Inorganic carbon and pH dependency of *Trichodesmium*’s photosynthetic rates.

Running title: Photosynthetic rates as a function of pH and HCO$_3^-$.

TOBIAS G. BOATMAN$^{1*}$, NIALL M. MANGAN$^{2}$, TRACY LAWSON$^{1}$, RICHARD J. GEIDER$^{1}$

$^{1}$School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, UK

$^{2}$Department of Engineering Sciences and Applied Mathematics, Northwestern University, Evanston, Illinois, 60208, USA

*To whom correspondence should be addressed: Tobias G Boatman
tboatman@chelsea.co.uk (Tel: 02084 819009); niall.mangan@northwestern.edu; tlawson@essex.ac.uk; geider@essex.ac.uk

**Highlight**

*Trichodesmium*’s photosynthetic rates appear to be a function of CO$_2$, although numerical simulations of the carbon concentrating mechanism (CCM) show carboxylation to be mediated as a function of pH and HCO$_3^-$.

© The Author(s) 2018. Published by Oxford University Press on behalf of the Society for Experimental Biology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Abstract

We established the relationship between photosynthetic carbon fixation rates and pH, CO$_2$ and HCO$_3^-$ concentrations in the diazotroph Trichodesmium erythraeum IMS101. Inorganic $^{14}$C-assimilation was measured in TRIS-buffered ASW medium where the absolute and relative concentrations of CO$_2$, pH and HCO$_3^-$ were manipulated. First, we varied the total dissolved inorganic carbon concentration (TIC) (< 0 to ~ 5 mM) at constant pH, so ratios of CO$_2$ and HCO$_3^-$ remained relatively constant. Second, we varied pH (~ 8.54 to 7.52) at constant TIC, so CO$_2$ increased whilst HCO$_3^-$ declined. We found that $^{14}$C-assimilation could be described by the same function of CO$_2$ for both approaches but showed different dependencies on HCO$_3^-$ when pH was varied at constant TIC than when TIC was varied at constant pH. A numerical model of Trichodesmium’s CCM showed carboxylation rates are modulated by HCO$_3^-$ and pH. The decrease in Ci assimilation at low CO$_2$, when TIC was varied, is due to HCO$_3^-$ uptake limitation of the carboxylation rate. Conversely, when pH was varied, Ci assimilation declined due to a high-pH mediated increase in HCO$_3^-$ and CO$_2$ leakage rates, potentially coupled to other processes (uncharacterised within the CCM model) that restrict Ci assimilation rates under high-pH conditions.

Keywords: Trichodesmium, Cyanobacteria, Ocean acidification, CO$_2$, Carbon acquisition, Gross photosynthesis, Net photosynthesis, Carbon concentrating mechanism (CCM)
Introduction

Over the past 150 years atmospheric CO\textsubscript{2} concentrations have increased from pre-industrial levels (i.e. 280 µmol mol\textsuperscript{-1}) to a current value of about 400 µmol mol\textsuperscript{-1}, and are predicted to increase further to 650 µmol mol\textsuperscript{-1} by mid-century and to 750/1000 µmol mol\textsuperscript{-1} by the end of this century (Raven \textit{et al.}, 2005). Equilibration of CO\textsubscript{2} between the atmosphere and ocean is leading to increases in dissolved CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{-} and decreases in pH and CO\textsubscript{3}\textsuperscript{2-}. This process of ocean acidification will reduce the pH from average preindustrial levels of 8.2 to about 7.9 by the end of the century (Zeebe \textit{et al.}, 1999; Zeebe and Wolf-Gladrow, 2001). To date, there are still many uncertainties as to the magnitude of biological responses of key organisms to these chemical changes.

One group of organisms of particular importance are the diazotrophic cyanobacteria (photosynthetic dinitrogen-fixers), notably because of their significant contribution to marine primary productivity by converting N\textsubscript{2} into NH\textsubscript{4}\textsuperscript{+}, thus providing "new" nitrogen to the oceans. The filamentous cyanobacterium \textit{Trichodesmium} is a colony forming species which fixes nitrogen in an area corresponding to half the Earth’s surface (Davis and McGillicuddy, 2006), and is estimated to account for more than half of the new (combined) nitrogen production in many parts of the oligotrophic tropical and subtropical oceans (Capone \textit{et al.}, 2005).

Cyanobacteria, \textit{Trichodesmium} sp. included, achieve high photosynthetic rates despite i), the slow diffusion of CO\textsubscript{2} in water (10\textsuperscript{4} times slower than in air) ii), a slow chemical equilibrium between HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{2} within the 7 – 8.5 pH range and iii), a low affinity of Rubisco for CO\textsubscript{2} relative to ambient CO\textsubscript{2} concentrations. Cyanobacteria employ an intracellular carbon concentrating mechanism (CCM) (Badger and Price, 2003; Badger \textit{et al.}, 2006; Kranz \textit{et al.}, 2010), where enhanced primary productivity significantly outweighs the metabolic costs of CCM activity (Price \textit{et al.}, 2008). The CCM benefits cyanobacteria by reducing photorespiration (Kaplan and Reinhold, 1999; Schwarz \textit{et al.}, 1995), aids in the dissipation of excess light energy as well as maintaining an optimal intracellular pH (Badger \textit{et al.}, 1994; Kaplan and Reinhold, 1999). The consensus is that upregulation of CCM activity in response to a low-CO\textsubscript{2} environment involves two components: firstly, an increase in the transport of inorganic carbon (Ci) from the environment into the cell via a suite of Ci transporters which could involve using ATP (BCT1 HCO\textsubscript{3}\textsuperscript{-} transporter), NADPH or reduced ferredoxin (CO\textsubscript{2} conversion from passive diffusion) or coupling to an electrochemical Na\textsuperscript{+} gradient (SbtA or
BicA HCO$_3^-$ transport) to provide the energy for Ci uptake (Badger et al., 2002; Badger and Price, 2003); and secondly, an increased ability to reduce CO$_2$ leakage from around the site of carboxylation, achieved via arrangement of the molecular components of the carboxysome structure and a CO$_2$ uptake system located on the thylakoid layer, preventing the efflux of leaked CO$_2$ to the outer cytosolic layer (Price et al., 2008).

