The physiological cost of diazotrophy for *Trichodesmium erythraeum* IMS101

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Abstract

*Trichodesmium* plays a significant role in the oligotrophic oceans, fixing nitrogen in an area corresponding to half of the Earth’s surface, representing up to 50% of new production in some oligotrophic tropical and subtropical oceans. Whilst *Trichodesmium* blooms at the surface exhibit a strong dependence on diazotrophy, colonies at depth or at the surface after a mixing event could be utilising additional N-sources. We conducted experiments to establish how acclimation to varying N-sources affects the growth, elemental composition, light absorption coefficient, N\textsubscript{2} fixation, PSII electron transport rate and the relationship between net and gross photosynthetic O\textsubscript{2} exchange in *T. erythraeum* IMS101. To do this, cultures were acclimated to growth medium containing NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} (replete concentrations) or N\textsubscript{2} only (diazotrophic control). The light dependencies of O\textsubscript{2} evolution and O\textsubscript{2} uptake were measured using membrane inlet mass spectrometry (MIMS), while PSII electron transport rates were measured from fluorescence light curves (FLCs). We found that at a saturating light intensity, *Trichodesmium* growth was ~10% and 13% lower when grown on N\textsubscript{2} than with NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}, respectively. Oxygen uptake increased linearly with net photosynthesis across all light intensities ranging from darkness to 1100 μmol photons m\textsuperscript{-2} s\textsuperscript{-1}. The maximum rates and initial slopes of light response curves for C-specific gross and net photosynthesis and the slope of the relationship between gross and net photosynthesis increased significantly under non-diazotrophic conditions. We attribute these observations to a reduced expenditure of reductant and ATP for nitrogenase activity under non-diazotrophic conditions which allows NADPH and ATP to be re-directed to CO\textsubscript{2} fixation and/or biosynthesis. The energy and reductant conserved through utilising additional N-sources could enhance *Trichodesmium*’s productivity and growth and have major implications for its role in ocean C and N cycles.

Introduction

In marine ecosystems, phytoplankton primary production is often limited by the bioavailability of fixed N [1–3], where N-sources (e.g. NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-}, NH\textsubscript{4}\textsuperscript{+}, urea etc) are quickly depleted by fast growing phytoplankton [4]. A significant fraction (~25 Tg N yr\textsuperscript{-1}) of N in the euphotic zone is lost via sedimentation to the deep ocean as particulate organic nitrogen (PON), making...
NO$_3^-$ concentrations higher at greater depth [5–7]. Whilst areas of upwelling transport NO$_3^-$ into the euphotic zone, there are vast regions of the oligotrophic open oceans that are dependent on the input of new N from N$_2$-fixing cyanobacteria. Among the most important marine diazotrophs are *Trichodesmium* sp., which can form extensive surface blooms in the tropical and subtropical oceans [8–12].

Previous studies have highlighted *Trichodesmium*’s capacity to assimilate various forms of combined N-sources [13–17]. It is commonly assumed that *Trichodesmium* obtains most of its nitrogen quota from N$_2$ fixation, however field-based measurements of N$_2$ fixation show wide temporal and spatial variability [18]. The causes of this variability remain unclear, but environmental factors such as the availability of combined nitrogen may be a contributing factor.

**Diazotrophy**

Diazotrophic cyanobacteria are able to meet their daily nitrogen quota by fixing dinitrogen (N$_2$).

$$N_2 + 16\text{ATP} + 8\text{H}^+ + 8e^- \rightarrow 2\text{NH}_3 + H_2 + 16\text{ADP} + 16\text{Pi} \quad (1)$$

While N$_2$ fixation is an extremely energy demanding process, *Trichodesmium* incurs additional costs related to the protection of nitrogenase from the irreversible inhibition of photosynthetically evolved O$_2$ [9, 19, 20]. The separation of O$_2$ evolution and N$_2$ fixation is regulated over a diurnal cycle of N$_2$ fixation and photosynthesis [21], involving daily synthesis and degradation of nitrogenase [22, 23] and alternation of photosynthetic activity states [24]. Temporal separation occurs over short timescales, where peak rates of photosynthesis (~ 10 am) and N$_2$ (~ 12 pm) fixation vary over a diel period. Spatial separation occurs via diazocytes, which are reversibly specialised cells for nitrogen fixation [25, 26]. Diazocytes contain the necessary proteins to perform photosynthetic CO$_2$ fixation and N$_2$ fixation. However, it has been suggested that when fixing N$_2$, cells increase cyclic electron transport around PSI to enhance ATP synthesis [21, 24], thus allowing the cells to meet the energetic demands of N$_2$ fixation (Eq 1).

