 $\mu$mol L$^{-1}$ HCO$_3^-$. These diurnal variations in HCO$_3^-$ transport efficiency occurred in all treatments but were more distinct under high pCO$_2$.

Changes in uptake kinetics, as in the HCO$_3^-$ uptake system, may be caused by variations in the reductive state of photosynthetic electron transport carriers, which affect the balance between cyclic and linear electron transport and thus the energy supply for transporters (Li and Canvin 1998). With respect to diurnal changes in *Trichodesmium*, the electron flow can also be altered by the up-regulation of the Mehler reaction (Kana 1993; Milligan et al. 2007). As an O$_2$-consuming process, it can additionally affect the [O$_2$]:[CO$_2$] ratio in the proximity of RubisCO, which has been suggested to be another trigger for the regulation of CCMs (Kaplan et al. 2001). Consequently, changes in the redox state of the photosynthetic electron transport carriers as well as the low [O$_2$]:[CO$_2$] ratios during midday could have contributed to the observed down-regulation of the HCO$_3^-$ uptake efficiency. The highly induced HCO$_3^-$ uptake systems at the beginning of the photoperiod may have been triggered by light and the excess of electrons. An up-regulated CCM and consequently efficient Calvin cycle provides the best mechanism to drain electrons (photochemical quenching) and avoid photodamage, similar to the response observed in *Chlamydomonas reinhardtii* (Marcus et al. 1986). The frequently excessive HCO$_3^-$ uptake observed may further provide a means to efficiently dissipate excess light energy (Tchernov et al. 1997). These and possibly other quenching mechanisms are important for *Trichodesmium*, since it thrives in low latitudes close to the surface, with high average irradiance.

Leakage—The efficiency of a CCM not only depends on the kinetics of the active carbon uptake systems but also on the loss of Ci via efflux. Leakage (ratio of Ci efflux to total Ci uptake) will increase the energetic costs of a CCM and/or decrease its capability to reach carbon saturation (Raven and Lucas 1985). Consequently, to increase the overall CCM efficiency, it is necessary to minimize the leakage. Following the approach by Badger et al. (1994), the MIMS was used to estimate leakage.

The MIMS approach yielded similar estimates for leakage in all pCO$_2$ treatments, yet the photoperiod imposed strong changes in leakage, with values as high as 0.55 during midday (Fig. 6). These high values were the result of increasing efflux combined with the down-regulation of total Ci uptake (Fig. 5; Table 2). As argued already, such high leakage might help to dissipate excess energy at times when PSII and Calvin cycle activity are down-regulated in *Trichodesmium*. Such modification of leakage will most likely be associated with a CO$_2$-trapping mechanism. It has been suggested that CO$_2$ efflux from the carboxysome is converted back to HCO$_3^-$ by the PSII-associated NDH-CO$_2$ uptake system (Price et al. 2008). The diurnal changes in PSII activity (Berman-Frank et al. 2001b) may therefore directly regulate the effective leakage of the cell and thus explain most of the diurnal variation we observed in *Trichodesmium*. It should be noted, however, that the CO$_2$ efflux estimated according to Badger et al. (1994) is based on the assumptions that the rate of diffusive CO$_2$ efflux in the light is well represented by the first seconds upon darkening. Despite shortcomings in methodology, our data indicate that higher leakage, for instance during midday, reflect a down-regulation of the overall CCM activity, which is consistent with the lower affinities of the Ci uptake system during these times (Table 2; Fig. 5).

Ecological and biogeochemical implications—Diazotrophic cyanobacteria like *Trichodesmium* support a large fraction of biological productivity in tropical and subtropical areas and exert, over long timescales, a significant influence on global carbon cycles by providing a major source of reactive N to the water column (Falkowski and Raven 2007). Despite its global importance, studies have only recently begun to investigate the effect of elevated CO$_2$ on species such as *Trichodesmium* (Barcelos e Ramos et al. 2007; Hutchins et al. 2007; Levitan et al. 2007). This study, consistent with previous investigations, showed a strong increase in photosynthesis and N$_2$ fixation under elevated CO$_2$ levels. To the extent that we can extrapolate these laboratory experiments to the real ocean, the marine N$_2$ fixation by *Trichodesmium* could increase from present-day to future pCO$_2$ level by 40% (the present study) or even up to 400% (Levitan et al. 2007). Similarly, high sensitivity to changes in carbonate chemistry has been observed in photosynthesis (Barcelos e Ramos et al. 2007; Hutchins et al. 2007; Levitan et al. 2007; present study). The magnitude of these CO$_2$ effects would, if representative for the natural environment, have large implications for the future ocean.

The relevance of marine N$_2$ fixation is also expected to increase owing to the projected expansion of oligotrophic regions to higher latitudes as a result of surface ocean warming and increased stratification (Boyd and Doney 2002; Breitbarth et al. 2007). Elevated N$_2$ fixation in a future ocean will likely influence phytoplankton in terms of productivity and species composition, and thereby alter the microbial food web (Mulholland et al. 2006). In summary, CO$_2$-related effects on photosynthesis and N$_2$ fixation as well as the overall changes in the ecosystem would provide a negative feedback on the increase in atmospheric CO$_2$. Significant uncertainties remain, however, as to the degree of sensitivity to CO$_2$ and the modification of these responses by other environmental factors (e.g., P or Fe limitation). Moreover, it is still unknown whether the
observed responses in *Trichodesmium* can be generalized to include other important diazotrophic cyanobacteria.

The present study has taken a first step toward understanding the underlying processes behind strong CO\(_2\) sensitivity by photosynthesis and N\(_2\) fixation in *Trichodesmium*. This diazotrophic organism was found to operate an efficient CCM based almost entirely on direct HCO\(_3^−\) uptake. Consequently, a direct effect of elevated CO\(_2\) on Rubisco carboxylation efficiency is unlikely (i.e., higher active or diffusive CO\(_2\) uptake would increase internal CO\(_2\)/O\(_2\) concentrations) or at least not the main reason for the CO\(_2\) sensitivity observed. Instead, owing to the observed plasticity in CCM regulation, *Trichodesmium* may be able to optimize the allocation of resources (e.g., ATP and NADPH) between the CCM and other processes like N\(_2\) fixation. Such a resource allocation would explain the influence of CO\(_2\) on nitrogenase activity. In view of the potential ecological and biogeochemical implications, investigation into the regulation of photosynthesis, CCMs, and N\(_2\) fixation in *Trichodesmium* and other important diazotrophs is clearly a research priority.

**References**


Carbon acquisition by Trichodesmium


Edited by: John Albert Raven

Received: 23 June 2008
Amended: 04 December 2008
Accepted: 05 December 2008