Both $^{14}$C isotope disequilibrium experiments and simultaneous measurements of CO$_2$ and O$_2$ exchanges during sequentional light-dark transitions indicate that HCO$_3^-$ contributes > 90% of the Ci assimilation by T. erythraeum IMS101 (Eichner et al., 2015; Kranz et al., 2009). This preference for HCO$_3^-$ is consistent with the evidence that Trichodesmium lacks a plasma membrane-bound extracellular carbonic anhydrase (eCA) (Badger et al., 2006; Price et al., 2008). Furthermore, the T. erythraeum genome indicates the presence of both a plasma membrane HCO$_3^-$ transporter (BicA) and an intracellular system for conversion of CO$_2$ to HCO$_3^-$ (NDH-I4) (Price et al., 2008). These two modes of the CCM result in the accumulation of HCO$_3^-$ in the cytosol, which diffuses to the carboxysome. Inorganic carbon uptake by Trichodesmium involves the uptake of HCO$_3^-$ by the BicA transporter. This transporter has a $K_m$ of 40-100 $\mu$M HCO$_3^-$, which is well below the typical concentration of HCO$_3^-$ in seawater (~ 2000 $\mu$M) (Badger et al., 2006). Following transport into the cell, C-fixation in Trichodesmium, like other cyanobacteria spp., occurs within carboxysomes where HCO$_3^-$ is converted to CO$_2$ via a carbonic anhydrase followed by fixation of CO$_2$ by ribulose bisphosphate carboxylase oxygenase (Rubisco). Carboxysomes provide microenvironments where CO$_2$ is elevated to compensate for the low affinity of cyanobacterial Rubiscos for CO$_2$ ($K_m$CO$_2$ > 150 mM) (Badger and Andrews, 1987). In Trichodesmium, CO$_2$ that leaks from carboxysomes can be converted to HCO$_3^-$ by the plasma membrane bound NDH-I4 protein, thus reducing the efflux of CO$_2$ from the cell, but at a cost of consuming reducing equivalents (NADPH or reduced Fd) (Price et al., 2008). Despite having a mechanism for intracellular recycling of CO$_2$, efflux is reported to account for the loss of up to 50% of HCO$_3^-$ uptake in Trichodesmium (Eichner et al., 2015; Kranz et al., 2010).

As reviewed in Boatman et al. (2017), the majority of previous studies show an increase (albeit not all statistically significant) in T. erythraeum IMS101 growth to future CO$_2$ concentrations (~ 750/1000 $\mu$mol mol$^{-1}$). This mirrors the increased productivity (CO$_2$ and N$_2$ fixation) and changing elemental composition in response to future CO$_2$ concentrations (~ 750/1000 $\mu$mol mol$^{-1}$), although the magnitudes of these responses differs between studies (Table S1). The increased productivity of T. erythraeum IMS101 with increased CO$_2$ is likely
attributable to a decrease in the energy required for operation of the CCM, allowing more energy (ATP) and reductant (NADPH) to be reallocated to N₂ fixation, CO₂ fixation and biosynthesis (Kranz et al., 2011).

Given *Trichodesmium*’s significant contribution to carbon and nitrogen biogeochemical cycles and the predicted changes to Ci speciation over the coming decades due to ocean acidification, we performed a systematic experiment to assess how the kinetics of Ci assimilation of *T. erythraeum* IMS101 were affected by acclimation to varying CO₂ concentrations (µmol mol⁻¹). We ensured that the Ci chemistry and all other growth conditions were well defined, with cultures fully acclimated over long-time periods to achieve balanced growth. We assessed how the rate of Ci assimilation was related to CO₂ or HCO₃⁻ concentrations in experiments where Ci speciation was modulated by varying pH and TIC. These assays of photosynthetic performance showed that i) *Trichodesmium* productivity was influenced by high-pH when TIC was held at a saturating concentration, indirectly making the rate of Ci assimilation a saturating function of CO₂ concentration and ii), maximum rates of CO₂ fixation declined and affinity for CO₂ increased when *Trichodesmium* was acclimated to a low-CO₂ concentration. We discuss how these responses can be attributed to decreases in the cost of operating a CCM at future CO₂ conditions.

**Materials and methods**

*T. erythraeum* IMS101 was semi-continuously cultured to achieve fully acclimated balanced growth at three target CO₂ concentrations (180, 380 and 720 µmol mol⁻¹), saturating light intensity (400 µmol photons m⁻² s⁻¹), 12:12 light:dark (L:D) cycle and optimum growth temperature (26 °C ± 0.7 °C) for ~ 5 months (~ 80 generations).

**Experimental setup**

*T. erythraeum* IMS101 was grown using YBCII medium (Chen et al., 1996) at 1.5 L volumes in 2 L pyrex bottles that had been acid-washed and autoclaved prior to culturing. Daily growth rates were quantified from changes in baseline fluorescence (F₀) measured between 09:00 to 10:30 on dark-adapted cultures (20 minutes) using a FRRfII FastAct Fluorometer System (Chelsea Technologies Group Ltd, UK). Cultures were deemed fully acclimated and
in balanced growth when both the slope of the linear regression of ln \( F_o \) and the ratio of live cell to acetone extracted (method detailed below) baseline fluorescence (\( F_o \)) were constant following every dilution with fresh YBCII medium. Cultures were kept at the upper section of the exponential growth phase through periodic dilution with new growth media at 3 to 5 day intervals. Illumination was provided side-on by fluorescent tubes (Sylvania Luxline Plus FHQ49/T5/840). Cultures were constantly mixed using magnetic PTFE stirrer bars and aerated with a filtered (0.2μm pore) air mixture at a rate of ~ 200 mL s\(^{-1}\). The CO\(_2\) concentration was regulated (± 2 µmol mol\(^{-1}\)) by mass flow controllers (Bronkhorst, Newmarket, UK) where CO\(_2\)-free air was supplied by an oil free compressor (Bambi Air, UK) via a soda lime gas-tight column which was mixed with a 10% CO\(_2\) in-air mixture from a gas cylinder (BOC Industrial Gases, UK). The CO\(_2\) concentration in the gas phase was continuously monitored and recorded by an infra-red gas analyser (Li-Cor Li-820, Nebraska USA), calibrated weekly by a standard gas (BOC Industrial Gases).

