RESEARCH PAPER

CO₂ modulation of the rates of photosynthesis and light-dependent O₂ consumption in *Trichodesmium*

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Abstract

As atmospheric CO₂ concentrations increase, so too does the dissolved CO₂ and HCO₃⁻ concentrations in the world's oceans. There are still many uncertainties regarding the biological response of key groups of organisms to these changing conditions, which is crucial for predicting future species distributions, primary productivity rates, and biogeochemical cycling. In this study, we established the relationship between gross photosynthetic O₂ evolution and light-dependent O₂ consumption in *Trichodesmium erythraeum* IMS101 acclimated to three targeted pCO₂ concentrations (180 µmol mol⁻¹=low-CO₂, 380 µmol mol⁻¹=mid-CO₂, and 720 µmol mol⁻¹=high-CO₂). We found that biomass- (carbon) specific, light-saturated maximum net O₂ evolution rates (PₙC,max) and acclimated growth rates increased from low- to mid-CO₂, but did not differ significantly between mid- and high-CO₂. Dark respiration rates were five times higher than required to maintain cellular metabolism, suggesting that respiration provides a substantial proportion of the ATP and reductant for N₂ fixation. Oxygen uptake increased linearly with gross O₂ evolution across light intensities ranging from darkness to 1100 µmol photons m⁻² s⁻¹. The slope of this relationship decreased with increasing CO₂, which we attribute to the increased energetic cost of operating the carbon-concentrating mechanism at lower CO₂ concentrations. Our results indicate that net photosynthesis and growth of *T. erythraeum* IMS101 would have been severely CO₂ limited at the last glacial maximum, but that the direct effect of future increases of CO₂ may only cause marginal increases in growth.

Keywords: Carbon fixation, CO₂, cyanobacteria, gross photosynthesis, net photosynthesis, ocean acidification, *Trichodesmium*.

Introduction

The ocean is one of the largest readily exchangeable reservoirs of inorganic carbon on Earth and is a major sink for anthropogenic CO₂ emissions (Sabine et al., 2004). The ocean's capacity to sequester atmospheric CO₂ is strongly mediated by biological processes (Raven and Falkowski, 1999), where organic matter production and export drive CO₂ sequestration. This is important as future emission scenarios predict that atmospheric CO₂ will increase from present concentrations (~400 µmol mol⁻¹) to 750 µmol mol⁻¹ or 1000 µmol mol⁻¹ by the end of this century (Raven et al., 2005). This will lead to an increase in the total dissolved inorganic carbon (TIC) in the surface ocean, reducing the pH from an average value of ~8.2 (pre-industrial) to ~7.9 (estimated for 2100) (Zeebe et al., 1999; Zeebe and Wolf-Gladrow, 2001). Ocean acidification therefore favours an increase in seawater CO₂ and HCO₃⁻ concentration and a decrease in pH and CO₃²⁻.
There are still many uncertainties regarding the biological response of key groups of organisms to these changing conditions, which is crucial for predicting future species distributions, primary productivity rates, and biogeochemical cycling. One group of great importance are diazotrophic cyanobacteria (photosynthetic dinitrogen fixers), as they contribute significantly to overall marine primary productivity by providing new nitrogen to many oligotrophic areas of the oceans. The filamentous cyanobacteria *Trichodesmium* are a colony-forming species that forms extensive surface blooms in the tropical and subtropical oceans (Carpenter and Capone, 1992; Capone et al., 1997; Campbell et al., 2005). *Trichodesmium* plays a significant role in the N cycle of the oligotrophic oceans; fixing nitrogen in an area corresponding to half of the Earth’s surface (Davis and McGillicuddy, 2006) and representing up to 50% of new production in some oligotrophic tropical and subtropical oceans (Capone, 2005). The annual marine N₂ fixation is currently estimated at between 100 Tg and 200 Tg N per year (Gruber and Sarmiento, 1997; Karl et al., 2002), of which *Trichodesmium* spp. contribute between 80 Tg and 110 Tg of fixed N₂ to open ocean ecosystems (Capone et al., 1997).

Cyanobacteria have performed oxygenic photosynthesis for ~2.7 billion years (Buick, 2008). During that time, CO₂ concentrations have declined and O₂ concentrations increased, thus exerting an evolutionary pressure to form a mechanism to reduce the impact of photorespiration on photosynthetic CO₂ fixation. Despite cyanobacterial Rubisco having a relatively low affinity for CO₂, cyanobacteria achieve high photosynthetic rates by virtue of an intracellular carbon-concentrating mechanism (CCM), which thereby reduces the diversion of energy into oxygenation of ribulose-1,5-bisphosphate (RuBP), the first step in photorespiration (Schwarz et al., 1995; Kaplan and Reinhold, 1999). In addition, the CCM can aid in the dissipation of excess light energy as well as maintaining an optimal intracellular pH (Badger et al., 1994; Kaplan and Reinhold, 1999).

Cyanobacteria have a unique ability to perform both photosynthesis and respiration simultaneously in the same cellular compartment (Nagarajan and Pakrasi, 2001). The thylakoid membranes of cyanobacteria contain both respiratory and photosynthetic electron transport chains, sharing the plastoquinone compartment (Nagarajan and Pakrasi, 2001). The thylakoid membranes of cyanobacteria achieve high photosynthetic rates because of their thylakoid concentration of carbon (CCM), which thereby reduces the diversion of energy into oxygenation of ribulose-1,5-bisphosphate (RuBP), the first step in photorespiration (Schwarz et al., 1995; Kaplan and Reinhold, 1999). In addition, the CCM can aid in the dissipation of excess light energy as well as maintaining an optimal intracellular pH (Badger et al., 1994; Kaplan and Reinhold, 1999).