**Uptake of additional N-sources**

Like other facultative diazotrophic cyanobacteria spp., *Trichodesmium* can exploit other forms of nitrogen including NH$_4^+$, NO$_3^-$, urea and amino acids [16, 27]. These N compounds are transported into the cell via permeases, metabolised to NH$_4^+$ and then incorporated into carbon skeletons through the glutamine synthetase (GS) and glutamine 2-oxoglutarate aminotransferase (GOGAT) pathways. This process is mediated by nitrate reductase (Eq 2) and nitrite reductase (Eq 3).

$$\text{NO}_3^- + 2e^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \quad (2)$$

$$\text{NO}_2^- + 6e^- + 8\text{H}^+ \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O} \quad (3)$$

For cyanobacteria, nitrate reductase is located in the cytosol and uses NADPH to catalyse the transfer of two electrons. The NO$_3^-$ formed by nitrate reductase is further reduced to NH$_4^+$ via the transfer of six electrons. Thus, the reduction of NO$_3^-$ to NH$_4^+$ can be expressed as;

$$\text{NO}_3^- + 8e^- + 10\text{H}^+ \rightarrow \text{NH}_4^+ + 3\text{H}_2\text{O} \quad (4)$$

Amino acids are synthesised from ammonia (NH$_3$) via the GS-GOGAT pathway. The initial GS pathway requires ATP and glutamate as a substrate;

$$\text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + \text{Pi} \quad (5)$$
where glutamine is subsequently transformed to 2-oxoglutarate and reduced using NADPH, forming two moles of glutamate.

\[
2 \text{- Oxoglutarate } + \text{Glutamine} \rightarrow 2(\text{Glutamate})
\]  

Thus, for every mole of glutamate produced, one mole each of NH\textsubscript{3}, NADPH, ATP and 2-oxoglutarate are required. Additionally, ATP is required for the active transport of inorganic NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-} into the cell [28]. Different N-sources require different amounts of energy and reductant and as such can be ordered into a hierarchy of energy requirements; where diazotrophy requires the highest investment of electrons and ATP, followed by NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-} and then NH\textsubscript{3}.

**Utilising additional N-sources**

Global warming is increasing sea surface temperatures (SSTs) which is enhancing water stratification and decreasing vertical mixing [29], potentially increasing the area of N-limited oceans. Whilst detrimental to many phytoplankton, a reduced flux of NO\textsubscript{3}\textsuperscript{-} into the upper mixed layer will increase the competitive advantage of diazotrophs for other limiting nutrients (i.e. Fe or P). *Trichodesmium* colonies have been observed migrating to the nutricline [30, 31] to facilitate the luxury uptake of polyphosphates before returning to the surface. Whilst at these depths, cells are exposed to NO\textsubscript{3}\textsuperscript{-} concentrations greater than those at the surface. As such, *Trichodesmium* colonies may be assimilating and storing (i.e. cyanophycin granules) more combined N than the blooms frequently measured on the surface [32]. This could have major implications for growth rates, primary productivity and biogeochemical cycles [33].

Our approach comprises a systematic experiment where *T. erythraeum* IMS101 was grown over long durations, at three N-source treatments, with controlled and well-defined growth conditions, ensuring fully acclimated, balanced growth had been achieved. Our aims were to assess the response of *T. erythraeum* IMS101 growth, light dependency of gross and net O\textsubscript{2} photosynthesis, PSII electron transport rates and elemental composition to different N-sources; investigating the physiological cost of performing diazotrophy.

**Materials and methods**

*T. erythraeum* IMS101 was semi-continuously cultured to achieve fully acclimated balanced growth at three N-source treatments (N\textsubscript{2}, NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}), at a targeted 380 µatm CO\textsubscript{2} concentration, saturating light intensity (400 µmol photons m\textsuperscript{-2} s\textsuperscript{-1}), 12:12 light:dark (L:D) cycle and optimal temperature (26 °C ± 0.2) (3 treatments in total) for ~ 2 months (~ 30 generations).

**Experimental setup**

Cultures were acclimated to the CO\textsubscript{2} and light intensity for ~ 4 months (~ 60 generations) under diazotrophic conditions before the addition of NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-}. Cultures were gradually enriched over a 2/3-week period by increasing the dilution ratio of YBCII media containing NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-} (100 µM).