The Ci chemistry was measured prior to the dilution of each culture with fresh media; where pH and TIC were measured directly, while the bicarbonate (HCO\(_3^-\)), carbonate (CO\(_3^{2-}\)) and CO\(_2\) concentrations were calculated via CO2SYS using the same constants as described in Boatman \textit{et al}. (2017) (Supplementary Information: I).

\begin{itemize}
\item \textit{Elemental stoichiometry}
\end{itemize}

Samples for elemental composition and CO\(_2\) response curves were collected at the same time of day between 4 and 6 hours into the photo-phase of the L:D cycle. Samples for determination of particulate organic carbon (POC), particulate nitrogen (PN) and particulate phosphorus (PP) were collected with each CO\(_2\) response curve, where each sample was a biological replicate culture. Three 100 mL aliquots from each culture were vacuum-filtered onto pre-combusted 25 mm (0.45 μm pore) glass fibre filters for measurements of POC, PN and PP. The POC and PN filters were placed in 1.8 mL cryovials (lids off) and dried at 60 °C. The POP filters were rinsed with 2 mL of sodium sulphate (0.1 M), placed in a glass 20 mL scintillation vial, 2 mL of magnesium sulphate (0.017 M) added and dried at 60 °C. POC was quantified using a TC analyser (Shimadzu TOC-V Analyser & SSM-5000A Solid Sample Combustion Unit), PN by the method of Bronk and Ward (2000) and PP by the method of Solorzano and Sharp (1980).
Inorganic carbon fixation response curves

The dependencies of CO$_2$ fixation on CO$_2$ and HCO$_3^-$ were obtained from experiments that involved (i) varied TIC with fixed pH and (ii) varied pH with fixed TIC (Supplementary Information: II and III) in TRIS-buffered YBCII medium using the $^{14}$C uptake technique (Nielsen and Jensen, 1957).

Prior to each experiment, 1 L of bicarbonate-free YBCII medium was aerated overnight with CO$_2$-free air (soda lime column). A 200 mL sample from each culture (triplicate cultures) was gravity filtered onto a cyclopore (1 µm pore) 47 mm filter (Whatman 60750) and gently re-suspended into 50 mL of CO$_2$-free YBCII medium. Exactly 5 mL of concentrated culture was pipetted into each tube of the TIC or pH gradients (35 mL total volume per tube) and gently inverted to evenly distribute the trichomes. The remaining culture was used for T$_0$ measurements, requiring an additional three test tubes (one per culture). During sample preparation, test tubes were maintained at growth temperature (26 °C) and a low light intensity (< 10 µmol photons m$^{-2}$ s$^{-1}$).

To characterise the Ci chemistry, exactly 20 mL of culture from each treatment was filtered through a swinnex filter (25 mm, 0.45µm pore, glass fibre filter); 15 mL into plastic centrifuge tube (no headspace) for TIC analysis (Shimadzu TOC-V Analyser & ASI-V Autosampler), and 5 mL into a plastic cryogenic vial (Sigma-Aldrich V5257-250EA) (no headspace) for pH analysis.

To measure chlorophyll $a$ concentrations, a 1 mL sample from each treatment was pipetted into 9 mL of 100% acetone and left in the freezer (-20 °C) overnight (Welschmeyer, 1994). The sample was vortex mixed and left in the dark (~ 30 minutes) for cell debris to precipitate and the solution to equilibrate to room temperature. A 2 mL aliquot was used to measure baseline fluorescence ($F_o$) using a FRRfIII FastAct Fluorometer System (Chelsea Technologies Group Ltd, UK) with the same parameters as live cultures. Chlorophyll $a$ concentrations were calculated from a calibration curve derived from a dilution series measured on a chlorophyll $a$ standard (Sigma-Aldrich C5753).

To assess whether cells had been affected by i), concentration via filtration and resuspension and ii), exposure to the range of TIC and pH gradients over the course of the $^{14}$C incubations, 2 mL aliquots of culture from each treatment were dark acclimated (~ 20 minutes) and the
photosynthetic efficiency of PSII ($F_{v}/F_{m}$) measured using a FRRfIII FastAct Fluorometer System (Chelsea Technologies Group Ltd, UK) (Fig. S1).

Finally, 10 mL of culture from each treatment was pipetted into 12 mL glass (PTFE capped) test tubes and used for $^{14}$C incubations. A $^{14}$C spike solution was prepared by pipetting 45 µL of a $^{14}$C labelled sodium bicarbonate solution (NaH$^{14}$CO$_3$) with a specific activity of 52 mCi mmol$^{-1}$ (Perkin Elmer, USA) into 8 mL of bicarbonate-free YBCII media. Exactly 250 µL of the spike was added to each tube culture. The T$_0$ tubes were immediately filtered through swinnex filters containing (0.45 µm pore) 25mm diameter glass fibre filters and placed in scintillation vials and acidified (500 µL of 3 M HCl). To determine the total activity (TC), precisely 20 µL of the spike was added into three scintillation vials already containing 4.5 mL of scintillation cocktail (Gold LLT) and 200 µL of phenylethylamine. The TC vial caps were screwed tight immediately. The spiked test tubes were placed within a custom-made water-jacketed incubator and maintained at 26 °C and saturating light intensity (400 ± 6 µmol photons m$^{-2}$ s$^{-1}$) (The Optoelectronic Manufacturing Corporation Ltd. 1ft T5 Daylight, UK). The incubations lasted between 60 and 90 minutes and occurred between 4 to 6 hours into the photophase of the L:D cycle. The $^{14}$C incubations were repeated in the dark, using black-coated (Plasti-Kote paint) test tubes. Dark $^{14}$C uptake rates were 8.25% (± 0.46) and 7.05% (± 0.25) of the maximum light-saturated $^{14}$C uptake rates for the TIC and pH response curves, respectively. Dark $^{14}$C uptake rates exhibited no response to varying TIC or pH and were used to correct the light-dependent rates of photosynthesis (Li and Dickie, 1991).