Cyanobacteria have a unique ability to perform both photosynthesis and respiration simultaneously in the same cellular compartment (Nagarajan and Pakrasi, 2001). Thylakoids of cyanobacteria contain both respiratory and photosynthetic electron transport chains, sharing the plastoquinone and plastocyanin pools and the Cyt b₆f complex. In contrast, the cytoplasmic membrane is only capable of performing respiratory electron transport (Nagarajan and Pakrasi, 2001). Thus, it is common in cyanobacteria for respiratory electron transport to be inhibited at low light intensities as photosynthesis increases in the thylakoid membranes (Kana, 1992). However, in *Trichodesmium* there remains the possibility that photosynthetic and respiratory metabolism differs between diazocytes (where N₂ fixation occurs) and other cells within a trichome.

Previous studies report an increase in growth and productivity (CO₂ and N₂ fixation) of *T. erythraeum* IMS101 as well as changing elemental composition in response to future CO₂ concentrations (~750–1000 µmol mol⁻¹) (Barcelos e Ramos et al., 2007; Levitan et al., 2007, 2010a; Kranz et al., 2010; Spungin et al., 2014; Hutchins et al., 2015; Boatman et al., 2017, 2018a, b), although, as discussed in Boatman et al. (2018a), the magnitude of the responses often differs between studies. Due to the significant contribution that *Trichodesmium* makes to biogeochemical cycles and the predicted change in inorganic carbon (Ci) speciation over the coming decades, we performed a systematic experiment to assess how the photosynthetic physiology of *T. erythraeum* IMS101 was affected by acclimation to varying CO₂. We ensured that the Ci chemistry and all other growth conditions were well defined, with cultures fully acclimated over long time periods (~5 months) to achieve balanced growth. We assessed the dark respiration, light absorption, and the light dependencies of gross O₂ evolution and O₂ consumption across different CO₂ conditions. We discuss how the responses that we observed may be related to N₂ fixation and changes in the cost of operating the CCM.

**Materials and methods**

*T. erythraeum* IMS101 was semi-continuously cultured to achieve fully acclimated balanced growth at three target pCO₂ concentrations (180, 380, and 720 µmol mol⁻¹), under saturating light intensity (400 µmol photons m⁻² s⁻¹), a 12/12 h light/dark (L/D) cycle, and an optimum growth temperature (26 ± 2 °C) for ~5 months (~40, 70, and 80 generations at low-, mid-, and high-CO₂, respectively).

**Experimental set-up**

Cultures of *T. erythraeum* IMS101 were grown in standard YBCII medium (Chen et al., 1996) under diazotrophic conditions (N₂ only) in 1.5 litre volumes in 2 litre Pyrex bottles that had been acid-washed and autoclaved prior to culturing. 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 (0.2 µm pore) air mixture at a rate of ~200 ml s⁻¹. The CO₂ concentration was regulated ±2 µmol mol⁻¹ by mass-flow controllers (Branhkorst, Newmarket, UK) and O₂-free air was supplied by an oil-free compressor (Bambi Air, UK) via a soda-lime gas-tight column that was mixed with a 10% CO₂-in-air mixture from a gas cylinder (BOC Industrial Gases, UK). The CO₂ concentration in the gas phase was continuously monitored by an infra-red gas analyser (Li-Cor Li-820, Lincoln, NE, USA), calibrated weekly against a standard gas (BOC Industrial Gases).

Cultures were kept at the upper section of the exponential growth phase through periodic dilution with new growth media at 3–5 d intervals. Daily growth rates were quantified from changes in baseline fluorescence (F₀) measured between 09.00 h and 10.30 h on dark-adapted cultures (20 min) using a FRFRlI FastAct Fluorometer System (Chelsea Technologies Group Ltd, UK). As detailed in Boatman et al. (2018a), cultures were deemed fully acclimated and in balanced growth when both the slope of the linear regression of ln(Fᵣ) and the ratio of live-cell to acetone-extracted Fᵣ were constant following every dilution with fresh YBCII medium.

The Ci chemistry was measured prior to the dilution of each culture with fresh media, where exactly 20 ml of culture from each treatment was filtered through a swinnex filter (25 mm, 0.45 µm pore, glass fibre filter): 15 ml into a plastic centrifuge tube (no headspace) for TIC analysis (Shimadzu TOC-V Analyser & ASI-Autosampler), and 5 ml into a plastic cryogenic vial (Sigma-Aldrich V5257-250EA; no headspace) for pH analysis. The bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), and CO₂ concentrations were calculated via CO₂SYS as described in Boatman et al. (2017). Overall, the CO₂ drawdown in the cultures ranged between 49 µmol mol⁻¹ and 90 µmol mol⁻¹ for all CO₂ treatments (Table 1) and exhibited a negligible CO₂ drift over a diurnal cycle (see Supplementary Fig. S1 at JXB online).