*T. erythraeum* IMS101 was grown using YBCII medium [34] at 1.5 L volumes in 2 L pyrex bottles that were acid-washed and autoclaved prior to culturing. Daily growth rates were quantified from changes in baseline fluorescence (F\textsubscript{o}) measured between 09:00 to 10:30 on dark-adapted cultures (20 minutes) using a FRRHII FastAct Fluorometer System (Chelsea Technologies Group Ltd, UK). Cultures were regarded as fully acclimated and in balanced growth when both the slope of the linear regression of ln F\textsubscript{o} versus time and the ratio of live cell to acetone extracted (method detailed below) baseline fluorescence (F\textsubscript{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 3–5 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 (0.2 µm pore) air mixture at a rate of ~200 mL s⁻¹. The CO₂ concentration was regulated (±2 µatm) by mass flow controllers (Bronkhorst, Newmarket, UK). CO₂-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% CO₂ in-air mixture from a gas cylinder (BOC Industrial Gases, UK). The CO₂ concentration 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).

Throughout all culturing, the inorganic carbon chemistry (S1 File) and dissolved inorganic NH₄⁺ and NO₃⁻ concentrations (S2 File) were determined prior to diluting with fresh media. Samples for elemental composition, photosynthesis-light response curves, fluorescence light curves (FLC), in vivo light absorption and acetylene reduction assays were collected at the same time of day, approximately 4 and 6 hours into the photo-phase of the L:D cycle.

Measuring O₂ exchange by membrane inlet mass spectrometry (MIMS)

Light dependent rates of O₂ production and consumption were measured with a membrane inlet mass spectrometer (MIMS), using an ¹⁸O₂ technique modified from McKew et al. [35] (S3 File). MIMS measurements consisted of three biological replicates per treatment (S4 File). Chlorophyll a concentrations at the point of sampling ranged from 80 to 245 µg Chla L⁻¹.

Changes in ¹⁶O₂ and ¹⁸O₂ and thus O₂ consumption (U₀) and O₂ evolution (E₀) were calculated using the following equations [36];

\[
U_0 = - \left( 1 + \frac{1^{16}O_2}{1^{18}O_2} \right) \cdot \frac{\Delta^{18}O_2}{\Delta t} \tag{7}
\]

\[
E_0 = \frac{\Delta^{18}O_2}{\Delta t} - \left( \frac{1^{16}O_2}{1^{18}O_2} \right) \cdot \frac{\Delta^{18}O_2}{\Delta t} \tag{8}
\]

where \(U_0\) is the rate of O₂ consumption calculated from the decrease of \(^{18}O_2\) over time (i.e. \(\Delta^{18}O_2/\Delta t\)), which takes into account the relative concentration of \(^{18}O_2\) compared to \(^{16}O_2\) (i.e. \(1 + ^{16}O_2/^{18}O_2\)) and \(E_0\) is the rate of O₂ evolution calculated from the increase in \(^{16}O_2\) over time (\(\Delta^{16}O_2/\Delta t\)), where the decline of \(^{18}O_2\) (i.e. \(\Delta^{18}O_2/\Delta t\)) and \(^{18}O_2\) is corrected for relative to the concentration of \(^{16}O_2\). Chlorophyll a- and C-specific rates were obtained by dividing \(U_0\) and \(E_0\) by the concentration of Chla and particulate organic carbon, respectively. Rates were multiplied by 1.073 to spectrally correct to the culturing LEDs (S1 Fig).

Photosynthesis-light (P-E) curves for gross (\(E_0^{\text{Chl}(C)}\)) and net photosynthesis (\(P_{\text{net}}^{\text{Chl}(C)} = E_0^{\text{Chl}(C)} - U_0^{\text{Chl}(C)}\)) were fitted to the equations from Platt and Jassby [37];

\[
E_0^{\text{Chl}(C)} = \frac{E_{\text{max}}^{\text{Chl}(C)}}{1 + e^{-\left(\frac{E_{\text{max}}^{\text{Chl}(C)}}{E_{\text{sat}}^{\text{Chl}(C)}}\right) \cdot \frac{E}{E_{\text{sat}}^{\text{Chl}(C)}}}} \tag{9}
\]

\[
P_{\text{net}}^{\text{Chl}(C)} = P_{\text{max}}^{\text{Chl}(C)} \cdot \left[ 1 - e^{-\left(\frac{P_{\text{max}}^{\text{Chl}(C)}}{P_{\text{sat}}^{\text{Chl}(C)}}\right) \cdot \frac{E}{E_{\text{sat}}^{\text{Chl}(C)}}} \right] + R_d^{\text{Chl}(C)} \tag{10}
\]

where \(E_{\text{max}}^{\text{Chl}(C)}\) and \(P_{\text{max}}^{\text{Chl}(C)}\) are the maximum gross and net O₂ evolution rates; \(\alpha_g^{\text{Chl}(C)}\) and \(\alpha_p^{\text{Chl}(C)}\) are the maximum gross and net photosynthesis rates; \(E_{\text{sat}}^{\text{Chl}(C)}\) and \(P_{\text{sat}}^{\text{Chl}(C)}\) are the maximum light saturation constants; and \(R_d^{\text{Chl}(C)}\) is the dark respiration rate.
and $\alpha_n^{Chl(C)}$ are the initial light-limited slopes for gross and net photosynthesis; $R_d$ is the dark respiration rate; and $E$ is the light intensity ($\mu$mol photons m$^{-2}$ s$^{-1}$). Curve fitting was performed on each replicate separately to calculate mean (± S.E.) curve fit parameterisations (Sigmaplot 11.0).