To terminate $^{14}$C uptake, samples were filtered through glass fibre (0.45 µm pore) 25 mm filters (Fisherbrand FB59451, UK) using a bespoke 30-funnel filtration manifold. Test tubes and filters were rinsed twice with 5 mL of YBCII media, before the filters were placed into scintillation vials. The vials were acidified (500 µL of 3 M HCl) overnight along with the T$_0$ samples. Exactly 4.5 mL of scintillation cocktail (Gold LLT) was added to the acidified vials and the caps tightened. Ensuring that the scintillation cocktail and filtered samples were well mixed, the vials were placed within a scintillation counter and the disintegrations per minute (DPM) of each vial measured (20 minutes per vial). The CO$_2$ fixation rates were calculated using the following equation:

$$C_{\text{fixation}} = \left( \frac{DPM_{(S)} - DPM_{(T_0)}}{DPM_{(TC)}} \right) \cdot \left( \frac{V_{(TC)}}{V_{(S)}} \right) \cdot \left( \frac{[TCO_2]}{t} \right) \cdot 1.05 \quad (1)$$
where the disintegrations per minute (DPM) were measured for the sample (S), initial (T₀) and total activity (TC) vials; TIC (mmol L⁻¹) is the mean concentration of TIC within the sample over the course of the incubation (inclusive of the NaH¹⁴CO₃ spike); V_(TC) and V_(S) are the volumes of the sample and TC vials, respectively; t is the experimental incubation time (h⁻¹); and 1.05 is a radioisotope discrimination factor (¹²C:¹⁴C). Note that mean T₀ and TC values were used when calculating the C-fixation rates (n = 3).

Inorganic carbon fixation rates were normalised to a POC basis and the CO₂ response curves fitted to a Michaelis-Menten function;

\[
V_C = \frac{(V_m^C \cdot \text{CO}_2)}{(K_m + \text{CO}_2)}
\]

(2)

where \(V_C\) is the organic C-specific rate of CO₂ fixation, \(V_m^C\) is the maximum rate of CO₂ fixation and \(K_m\) is the half saturation constant. Curve fitting was performed on individual replicates to calculate mean (± S.E.) curve fit parameterisations (Sigmaplot 11.0), as well on the combined data where all replicates of the varied TIC (fixed pH) and varied pH (fixed TIC) data was combined per CO₂ treatment.

**Spectrophotometric chlorophyll a analysis**

Samples for spectrophotometric determination of chlorophyll a were collected with each CO₂ response curve and were used to normalise productivity rates as well as to calculate Chl a:C. A 100 mL sample from each culture was vacuum-filtered onto a 25 mm (0.45 µm pore) glass fiber filter (Fisherbrand FB59451, UK) and extracted in 5 mL of 100% methanol. Filters were homogenised and extracted overnight at -20 °C, before being centrifuged at 10,000 rpm for 10 minutes and a 3 mL aliquot of the supernatant added to a quartz cuvette. The absorption spectrum (400 to 800 nm) was measured using a (Hitachi U-3000, Japan) spectrophotometer and the Chl a concentration (µg L⁻¹) calculated using the following equation (Ritchie, 2008);

\[
\text{Chl } a = \left[ \frac{(12.9447 \cdot (\text{Abs}^{665} - \text{Abs}^{750})) \cdot V_(E)}{V(E)} \right] \cdot 1000
\]

(3)

where \(\text{Abs}^{665}\) and \(\text{Abs}^{750}\) are the baseline-corrected optical densities of the methanol extracted sample at 665 and 750 nm; \(V(E)\) is the volume of the solvent used for extraction (i.e. 5 mL);
V_{(F)} is the volume of culture filtered (i.e. 100 mL); and 12.9447 is a cyanobacteria-specific Chl $a$ coefficient for 100% methanol extraction.

Modelling the CCM

The CO$_2$ and HCO$_3^-$ fluxes and concentrations in an idealised *Trichodesmium* cell were calculated using the numerical model from Mangan *et al.* (2016) and Mangan and Brenner (2014). The aim was to offer a qualitatively informative view of the CCM system, without attempting to match carboxylation rates or fluxes to the experimental system or rescale the results from the idealised cell to what would be expected from the experimental data. With the exception of a few key parameter values (Table 2), the model is equivalent to that reported in Mangan *et al.* (2016). The main changes between the idealised *Trichodesmium* cell and previous models are the increase in cell and carboxysome size to be consistent with reported values for *T. erythraeum*, changing the RuBisCO kinetic constants, using pH and external CO$_2$ and HCO$_3^-$ concentrations similar to those in the $^{14}$C incubations, updating the pKa$_{eff}$ for HCO$_3^-$ to CO$_2$ to match that used in the CO2SYS calculation and re-calculating the HCO$_3^-$ uptake rate to support internal inorganic carbon concentrations of ~ 30 mM. We scaled the RuBisCO concentration by the carboxysome volume, so that the activity per volume remained the same. Similarly, we scaled the amount of carbonic anhydrase by the carboxysome surface area, so that the activity per area remained the same. The carbonic anhydrase activity was sufficient to equilibrate CO$_2$ and HCO$_3^-$ to $K'_{eq} = \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} = 10^{-pK_{eff} + pH}$. We set the carbonic anhydrase K$_{ca}$ value to preserve the correct equilibrium value for the internal pH.

Results

Inorganic carbon chemistry, growth rate and cell composition

Overall the CO$_2$ drawdown in the cultures ranged between 57 to 78 $\mu$mol mol$^{-1}$ for all CO$_2$ treatments (Table 1) and exhibited a negligible CO$_2$ drift over a diurnal cycle (Fig. S2). Dissolved inorganic NH$_4^+$ concentrations in the growth medium were ~ 1 $\mu$M, while NO$_3^-$ concentrations were ~ 0.3 $\mu$M, which is below the 1 $\mu$M detection limit.
Balanced growth rates increased from ~0.2 d\(^{-1}\) at low-\(\text{CO}_2\) to ~0.34 d\(^{-1}\) at mid-\(\text{CO}_2\) and ~0.36 d\(^{-1}\) at high-\(\text{CO}_2\) (Table 3). The dark adapted photochemical efficiencies of PSII \(F_v/F_m\) were proportionate to the growth \(\text{CO}_2\), increasing from 0.27 at low-\(\text{CO}_2\) to ~0.31 at mid-\(\text{CO}_2\) and ~0.34 at high-\(\text{CO}_2\) (Table 3). The particulate C:N ratio was independent of \(\text{CO}_2\), while the C:P and N:P ratios increased with increasing \(\text{CO}_2\) (Table 3). Both Chl \(a\):C and Chl \(a\):N ratios were about 30-40% higher at mid-\(\text{CO}_2\) than at low or high-\(\text{CO}_2\).  