**Gross and net O₂ exchange**

Light-dependent rates of O₂ production and consumption were measured on four biological replicates per CO₂ treatment, using a membrane inlet mass spectrometer (MIMS) and an ¹⁸O₂ technique modified from McKew et al. (2013).
The growth conditions (±SE) achieved for T. erythraeum IMS101 when cultured at three target gas phase ChlCO₂ 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-CO₂</th>
<th>Mid-CO₂</th>
<th>High-CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>8.461</td>
<td>8.175</td>
<td>7.905</td>
</tr>
<tr>
<td>H⁺</td>
<td>mM</td>
<td>3.5 (0.1)</td>
<td>6.7 (0.1)</td>
<td>12.5 (0.2)</td>
</tr>
<tr>
<td>A(µ)</td>
<td>µM</td>
<td>2427 (32)</td>
<td>2490 (51)</td>
<td>2444 (42)</td>
</tr>
<tr>
<td>TCO₂</td>
<td>µM</td>
<td>1797 (30)</td>
<td>2076 (44)</td>
<td>2204 (37)</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>µM</td>
<td>1356 (30)</td>
<td>1773 (37)</td>
<td>2008 (32)</td>
</tr>
<tr>
<td>CO₂</td>
<td>µM</td>
<td>436 (3)</td>
<td>295 (8)</td>
<td>179 (5)</td>
</tr>
<tr>
<td>CO₂</td>
<td>µM</td>
<td>3.3 (0.2)</td>
<td>8.2 (0.2)</td>
<td>17.4 (0.3)</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>mM</td>
<td>1.00 (0.12)</td>
<td>1.06 (0.08)</td>
<td>1.02 (0.06)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>mM</td>
<td>0.33 (0.05)</td>
<td>0.36 (0.02)</td>
<td>0.32 (0.02)</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>76</td>
<td>32</td>
<td>28</td>
</tr>
</tbody>
</table>

Individual pH values were converted to a H⁺ concentration, allowing a mean pH value to be calculated. Dissolved inorganic NH₄⁺ was determined using the phenol-hypochlorite method as described by Solorzano (1969), while dissolved inorganic NO₃⁻ was determined using the spectrophotometric method as described by Collos et al. (1999).

MIMS samples were prepared by placing 300 ml of culture in a large gas-tight syringe, and gently bubbled with N₂ gas for ~20 min to reduce the [²¹⁸O]₂ concentration. The headspace was removed, and 2 ml of [²¹⁸O]₂ gas (CK Gas Products, UK; 99% purity) was added and mixed by continuously inverting the syringe for ~20 min. During this process, the culture was maintained at a low light intensity (~10 µmol photons m⁻² s⁻¹) and at growth temperature (26 °C). Samples were incubated using a series of 6 ml glass stopper, gas-tight test tubes, which were cleaned with detergent, acid-washed (10% HCl for 1 d), and rinsed with deionized water (Millipore Milli-Q Biocel, ZMQS60FOI) prior to use. Glass beads were placed inside each test tube, allowing the sample to be mixed throughout the incubation. The [²¹⁸O]₂-enriched culture was quickly dispensed into the gas-tight glass test tubes, sealed using ground glass stoppers (no headspace), and immediately placed into a temperature-controlled (26 °C) incubator. A white light-emitting diode (LED) block (Iso Light 400, Technologica, Essex, UK) was positioned at one end of the incubator, generating light intensities ranging from 10 µmol photons m⁻² s⁻¹ to 1100 µmol photons m⁻² s⁻¹. For each replicate, 24 test tubes were incubated across the light gradient, a minimum of 10 test tubes were used to determine the initial concentration of O₂ isotopes, and an additional four test tubes were incubated in the dark (26 °C) to determine dark respiration rates. All physiological and elemental analyses were performed using methods described elsewhere (Ritchie, 2008).

Changes in [²¹⁸O]₂ and [²¹⁹O]₂ and thus O₂ consumption (U₀) and O₂ evolution (E₀) were calculated using the following equations (Radmer and Kok, 1976):

\[
U_0 = -\left(1 + \frac{1}{2} \frac{16O_2}{18O_2}\right) \frac{\Delta 18O_2}{\Delta t}
\]

(1)

\[
E_0 = \frac{\Delta 16O_2}{\Delta t} - \frac{1}{2} \frac{16O_2}{18O_2} \frac{\Delta 18O_2}{\Delta t}
\]

(2)

where U₀ is the rate of O₂ consumption and E₀ is the rate of gross O₂ evolution. C-specific rates were obtained by dividing U₀ and E₀ by the concentration of particulate organic carbon (POC). Rates were also normalized to Chl a and particulate organic nitrogen (PON), and are presented in Supplementary Figs S2 and S3.

The P–E curves for gross (E₀C) and net O₂ exchange (PₐC=É₀C−U₀C) were fitted to the following equations from Platt and Jassby (1976):

\[
E_{0C} = E_{0C,max} \cdot \left[1 - e^{-\frac{\alpha_{EC} \cdot E}{E_{0C,max}}}\right]
\]

(3)

\[
P_{aC} = P_{aC,max} \cdot \left[1 - e^{-\frac{\alpha_{EC} \cdot E}{P_{aC,max}}}\right] + R_{aC}
\]

(4)

where E₀C,max and PₐC,max are the carbon-specific maximum gross and net O₂ evolution rates; αEC and αaC are the carbon-specific initial light-limited slopes for gross and net photosynthesis; RₐC is the dark respiration rate; and E is the light intensity (µmol photons m⁻² s⁻¹). Curve fitting was performed on each biological replicate separately to calculate mean (±SE) curve fit parameterizations (SigmaPlot 11.0).

The maximum quantum efficiencies of gross (ϕₐ,nC) and net (ϕₐ,nC) O₂ evolution were calculated as follows:

\[
\phi_{EC,n} = \frac{\alpha_{EC}}{a_{EC,eff}}
\]

(5)

\[
\phi_{a,n} = \frac{\alpha_{EC}}{a_{EC,eff}}
\]

(6)

where the C-specific initial slope for gross (αEC) or net (αaC) O₂ evolution, spectrally corrected to the culturing LEDs (Supplementary Fig. S6), was divided by the C-specific, spectrally corrected effective light absorption coefficient (aEC,eff).