**CO\(_2\)** response curves

Based on shape of the response curves, inorganic carbon (\(^{14}\text{C}\)) fixation rate was fit to a saturating function of the dissolved \(\text{CO}_2\) concentration in both the pH gradient and TIC gradient experiments (Fig. 1). Although a saturating function of \(\text{HCO}_3^-\) concentration was observed when TIC was varied at constant pH (Fig. 1A-C), \(\text{Ci}\) assimilation could not be described by the same kinetic constants when pH was varied at constant TIC (Fig. 1D-F).

The \(K_m\) for photosynthetic C-fixation increased from 0.8 µM in cultures acclimated to a low-\(\text{CO}_2\) to 2.2 µM and 3.2 µM in cultures acclimated to mid and high-\(\text{CO}_2\), respectively and were approximately 4 to 5-fold lower than the ambient \(\text{CO}_2\) concentrations in the cultures. The maximum organic carbon specific rate of C-fixation \(V_m^C\) was also higher in cells grown at mid-\(\text{CO}_2\) than at low-\(\text{CO}_2\), although the rates at mid and high-\(\text{CO}_2\) did not differ significantly (Table 4). The affinity for \(\text{CO}_2\) \((V_m^C/K_m)\) declined by about 40% with increasing \(\text{CO}_2\) (Table 4).

**Modelled response curves**

Without parameter fitting, the model of *Trichodesmium*’s CCM produced behaviors consistent with the experimental data when either external TIC (i.e \(\text{HCO}_3^-\)) was varied at a fixed pH or when pH was varied at a fixed TIC (Fig. 2A,B). Assuming \(\text{HCO}_3^-\) is the dominant form of inorganic carbon taken up by the cell (Eichner *et al.*, 2015; Kranz *et al.*, 2009), *Trichodesmium* exhibited a significant response to changes in external pH and \(\text{CO}_2\) concentrations. The decrease in carboxylation rate with decreasing external \(\text{CO}_2\) was due to a decrease in \(\text{HCO}_3^-\) uptake (when TIC was varied) or an increase in \(\text{HCO}_3^-\) and \(\text{CO}_2\) leakage out of the cell (when pH was varied) (Fig. S3). Modelled carboxylation rates from both
numerical simulations exhibited a smooth function of \( \text{HCO}_3^- \) uptake, \( \text{HCO}_3^- \) leakage and \( \text{CO}_2 \) leakage (Fig. 2C).

The \( V_m^C \) of the pH gradient and TIC gradient experiments were not significantly different (Table S3). However, the maximum carboxylation rates from the simulations were significantly different (Fig. 2); principally because the external \( \text{HCO}_3^- \) concentration used in the pH-dependent simulation (chosen to be the same as the experiment) was not sufficient to saturate RuBisCO. It is possible that the \( K_m \) assumed for RuBisCO was set too high, or the internal pH, geometry, or \( \text{HCO}_3^- \) uptake values are substantially different. Note that we are simulating values beyond the range of those in the experiments, so such a discrepancy is magnified.

**Discussion**

Amongst our key findings are: (i) The acclimated growth rate increased from low- to mid-\( \text{CO}_2 \) but did not increase significantly between mid- and high-\( \text{CO}_2 \) treatments, suggesting that the positive effect of elevated \( \text{CO}_2 \) on *Trichodesmium* carbon assimilation over the coming decades may only be slight. (ii) The maximum rate (\( V_m^C \)) and the half saturation constant (\( K_m \)) for C-fixation increased with growth \( \text{CO}_2 \), but the affinity for \( \text{CO}_2 \) (\( V_m^C/K_m \)) declined, which is likely attributed to *Trichodesmium*’s CCM activity. (iii) Measured inorganic C-fixation rate in *Trichodesmium* could be described as a saturating function of \( \text{CO}_2 \) both when \( \text{CO}_2 \) was manipulated by varying pH at constant TIC and when \( \text{CO}_2 \) was manipulated by varying TIC at constant pH. (iv) A mechanistic model of *Trichodesmium*’s CCM indicates that the former is due to \( \text{HCO}_3^- \) uptake limitation of carboxylation rate, whereas the latter is due to a high-pH mediated increase in \( \text{HCO}_3^- \) and \( \text{CO}_2 \) leakage, potentially coupled to other unknown processes operating outside of the paratermised model that are restricting Ci assimilation rates at high-pH. Such processes may involve the direct effect of pH on membrane conformation, membrane transport processes or metabolic functions.
Effect of acclimation to variation of inorganic chemistry on growth rates and elemental stoichiometry

Increased growth rate from low- (180 µmol mol\(^{-1}\)) to mid- (380 µmol mol\(^{-1}\)) and high-CO\(_2\) (720 µmol mol\(^{-1}\)) was similar to previous findings (Barcelos e Ramos et al., 2007; Boatman et al., 2017). Growth rate at high CO\(_2\) was 8% greater than at mid-CO\(_2\), but this difference was not statistically significant. The magnitude of this increase at high CO\(_2\) is comparable to several recent studies, which report growth rate increases of between 7 to 26% with increases of CO\(_2\) beyond 400 µmol mol\(^{-1}\) (Barcelos e Ramos et al., 2007; Boatman et al., 2017; Garcia et al., 2011; Hutchins et al., 2007; Kranz et al., 2010; Levitan et al., 2007).

The observed increase in C:P and N:P are consistent with previous findings (Barcelos e Ramos et al., 2007; Kranz et al., 2010; Levitan et al., 2010), where changes can be ascribed to increases in cellular N and C incorporation, with P quotas relatively unaffected by CO\(_2\) (Hutchins et al., 2007; Kranz et al., 2010). In contrast, the C:N and thus the balance between CO\(_2\) fixation and N\(_2\) fixation was not significantly affected by the growth CO\(_2\). Similarly, Levitan et al. (2007) found C:N varied only slightly (from 6.5 to 7.0) across growth CO\(_2\) concentrations from 250 to 900 µmol mol\(^{-1}\).

We report C-specific rates here as these are most directly related to changes in specific growth rate because both rates can be expressed in equivalent units of inverse time (e.g. h\(^{-1}\) or d\(^{-1}\)). However, we note that due to differences in the Chl \(\alpha\):C ratio, chlorophyll \(\alpha\)-specific rates showed a different pattern, increasing progressively from low- through mid- to high-CO\(_2\) (Table S2, Fig. S4). A reduction in Chl \(\alpha\):C decreases the energy demands associated with synthesis of the photosynthetic apparatus and is dictated by the total demands for reductant (NADPH) and high-energy phosphate bonds (ATP) (Geider et al., 2009), the minimum turnover times for PSII (\(\tau_{\text{PSII}}\)) and PSI (\(\tau_{\text{PSI}}\)) and the minimum pigment content required for effective light absorption and energy transfer (\(a_{\text{min}}\)) (Behrenfeld et al., 2008). We suggest that the reduced Chl \(\alpha\):C at low CO\(_2\) relative to mid CO\(_2\) is likely due to the cost of up-regulating the CCM, whereas the reduced Chl \(\alpha\):C at high CO\(_2\) may be due to an increase in carbohydrate storage granules relative to the mid CO\(_2\) treatment (Table 3).
**CO₂ response curves**

Growth rates reported here are comparable to the 2 µM EDTA, iron replete (unchelated) treatments in Boatman *et al.* (2017), as well as 20 µM EDTA, iron replete (chelated) cultures (Boatman *et al.*, Unpublished); suggesting that cultures were not exposed to toxic concentrations of certain trace metals (e.g. copper) caused from low trace metal buffering capacity as reported by Hong *et al.* (2017). Furthermore, dissolved inorganic NH₄⁺ concentrations were consistently around 0.3 µM (Table 1). We are therefore confident that the observed positive effect of ocean acidification on growth and primary productivity is driven by the increased CO₂ concentration, rather than a consequence of a pH induced shift of the NH₃/NH₄⁺ equilibrium. We measured CO₂ response curves at one time of day (4 to 6 hours into the photo-phase of a 12:12 L:D cycle) and as such cannot extrapolate to a diel response given the reports of temporal separation of photosynthesis and N₂ fixation in *Trichodesmium* (Berman-Frank *et al.*, 2001).

The mechanistic model of Mangan *et al.* (2016) indicates that the CO₂ response we observed when the TIC was varied (pH fixed) was caused by HCO₃⁻ limitation, where HCO₃⁻ uptake limits the rate of carboxylation. Conversely, the CO₂ response we observed when pH was varied (TIC fixed) is a function of the pH dependency of HCO₃⁻ and CO₂ leakage, which in turn could lead to CO₂ limitation of C-fixation and/or diversion of reducing equivalents from powering CO₂ fixation via the Calvin cycle to powering the conversion of CO₂ to HCO₃⁻ by the NDH-I₄ complex. The model of *Trichodesmium*’s CCM shows the relative importance of leakage, which is notably sensitive to certain parameters in the system such as internal pH, RuBisCO activity, cell size and carboxysome size.

Previous studies have shown a notable response in CCM activity to changes in CO₂; for example a two-fold lower DIC half saturation concentration in cells acclimated to 150 µmol mol⁻¹ (pH₉BS 8.56) compared with 370 µmol mol⁻¹ (pH₉BS 8.26) (Kranz *et al.*, 2009). Our experimental observations indicate that Ci assimilation (Vᵢ) is well described by a CO₂ response curve, but not by a single HCO₃⁻ response curve (Fig. 1). Here we offer an explanation as to the response of Vᵢ to HCO₃⁻ concentration in the experiments where we varied pH(Total) from 7.65 to 8.5 at constant TIC.

Based on the numerical simulations, carboxylation rates across an external pH gradient ranging from 7.5 to 8.5 exhibit a clear linear response, which cannot be ascribed to a Michaelis-Menten function (Fig. S3). Conversely, our experimental data shows a clear and
significant decrease in Ci assimilation rates at low external CO₂/high-pH (Fig. 1). In addition, the Ci assimilation rates for the pH gradient and TIC gradient experiments, for all replicates of all three CO₂ treatments, exhibited similar inflection points to external CO₂ (Fig. S5). In order for the simulated system to exhibit a rate saturating response to external CO₂, CO₂ would have to be the dominant source of inorganic carbon. This would contradict all previous research showing that HCO₃⁻ accounts for > 90% of inorganic carbon uptake (Kranz et al., 2009; Kranz et al., 2010) and with the currently accepted mechanism of Ci assimilation in T. erythraeum IMS101 (Badger and Price, 2003).

Given how well the numerical simulations modelled carboxylation rates as a smooth function of HCO₃⁻ uptake, HCO₃⁻ leakage and CO₂ leakage (Fig. 2C), we propose that the linear pH-dependency on carboxylation rate predicted by the model is mechanistically correct, but that processes not captured by the model are contributing to the decrease in Ci assimilation rate at high-pH. Such factors could include a direct effect of high-pH on cell membrane properties and alteration in membrane conformation (Myklestad and Swift, 1998), or the influence of pH on membrane transport processes and metabolic functions involved in cellular pH regulation (Raven, 1981).

Interestingly, for the mid and high CO₂ treatments, a Michaelis-Menten function provided a better fit for the pH varied (TIC fixed) data than a linear regression. However, there was no significant difference between a linear or Michaelis-Menten function for the low CO₂ data, which suggests that full acclimation to a high-pH environment prior to the ¹⁴C incubations lessened the negative effect that high-pH had on Ci assimilation.

Based on our simulation, Trichodesmium’s actual carboxylation rate should be modelled as a function of HCO₃⁻ and pH. This is because the CO₂ concentration in a saturated HCO₃⁻/high-pH (i.e. 3.8 mM HCO₃⁻, pH = 8.4) environment could be equivalent to a limited HCO₃⁻/present pH (i.e. 1.9 mM HCO₃⁻, pH = 8.1) environment; which for the aforementioned reasons, will impose different constraints on leakage/uptake rates. That said, our experimental data clearly suggests that high-pH induced processes operating outside of the CCM are contributing to decrease Ci assimilation. Overall, this may allow Trichodesmium’s Ci assimilation rates to be ascribed as a function of CO₂ (Fig. 1, Fig. S4), which would be considerably simpler to implement in biogeochemical models of Trichodesmium growth and photosynthesis (Hutchins et al., 2013) than a HCO₃⁻ response curve in which the kinetic constants (K_m and V_m) are pH-dependent. Further experimental work is needed to assess
whether a CO$_2$ parameterisation is consistent across an extended range of pH and HCO$_3^-$ conditions than those used in our experiments.