Spectrophotometric Chl a and POC analysis

Samples for the determination of Chl a and POC were collected with each light–response curve, while PON was calculated from the measured POC using the CO₂-specific C:N ratio reported in Boatman et al. (2018a). For measurements of Chl a and POC, two 100 ml samples from each culture were vacuum-filtered onto pre-combusted 25 mm glass fibre filters (0.45 µm pore; Fisherbrand FB59451, UK). The first filter was dried at 60 °C and the POC quantified using a TC analyser (Shimadzu TOC-V Analyser & SSM-5000A Solid Sample Combustion Unit). The second filter was placed in 5 ml of 100% methanol, homogenized, and extracted overnight at ~20 °C, before being centrifuged at 12 000 g for 10 min, and a 3 ml aliquot of the supernatant added to a quartz cuvette. The absorption spectrum (400–800 nm) was measured using a (Hitachi U-3000, Japan) spectrophotometer and the Chl a concentration (µg l⁻¹) was calculated using the following equation (Ritchie, 2008):

\[
Chl a = \left[\frac{12.9447 \cdot (Abs_{665} - Abs_{750}) \cdot Vol_{F}}{Vol_I}\right] 1000
\]

(7)

where Abs₆₆₅ and Abs₇₅₀ are the baseline-corrected optical densities of the methanol-extracted sample at 665 nm and 750 nm; Volₚ is the volume of the solvent used for extraction (i.e. 5 ml); Vol₁ is the volume of culture filtered (i.e. 100 ml), and 12.9447 is a cyanobacteria-specific Chl a coefficient for 100% methanol extraction.

Supporting spectrophotometric measurements were made on live cells using an integrating sphere to determine the in vivo light absorption (Supplementary File S1). From this we determined biomass-specific (Chl a, C, and N) light absorption coefficients under the varying CO₂ treatments (Supplementary File S4), reconstructed the light absorption spectra from photosynthetic pigment spectra (Supplementary File SII; Supplementary Table S1), and calculated maximum quantum efficiencies of gross and net O₂ evolution (Table 3).
Results

Growth rate, cell composition, and light absorption

Balanced growth rates increased from 0.2 d⁻¹ at low-CO₂ to 0.34 d⁻¹ at mid-CO₂ and 0.36 d⁻¹ at high-CO₂ (Table 2). Chl a:C ratios were lowest under low-CO₂ conditions and were significantly higher in the mid-CO₂ treatment relative to the low- and high-CO₂ treatments (Table 2).

Light dependence of O₂ exchange

The C-specific maximum rate (E₀C,max) and initial slope (α₀C) of light-dependent gross photosynthesis were significantly higher in the mid-CO₂ treatment relative to the low- and high-CO₂ treatments (Table 3). Conversely, the light saturation parameter (Eₛ=E₀C,max/α₀C) for gross O₂ evolution (Table 3) and the maximum quantum efficiency of gross O₂ evolution (Φₘₐₓ=α₀C/αₘₚₐₑₚₜ) (Table 3) did not vary significantly amongst the CO₂ treatments due to co-variation of α₀C and E₀C,max.

Table 2. The mean (±SE) balanced growth rate and Chl a:C ratio for T. erythraeum IMS101 when acclimated to three target CO₂ 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>
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<th>Low-CO₂</th>
<th>Mid-CO₂</th>
<th>High-CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate</td>
<td>d⁻¹</td>
<td>0.198 (0.027)</td>
<td>0.336 (0.026)</td>
<td>0.361 (0.020)</td>
</tr>
<tr>
<td>Chl a:C</td>
<td>g mol⁻¹</td>
<td>0.032 (0.003)</td>
<td>0.089 (0.003)</td>
<td>0.086 (0.003)</td>
</tr>
</tbody>
</table>

Abbreviations: Chl a:C ratios are g/mol (n=9 at low-CO₂, n=6 at mid- and high-CO₂). Letters indicate significant differences between CO₂ treatments (one-way ANOVA, Tukey post-hoc test; P<0.05); where B is significantly greater than A, and C is significantly greater than B and A.

Table 3. The physiological parameters (±SE) of the C-specific light–response curves for the gross and net photosynthetic O₂ evolution of T. erythraeum IMS101 (n=4) measured using the MIMS light source

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Low-CO₂</th>
<th>Mid-CO₂</th>
<th>High-CO₂</th>
</tr>
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<tr>
<td>Gross O₂ evolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₀C,max (mmol O₂ (g C)⁻¹ h⁻¹)</td>
<td>1.875 (0.118)</td>
<td>3.795 (0.175) C</td>
<td>2.973 (0.158) B</td>
<td></td>
</tr>
<tr>
<td>Eₛ (µmol photons m⁻² s⁻¹)</td>
<td>277 (15)</td>
<td>250 (20)</td>
<td>281 (15)</td>
<td></td>
</tr>
<tr>
<td>α₀C (µmol O₂ (g C)⁻¹ h⁻¹ (µmol photons m⁻² s⁻¹)⁻¹)</td>
<td>6.78 (0.33) A</td>
<td>15.35 (0.82) C</td>
<td>10.58 (0.18) B</td>
<td></td>
</tr>
<tr>
<td>Φₘₐₓ (mol O₂ (mol photons)⁻¹)</td>
<td>0.037 (0.004)</td>
<td>0.042 (0.004)</td>
<td>0.045 (0.003)</td>
<td></td>
</tr>
<tr>
<td>Net photosynthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pₘₐₚₐₑₚₜ (mmol O₂ (g C)⁻¹ h⁻¹)</td>
<td>1.131 (0.061) A</td>
<td>2.534 (0.287) B</td>
<td>2.312 (0.140) B</td>
<td></td>
</tr>
<tr>
<td>Eₛ (µmol photons m⁻² s⁻¹)</td>
<td>300 (41)</td>
<td>270 (24)</td>
<td>270 (10)</td>
<td></td>
</tr>
<tr>
<td>αₘₚₐₑₚₜ (µmol O₂ (g C)⁻¹ h⁻¹ (µmol photons m⁻² s⁻¹)⁻¹)</td>
<td>3.94 (0.46) A</td>
<td>9.48 (1.02) B</td>
<td>8.57 (0.35) B</td>
<td></td>
</tr>
<tr>
<td>Rₘₚₐₑₚₜ (mol O₂ (g C)⁻¹ h⁻¹)</td>
<td>-0.600 (0.078)</td>
<td>-0.644 (0.132)</td>
<td>-0.659 (0.013)</td>
<td></td>
</tr>
<tr>
<td>Φₘₚₐₑₚₜ (mol O₂ (mol photons)⁻¹)</td>
<td>0.020 (0.002) A</td>
<td>0.026 (0.003) A</td>
<td>0.037 (0.002) B</td>
<td></td>
</tr>
<tr>
<td>Slopes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pₛ versus Eₛ</td>
<td>Dimensionless</td>
<td>0.571 (0.028) A</td>
<td>0.646 (0.046) A</td>
<td>0.791 (0.017) B</td>
</tr>
<tr>
<td>Uₛ versus Eₛ</td>
<td>Dimensionless</td>
<td>0.429 (0.028) B</td>
<td>0.354 (0.046) A</td>
<td>0.209 (0.017) A</td>
</tr>
<tr>
<td>Uₛ versus Pₛ</td>
<td>Dimensionless</td>
<td>0.701 (0.073) B</td>
<td>0.553 (0.111) B</td>
<td>0.254 (0.024) A</td>
</tr>
</tbody>
</table>