Conclusion

Climate change is driving ocean acidification, which results in higher CO$_2$ and HCO$_3^-$ concentrations and a decrease in pH. We observed systematic changes in the kinetics of inorganic carbon assimilation of *T. erythraeum* IMS101 in response to acclimation to CO$_2$ ranging from low-CO$_2$ (levels at the last glacial maximum) through mid-CO$_2$ (levels at the end of the 20th century) to high-CO$_2$ (levels predicted for 2050 to 2100). Extrapolating these responses to future scenarios of the natural environment should consider i), these findings were obtained using acclimation experiments whereas *Trichodesmium* may adapt to future conditions (Hutchins *et al.*, 2015); ii), variability may exist between strains and clades (Hutchins *et al.*, 2013); and iii), the additional effects of integrated abiotic variables (i.e. light and temperature) and nutrients (i.e. P and Fe) on *Trichodesmium* productivity (Walworth *et al.*, 2016).

In the context of the open-oceans, our results indicate that nutrient-replete net photosynthesis and growth rates of *T. erythraeum* IMS101 would have been severely CO$_2$ limited at the last glacial maximum relative to current conditions. However, future increases in CO$_2$ (i.e. 720 µmol mol$^{-1}$) may not significantly increase growth and productivity of IMS101, although we note that others report a stimulation of growth and photosynthesis by increasing CO$_2$ beyond current ambient concentrations (Hutchins *et al.*, 2007; Levitan *et al.*, 2010; Levitan *et al.*, 2007). However, we did observe that growth under high CO$_2$ will increase key stoichiometric ratios (N:P and C:P). Increases of N:P and C:P in *Trichodesmium*-dominated oceanic regimes may affect bacterial and zooplankton metabolism, the pool of bioavailable nitrogen, the depth at which sinking organic matter is remineralised and consequently carbon sequestration via the biological carbon pump (McGillicuddy, 2014; Mulholland *et al.*, 2004). These responses could serve as a negative feedback to climate change by increasing new N and C production and thereby increasing the organic carbon sinking to the deep ocean.
Acknowledgements

Tobias Boatman was supported by a UK Natural Environment Research Council PhD studentship (NE/J500379/1 DTB).
References


Table 1. The growth conditions (± S.E.) achieved for *T. erythraeum* IMS101 when cultured at three target gas phase CO$_2$ concentrations (Low = 180 µmol mol$^{-1}$, Mid = 380 µmol mol$^{-1}$ and High = 720 µmol mol$^{-1}$), saturating light intensity (400 µmol photons m$^{-2}$ s$^{-1}$) and optimal temperature (26 °C).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>Low CO$_2$</th>
<th>Mid CO$_2$</th>
<th>High CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Total</td>
<td>8.458</td>
<td>8.174</td>
<td>7.906</td>
</tr>
<tr>
<td>H$^+$</td>
<td>nM</td>
<td>3.5 (0.20)</td>
<td>6.7 (0.13)</td>
<td>12.4 (0.28)</td>
</tr>
<tr>
<td>A$_T$</td>
<td>µM</td>
<td>2431 (70)</td>
<td>2447 (54)</td>
<td>2442 (56)</td>
</tr>
<tr>
<td>TIC</td>
<td>µM</td>
<td>1800 (69)</td>
<td>2039 (46)</td>
<td>2201 (50)</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>µM</td>
<td>1362 (67)</td>
<td>1743 (39)</td>
<td>2005 (44)</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>µM</td>
<td>435 (16)</td>
<td>289 (9)</td>
<td>179 (6)</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>µM</td>
<td>3.3 (0.3)</td>
<td>8.1 (0.2)</td>
<td>17.3 (0.5)</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>µM</td>
<td>1.03 (0.14)</td>
<td>1.00 (0.08)</td>
<td>1.08 (0.06)</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>µM</td>
<td>0.34 (0.05)</td>
<td>0.32 (0.03)</td>
<td>0.30 (0.02)</td>
</tr>
</tbody>
</table>

Individual pH values were converted to a H$^+$ concentration, allowing a mean pH value to be calculated. Dissolved inorganic NH$_4^+$ was determined using the phenol-hypochlorite method as described by Solorzano (1969), while dissolved inorganic NO$_3^-$ was determined using the spectrophotometric method as described by Collos *et al.* (1999).
**Table 2. Key parameter values used in the numerical simulation of Trichodesmium’s CCM.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>Model Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell radius, $R_b$</td>
<td>$\mu m$</td>
<td>3</td>
</tr>
<tr>
<td>Carboxysome radius $R_c$</td>
<td>$\mu m$</td>
<td>0.15</td>
</tr>
<tr>
<td>RuBisCO reaction rate $k_{Rub}$</td>
<td>1/s per active site</td>
<td>1.92</td>
</tr>
<tr>
<td>RuBisCO $K_{CO2}$</td>
<td>$\mu M$</td>
<td>145</td>
</tr>
<tr>
<td>RuBisCO $K_{O2}$</td>
<td>$\mu M$</td>
<td>600</td>
</tr>
<tr>
<td>RuBisCO specificity, S</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Number of RuBisCO active sites</td>
<td>-</td>
<td>54000</td>
</tr>
<tr>
<td>Number of carbonic anhydrase active sites</td>
<td>-</td>
<td>900</td>
</tr>
<tr>
<td>Carbonic anhydrase ½ max constant for CO$<em>2$, $K</em>{ca}$</td>
<td>$\mu M$</td>
<td>104.7</td>
</tr>
<tr>
<td>Internal pH</td>
<td>-</td>
<td>8.3</td>
</tr>
<tr>
<td>$pK_{a_{eq}}$ for HCO$_3^-$:CO$_2$</td>
<td>-</td>
<td>5.84</td>
</tr>
<tr>
<td>Carboxysome permeability</td>
<td>cm/s</td>
<td>$3 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>HCO$_3^-$ uptake velocity, $j_c$</td>
<td>cm/s</td>
<td>$2.4 \cdot 10^{-7}$</td>
</tr>
<tr>
<td>CO$_2$ to HCO$_3^-$ conversion at membrane</td>
<td>cm/s</td>
<td>$0.6 \cdot 10^{-7}$</td>
</tr>
</tbody>
</table>