Abbreviations: E₀C,max the C-specific maximum gross O₂ evolution rate; Pₘₚₐₑₚₜ the C-specific maximum net O₂ evolution rate; Eₛ the light saturation parameter; α₀C and αₘₚₐₑₚₜ are the C-specific initial slopes of the light–response curve for net and gross photosynthesis; Φₘₐₓ and Φₘₚₐₑₚₜ are the maximum quantum efficiencies of gross and net O₂ evolution calculated using the absorption coefficients reported in Supplementary Table S1; Rₘₚₐₑₚₜ the C-specific dark respiration rate; Slope=the slope of the regression of Eₛ against Pₛ, Eₛ against O₂ uptake (Uₛ), and Uₛ against Pₛ. Letters indicate significant differences between CO₂ treatments (one-way ANOVA, Tukey post-hoc test; P<0.05); where B is significantly greater than A, and C is significantly greater than B and A.

Photosynthetic quotient

We calculated the photosynthetic quotient (PQ) as:

\[
PQ = \frac{Pₛ}{Vₙ} \tag{8}
\]

The C:N ratios reported by Boatman et al. (2018a) for the low-, mid-, and high-CO₂ treatments (7.9, 7.8, and 7.3 mol:mol, respectively) were not significantly different. As such, the CO₂ response of N-specific maximum rates and light-limited initial slopes of gross O₂ evolution were comparable with C-specific rates (Supplementary Fig. S2; Supplementary Table S2). The ~2-fold variability of E₀C,max and α₀C was largely due to differences in the Chl a:C ratio, with the Chl a-specific light absorption varying by only 1% across CO₂ treatments (Supplementary Table S1).

The C-specific dark respiration rate (Rₙ) varied by ~10% amongst CO₂ treatments (Table 3). The maximum net O₂ evolution rate (PₙC,max) approximately doubled from the low-CO₂ to the mid- and high-CO₂ treatments, but did not differ between mid- and high-CO₂, with the initial slope (αₙC) showing a similar pattern to PₙC,max (Table 3). Similar responses were observed for the maximum rate (VₙC,max) and initial slope (Affinity) of the CO₂ dependency of C fixation as reported in Boatman et al. (2018a).

The relationship between Pₛ and Eₛ was linear (Fig. 1), with the slope increasing by ~13% from low- to mid-CO₂ and by 22% from mid- to high-CO₂ (Table 4). This linear relationship indicates that light-dependent O₂ consumption (Uₛ) was a constant proportion of gross O₂ evolution (EₙC), independent of light intensity under each of the CO₂ treatments. Subtracting the slope from unity gives the ratio of light-dependent O₂ consumption to gross O₂ evolution, which declined significantly from 0.79 at high-CO₂ to 0.65 at mid-CO₂ and 0.57 at low-CO₂.
where \( P_{ac} \) is the C-specific net \( O_2 \) evolution rate \((E_{0} - U_{0})\) and \( V_C \) is the C-specific C-fixation rate reported by Boatman et al. (2018a), with both \( P_{ac} \) and \( V_C \) calculated at the growth light intensity (400 mmol photon m\(^{-2}\) s\(^{-1}\)) and growth CO\(_2\) concentration (Table 1). The \( PQ \) calculated in this way (Table 4) did not vary systematically amongst the CO\(_2\) treatments, averaging about 1.15 mol \( O_2 \) mol CO\(_2\)\(^{-1}\).

A second value of \( PQ \) was calculated by dividing \( E_0 \) by \( V_C \) for C fixation under the corresponding conditions, which increased from 1.3 mol \( O_2 \) mol CO\(_2\)\(^{-1}\) in the high-CO\(_2\) treatment to \( \sim2.0 \) mol \( O_2 \) mol CO\(_2\)\(^{-1}\) for the low- and mid-CO\(_2\) treatments (Table 4), reflecting the increase in light-dependent \( O_2 \) consumption with decreasing CO\(_2\).

**Discussion**

**Effect of acclimation to variation of inorganic chemistry on growth rates and the Chl \( a/C \) ratio**

The increased growth rate from low- (180 \( \mu \)mol mol\(^{-1}\)) to mid- (380 \( \mu \)mol mol\(^{-1}\)) and high-CO\(_2\) (720 \( \mu \)mol mol\(^{-1}\)) was similar to previous findings (Barcelos e Ramos et al., 2007; Boatman et al., 2017, 2018b). Whilst not statistically significant, balanced growth rates were \( \sim10\% \) greater at high-CO\(_2\) than at mid-CO\(_2\). The magnitude of this increase is comparable with several recent studies, which show rates increasing by 7–26\% at similar CO\(_2\) concentrations (Barcelos e Ramos et al., 2007; Hutchins et al., 2007; Levitan et al., 2007; Kranz et al., 2010; Garcia et al., 2011; Boatman et al., 2017).

We observed that the Chl \( a/C \) ratio varied 1.7-fold, peaking in the mid-CO\(_2\) treatment (Table 2). This is in contrast to previous research which showed that both Chl \( a/C \) and growth rate were largely independent of CO\(_2\) in *Trichodesmium* (Kranz et al., 2009, 2010). One possible explanation for the difference between our study and previous research is that we grew *Trichodesmium* at a higher light intensity (400 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) in our experiments; 200 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) used by Kranz et al.), and that pigment synthesis was down-regulated in our low-CO\(_2\) treatment where CO\(_2\) limited growth rate. Down-regulation of pigment synthesis by CO\(_2\) limitation on growth was also observed previously for *Synechococcus* by Fu et al. (2007). In contrast, we hypothesize that the reduction in Chl \( a/C \) that we observed from mid-CO\(_2\) to high-CO\(_2\) may be due to the reduced cost of operating a CCM at high-CO\(_2\).
Table 4. The photosynthetic quotients (±SE) for T. erythraeum IMS101, calculated from the light-saturated, maximal rates of carbon-specific O2 evolution, and the C fixation rates

<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>( E_{\text{OC}}/V_{\text{C}} )</td>
<td>mol O2 (mol Chl)(^{-1} )</td>
<td>1.98 (0.10)</td>
<td>1.99 (0.04)</td>
<td>1.28 (0.04)</td>
</tr>
<tr>
<td>( P_{\text{R}}/V_{\text{C}} )</td>
<td>mol O2 (mol Chl)(^{-1} )</td>
<td>1.14 (0.07)</td>
<td>1.29 (0.11)</td>
<td>1.01 (0.05)</td>
</tr>
</tbody>
</table>

\( V_{\text{C}} \) was calculated as \( V_{\text{C}} = (V_{\text{C}} \text{max}) \times [\text{CO}_2]/([\text{K}_m] + [\text{CO}_2]) \) using the value of \( K_m \) from Boatman et al. (2018a) and [CO2] from Table 1. \( E_{\text{OC}} \) was calculated as \( E_{\text{OC}} = E_{\text{OC, max}} \times (1 - e^{-([\alpha \times E]/E_{\text{OC, max}})}) \) and \( P_{\text{R}} \) was calculated as \( P_{\text{R}} = P_{\text{R, max}} \times (1 - e^{-([\alpha \times E]/P_{\text{R, max}})}) \) using \( E = 400 \) μmol photons m\(^{-2} \) s\(^{-1} \) and values of \( E_k \) from Table 3.

Dark respiration and maintenance metabolic rate

Maintenance metabolism is a collection of key functions necessary to preserve cell viability that are commonly assumed to be independent of growth rate and as such can be estimated by extrapolating the relationship between light-limited growth rate and light intensity (\( E \)) to \( E = 0 \) (Geider and Osborne, 1989). We estimated a maintenance metabolic rate \( [0.034 \text{ d}^{-1} \times E/P_{\text{nC, max}}] \) using \( E = 400 \) μmol photons m\(^{-2} \) s\(^{-1} \) and values of \( E_k \) from Boatman et al. (2018a) as independent of growth rate and as such can be estimated from previous observations of \( \alpha \) and \( \text{E}_k \) from Boatman (2014). We suggest that the reduced Chl a:C ratio at low-CO2 relative to mid-CO2 is probably due to the cost of up-regulating the CCM, whereas the reduced Chl a:C ratio at high-CO2 relative to mid-CO2 may be due to an increase in carbohydrate storage granules.

In contrast to \( E_{\text{nC, max}} \), which clearly peaked in the mid-CO2 treatment, the maximum net O2 evolution rate \( (P_{\text{nC, max}}) \) increased from low- to mid-CO2 but was not statistically different between mid- and high-CO2 treatments (Table 3). The 2-fold increase of \( P_{\text{nC, max}} \) from low- to mid- and high-CO2 is consistent with the effect of CO2 on growth rate.

Dividing the rate of net O2 evolution (\( P_{\text{nC}} \)) by the rate of C fixation under comparable light and CO2 conditions gave values for the PQ that ranged from 1.0 mol O2 to 1.3 mol O2 evolved per mol CO2 fixed (Table 4). A PQ of 1.0 is expected if carbohydrate is the major product of photosynthesis as \( P_{\text{nC}} \) measures the light-driven electron transport from PSII to NADPH, which then feeds into CO2 assimilation by the Calvin cycle. A PQ >1.0 is expected if recent photosynthate is used in synthesis of compounds that are more reduced than carbohydrates (e.g. lipid) and/or that photosynthetically generated reductant is used to power N2 fixation and N assimilation into amino acids. Alternatively or in addition, a slightly higher PQ may be required if photosynthetically generated reductant is required for operation of the CCM, or for salvaging CO2 that leaks from carboxysomes by conversion of CO2 to HCO3\(^-\) by NDH-I4 (Price et al. 2008). This corroborates our findings, where under high-CO2, when the CCM is probably down-regulated, we observed a PQ value close to 1.0. Conversely at low- and mid-CO2, when more energy is required for the CCM, we observed a PQ value >1.0.