The cell radius was measured from a bioimage collected using fluorescence microscopy (Fig. S12). Kinetic constants of RuBisCO carboxylation ($K_{CO2}$), oxygenation ($K_{O2}$) and the specificity factor (S) for a form 1B cyanobacteria were abstracted from Badger et al. (1998).
Table 3. The mean (± S.E.) balanced growth rate, dark adapted photochemical efficiency of PSII ($F_v/F_m$), elemental stoichiometry and chlorophyll a to C and N ratios for T. erythraeum IMS101 when acclimated to three target CO$_2$ concentrations (Low = 180 µmol mol$^{-1}$, Mid = 380 µmol mol$^{-1}$ and High = 720 µmol mol$^{-1}$), saturating light intensity (400 µmol photons m$^{-2}$ s$^{-1}$) and optimal temperature (26 °C).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>Low CO$_2$</th>
<th>Mid CO$_2$</th>
<th>High CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate</td>
<td>d$^{-1}$</td>
<td>0.198 (0.027)</td>
<td>[A] 0.336 (0.026)</td>
<td>[B] 0.361 (0.020)</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>dimensionless</td>
<td>0.274 (0.025)</td>
<td>[A] 0.305 (0.020)</td>
<td>[B] 0.342 (0.037)</td>
</tr>
<tr>
<td>Elemental Stoichiometry</td>
<td>C:N mol:mol</td>
<td>7.9 (0.8)</td>
<td>7.8 (0.3)</td>
<td>7.3 (0.8)</td>
</tr>
<tr>
<td>C:P mol:mol</td>
<td></td>
<td>91.9 (6.3)</td>
<td>[A] 143.6 (6.3)</td>
<td>[B] 155.5 (13.5)</td>
</tr>
<tr>
<td>N:P mol:mol</td>
<td></td>
<td>11.9 (0.6)</td>
<td>[A] 18.4 (0.7)</td>
<td>[B] 21.8 (1.7)</td>
</tr>
<tr>
<td>Chl a:C g:mol</td>
<td></td>
<td>0.052 (0.003)</td>
<td>[A] 0.089 (0.003)</td>
<td>[C] 0.066 (0.003)</td>
</tr>
<tr>
<td>Chl a:N g:mol</td>
<td></td>
<td>0.401 (0.037)</td>
<td>[A] 0.693 (0.035)</td>
<td>[B] 0.474 (0.043)</td>
</tr>
</tbody>
</table>

Abbreviations; C:N, C:P and N:P ratios are mol:mol, Chl a:C and Chl a:N ratios are g:mol ($n$ = 9 at low-CO$_2$, $n$ = 6 at mid- and high-CO$_2$). Letters in parenthesis indicate significant differences between CO$_2$ treatments (One Way ANOVA, Tukey post hoc test; P < .05); where [B] is significantly greater than [A] and [C] is significantly greater than [B] and [A].
Table 4. The physiological parameters (± S.E.) of the C-specific C-fixation versus CO₂ concentration response curves for T. erythraeum IMS101. Data was fitted using the Michaelis-Menten model to obtain estimates of the half saturation constant (Kₘ) and maximum uptake rate (Vₘ⁵) for CO₂ assimilation using the combined data from all replicates from both experiments employing varied TIC at fixed pH and varied pH at fixed TIC for each CO₂ treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Low CO₂</th>
<th>Mid CO₂</th>
<th>High CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vₘ₅⁵</td>
<td>h⁻¹</td>
<td>0.011 (0.0002)</td>
<td>0.024 (0.0007)</td>
<td>0.026 (0.0008)</td>
</tr>
<tr>
<td>Kₘ⁵</td>
<td>µM CO₂</td>
<td>0.8 (0.1)</td>
<td>2.2 (0.3)</td>
<td>3.2 (0.4)</td>
</tr>
<tr>
<td>Affinity⁵ₜₚ</td>
<td>mM (CO₂)⁻¹ h⁻¹</td>
<td>13.3 (1.7)</td>
<td>10.9 (1.5)</td>
<td>8.0 (1.0)</td>
</tr>
</tbody>
</table>

Abbreviations; Vₘ₅, the C-specific maximum C-fixation rates; Kₘ, the half saturation constant; Affinity⁵ₜₚ, the C-specific initial slope of the Vₘ₅ vs CO₂ response curve.
Fig. 1. The CO$_2$ and HCO$_3^-$ response curves for inorganic C-fixation by *T. erythraeum* IMS101. C-fixation rates are normalised to a carbon (h$^{-1}$) basis. Data indicated by filled circles were obtained by varying TIC and HCO$_3^-$ (mmol L$^{-1}$) at a fixed pH of ~ 8.15 (A-C). Data indicated by unfilled circles were obtained by varying pH (~ 7.52 – 8.54) at a fixed TIC (D-F). Differences in the range of HCO$_3^-$ and CO$_2$ gradients between CO$_2$ treatments are due to variability in pipetting and not from instability in the Ci chemistry. For the CO$_2$ response, curve fitting was performed using all replicates from both the TIC and pH gradients. For the HCO$_3^-$ response, curve fitting was performed using data from the TIC gradient only. The CO$_2$ and HCO$_3^-$ response curves for individual experiments are shown in Fig. S6-S11.

Fig. 2. The numerically calculated carboxylation rates (µM s$^{-1}$) obtained from the model simulations for *T. erythraeum* IMS101 as a function of external CO$_2$ (A) and HCO$_3^-$ (B) concentrations; where TIC (i.e. HCO$_3^-$) was varied at a fixed pH = 8.15 (dashed lines) and pH was varied at a fixed HCO$_3^-$ = 1.9 mM (solid lines). Carboxylation rates are also plotted against the net HCO$_3^-$ uptake rate (C), where HCO$_3^-$ and CO$_2$ leakage rates were subtracted from the rate of gross HCO$_3^-$ transport.
Figure 1
Figure 2