Effect of acclimation to pCO2 on light-stimulated O2 consumption and the relationship between net and gross O2 evolution

We found that O2 consumption rates (\( U_{\text{0}} \)) of Trichodesmium increased markedly with light intensity, with \( U_{\text{0}} \) saturating at a similar light intensity to gross O2 evolution (Fig. 1). This suggests that light-driven \( U_{\text{0}} \) increased in parallel with gross O2 evolution and that dark respiration continued at similar rates in the light and dark. Previously, Kana (1993) observed a very slight decline in \( U_{\text{0}} \) between darkness and low-light intensity in natural Trichodesmium colonies, followed by parallel increases of \( U_{\text{0}} \) and O2 evolution (\( E_{\text{nC}} \)) with increasing light. Kana (1993) attributed the light-stimulated component of \( U_{\text{0}} \) to the Mehler reaction, as the addition of DCMU to illuminated cells caused \( U_{\text{0}} \) to decline to the rate observed in darkness.

The slope of the dependence of \( U_{\text{0C}} \) on \( E_{\text{nC}} \) decreased with increasing growth CO2 from 0.43 in cultures grown under low-CO2 to 0.21 in cultures grown under high-CO2 (Table 3). Thus, the light-driven component of \( U_{\text{0}} \) decreased from ~43% of \( E_{\text{nC}} \) in the low-CO2 culture to 21% of \( E_{\text{nC}} \) in the high-CO2 culture.

Effect of acclimation to pCO2 on gross photosynthesis and photosynthetic quotients

Carbon-specific rates are directly related to changes in the specific growth rate, as both rates can be expressed in equivalent units of inverse time (e.g. h\(^{-1} \) or d\(^{-1} \)). However, due to differences in the Chl a:C ratio (Table 2), Chl a-specific maximum rates (\( E_{\text{nC, max}} \)) and initial slopes (\( \alpha \text{Chl} \)) of light-dependent gross photosynthesis did not differ significantly between CO2 treatments (Supplementary Fig. S3; Supplementary Table S3), an observation that is consistent with the findings reported by Levitan et al. (2007) and Eichner et al. (2014).
culture. Pseudocyclic photophosphorylation coupled to the consumption of O2 associated with the Mehler reaction can provide ATP that may be used to support N2 fixation, CO2 fixation, or for operating a CCM (Miller et al., 1988). The linearity between U0 and E0 suggests that light-dependent O2 consumption may be required to balance the ratio of ATP production to NADPH production in the light reactions of photosynthesis. As the photon efficiency of ATP production by pseudocyclic photophosphorylation is the same as that of photophosphorylation driven by linear photosynthetic electron transport (LPET) (Baker et al., 2007), our results suggest that 26% more ATP than can be generated by LPET is required by cells growing under high-CO2, increasing to 55% more ATP in cells growing under mid-CO2 and 75% more ATP in cells growing under low-CO2. Much of the additional ATP that could be generated by pseudocyclic photophosphorylation may be accounted for by the ATP requirements for CO2 fixation by the Calvin cycle (1.5 ATP/e−) and N2 fixation (2.1 ATP/e−) being greater than the ratio of ATP to reducing equivalents generated by LPET (1.25 ATP/e−). At low-CO2, the increase in U0 may also be required to operate a CCM, as observed in the freshwater Synechococcus (Miller et al., 1988).

An estimate of the cost of operating the CCM

Stimulation of growth and productivity of T. erythraeum IMS101 in response to increasing CO2 is commonly attributed to reductions in the amount of energy required for establishing, maintaining, and operating a CCM (Hutchins et al., 2007, 2015; Levitan et al., 2007; Garcia et al., 2011). Two aspects for the cost of operating the CCM in Trichodesmium are those for HCO3− transport into the cell, which must balance CO2 fixation and CO2 leakage, and those for retaining inorganic C within the cell by converting CO2 that diffuses from the carboxysome to HCO3− via the NDH-I4 CO2 uptake/salvage system. The cost of HCO3− transport of ~1 ATP for each C transported into the cell (Raven et al., 2014) could be supplied by either cyclic photosynthetic electron transfer around PSI or pseudocyclic photosynthetic electron transfer linked to the Mehler reaction. As the ratio of CO2 leakage to gross inorganic C uptake has been found to be independent of pCO2 over the range of 150−1000 µmol mol−1 (Kranz et al., 2009, 2010), the ATP required for HCO3− transport will also be independent of pCO2.

Rather than fueling ATP production by pseudocyclic electron transport, light-dependent O2 consumption may be a consequence of operating the NDH-I4 CO2 uptake/salvage system. For Trichodesmium, the NDH-I4 protein is thought to reduce the efflux of CO2 from the cell, converting it to HCO3− but at a cost of consuming reducing equivalents [NADPH or reduced ferredoxin (Fd)] (Price et al., 2008).

The stoichiometry based on NADPH as the electron donor can be represented as:

\[2 \text{NADPH} + 2 \text{CO}_2 + \text{O}_2 \rightarrow 2 \text{HCO}_3^- + 2 \text{NADP}^+ \] (9)

If NDH-I4 activity is employed to minimize CO2 effluxes, then the rate of O2 consumption associated with this process would be expected to decrease with increases of extracellular CO2 concentration. As NADPH consumption by this mechanism is closely linked in time and space to NADPH production via LPET, this process would be inhibited by DCMU in a similar manner to the Mehler reaction.

Roles of photosynthetic and respiratory metabolism in N2 fixation

Increased pCO2 will not only stimulate CO2 fixation, but will also stimulate N2 fixation (dependent on carbon skeletons for sequestration of the ammonium produced) and growth in Trichodesmium (Barcelos e Ramos et al., 2007; Hutchins et al., 2007; Levitan et al., 2007, 2010b; Kranz et al., 2009, 2010). This is probably in response to energy relocation from the CCM (Badger et al., 2006; Kranz et al., 2011) toward CO2 and N2 fixation (Levitan et al., 2007; Kranz et al., 2011).

Diazocytes may use the light reactions of photosynthesis to provide some or most of the ATP required to support N2 fixation either through cyclic photophosphorylation associated with electron transfer around PSI or through pseudocyclic photophosphorylation involving LPET from H2O to O2 involving both PSII and PSI. However, if respiration provides the ATP for N2 fixation, then sugars and/or organic acids may be produced by photosynthesis in diazocytes prior to the initiation of N2 fixation (temporal separation of CO2 fixation from N2 fixation) or in other cells within the trichome and transported laterally into the diazocytes (spatial separation of CO2 fixation from N2 fixation).

Temporal separation of N2 fixation from photosynthetic O2 evolution may be achieved if glycogen that accumulates in diazocytes prior to the onset of N2 fixation provides the reducing equivalents and ATP to fuel N2 fixation, perhaps supplemented by high rates of cyclic photosynthetic electron transfer around PSI to generate ATP. Temporal separation is consistent with the pattern of CO2 fixation and N2 fixation observed in Trichodesmium where the former peaks earlier in the day than the latter (Berman-Frank et al., 2001). Spatial separation has been observed in heterocystous cyanobacteria where transport of sugars into heterocysts from surrounding cells can be respired to fuel N2 fixation (Wolk, 1968; Böhme, 1998). Although we are not aware of direct evidence for rapid transfer of metabolites amongst cells along a trichome, such a transfer would be consistent with observations of Finzi-Hart et al. (2009), showing that all cells within a Trichodesmium trichome show the same temporal pattern of accumulation and mobilization of cyanophycin granules and the same temporal pattern of labelling with 13CO2 and 15N.

Conclusion

In this study, we accredited the bell-shaped CO2 response of the C-specific maximum gross photosynthesis rates (E0Cmax) to the CCM, where the 2-fold increase in E0Cmax from low- to mid-CO2 supports the almost 2-fold increase in balanced growth rates...
and the decrease in $E_{\text{ic,max}}$ from mid- to high-$\text{CO}_2$ is due to less expenditure on the CCM whilst cells grow at a similar rate.

Our results indicate a significant decrease in the ratio of the rate of light-driven $\text{O}_2$ consumption to the rate of gross photosynthetic $\text{O}_2$ evolution with increasing $\text{CO}_2$, which probably arises from a reduced cost of operating the CCM. In addition, dark respiration appears to be sufficient to provide much of the energy required to support significant rates of N$_2$ fixation, even in the light.

We have not extrapolated our findings to a full day as light–response curves were measured at one time point and *Trichodesmium* exhibits pronounced diurnal variability in photosynthesis and N$_2$ fixation (Berman-Frank et al., 2001). In addition, extrapolating to future conditions in the natural environment should consider (i) the impact of adaptation of *Trichodesmium* to future conditions (Hutchins et al., 2015); (ii) strain and clade variability (Hutchins et al., 2013); and (iii) additional integrated effects of abiotic variables other than $\text{CO}_2$ (i.e. light intensity, temperature, and nutrients such as P and Fe) (Walworth et al., 2016).

In the context of open oceans, nutrient-replete P$_n$ and growth rates of *T. erythraeum* IMS101 would have been severely $\text{CO}_2$ limited at the last glacial maximum relative to current conditions. Future increases of $\text{CO}_2$ may not significantly increase growth and productivity of *Trichodesmium*, although increases in key stoichiometric ratios (N:P and C:P) as reported by Boatman et al. (2018a) may affect bacterial and zooplankton metabolism, the pool of bioavailable N, the depth at which sinking organic matter is remineralized, and consequently carbon sequestration via the biological carbon pump (Mulholland et al., 2004; McGillicuddy, 2014). These responses could serve as a negative feedback to climate change by increasing new N and C production, thereby increasing the organic carbon sinking to the deep ocean.

**Supplementary data**

Supplementary data are available at *JXB* online.

Table S1. The measured and modelled effective light absorption coefficients and relative photosynthetic pigment contribution to the total light absorption.

Table S2. The physiological parameters of the N-specific light–response curves for gross and net photosynthetic $\text{O}_2$ evolution of *T. erythraeum* IMS101.

Table S3. The physiological parameters of the Chl a-specific light–response curves for gross and net photosynthetic $\text{O}_2$ evolution of *T. erythraeum* IMS101.

Table S4. Values of the goodness of fit for the C-specific light–response curves for gross and net photosynthetic $\text{O}_2$ evolution of *T. erythraeum* IMS101.

Table S5. The Chl a-specific photosynthetic quotients of *T. erythraeum* IMS101.

Fig. S1. The inorganic carbon chemistry of *T. erythraeum* IMS101 cultures over the light period.

Fig. S2. The N-specific light–response curves for gross $\text{O}_2$ evolution, $\text{O}_2$ consumption, and net photosynthesis for *T. erythraeum* IMS101.

Fig. S3. The Chl a-specific light–response curves for gross $\text{O}_2$ evolution, $\text{O}_2$ consumption, and net photosynthesis for *T. erythraeum* IMS101.

Fig. S4. The measured and modelled in vivo light absorption spectra for *T. erythraeum* IMS101.

Fig. S5. The light compensation point of *T. erythraeum* IMS101 growth.

Fig. S6. The relative emission spectra of the culturing and MIMS LEDs and the spectral corrected light absorption spectra of *T. erythraeum* IMS101.

File S1. In vivo light absorption.

File S2. Modelling the in vivo light absorption from pigment absorption spectra.

File S3. Stoichiometry and energetics of N$_2$ fixation.

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**References**


Photosynthesis modulated by the inorganic carbonate system | 597