The maximum quantum efficiency of gross ($\phi_{mgross}$) and net ($\phi_{mnet}$) $O_2$ evolution was calculated as follows;

$$
\phi_m = \frac{\alpha_g^{Chl(C)}}{\alpha_{eff}^{Chl(C)}}
$$

where the C-specific initial slope for gross ($\alpha_g^{Chl(C)}$) or net ($\alpha_n^{Chl(C)}$) $O_2$ evolution was divided by the C-specific, effective light absorption coefficient ($\alpha_{eff}^{Chl(C)}$).

Measuring nitrogenase activity by acetylene reduction

Acetylene reduction rates were measured using gas chromatography (ATI Unicam 610 series). Gaseous samples were injected into the GC column head (60 °C), carried via $N_2$ gas through a Porapak N column (100 °C) to a flame ionising detector (100 °C). Peak areas of acetylene and ethylene were quantified by an integrated chromatograph data acquisition unit (Shimadzu C-R8A Integrator) and were converted into concentrations via an acetylene and ethylene standard curve performed with standard gases (Scientific and Technical Gases Ltd., UK). Triplicate 6 mL samples of each biological replicate culture were placed into 12 mL exetainer, screw capped glass vials (Labco Ltd, UK). Exactly 1.2 mL of the headspace was removed and replaced with a 1.2 mL sample of acetylene (BOC Industrial Gases, UK) (headspace = 20% acetylene). The vials were gently inverted for 1 minute before 250 $\mu$L of headspace was injected into the GC column for an initial measurement of acetylene and ethylene concentrations ($T_0$). Vials were incubated at 26 °C and 400 $\mu$mol photons m$^{-2}$ s$^{-1}$ in an aluminium temperature block and were gently inverted every 10 minutes to prevent trichomes from settling on the bottom or aggregating at the meniscus. After 1 hour, a second 250 $\mu$L gaseous headspace was injected into the GC column for the post-incubation measurement ($T_1$). Temperature and pressure was measured during each set of measurements and accounted for in the calculations. The rate of ethylene production was calculated with the assumption that the concentrations of acetylene and ethylene within the media were always in equilibrium to those in the headspace;

$$
\Delta C_2H_2 = \frac{C_2H_2(T_1) - C_2H_2(T_0)}{t \cdot V_{(i)}}
$$

where ($\Delta C_2H_2$) is the ethylene production rate ($\mu$mol $C_2H_4$ h$^{-1}$), $C_2H_2(T_0)$ and $C_2H_2(T_1)$ are the ethylene concentrations in the headspace at the start ($T_0$) and end ($T_1$) of the incubation, $V_{(i)}$ is the volume of gaseous sample injected into the GC column (L$^{-1}$) and $t$ is the incubation time (min).

$N_2$ fixation rates were calculated to a Chla ($\mu$mol $N_2$ (mg Chla)$^{-1}$ h$^{-1}$) and total carbon ($\mu$mol $N_2$ (mg C)$^{-1}$ h$^{-1}$) basis;

$$
N_2 \text{ fixation} = \left( \frac{\Delta C_2H_2}{Chl \ a(C)} \right) \cdot 0.25
$$

where $\Delta C_2H_2$ ($\mu$mol h$^{-1}$) is divided by the Chla or total carbon concentration (mg) and multiplied by 0.25 under the assumption that reduction of four moles of acetylene is equivalent to reduction of one mole of dinitrogen.
Fluorescence light curves (FLCs)

A 2 mL sample of each replicate culture was used to measure a fluorescence light curve (FLC) [38]. The FLCs were measured with a FRRFII FastAct Fluorometer System, using a white LED actinic light source (Chelsea Technologies Group Ltd, UK). Each FLC lasted 1 hour; comprising 12 light steps which ranged from 10 to 1600 μmol photon m⁻² s⁻¹, each lasting 5 minutes in duration. The FLCs provided measurements of the light absorption cross-section of PSII photochemistry (σPII'), the average time constant for the re-opening of a closed PSII reaction centre (τf') and the operating efficiency of PSII photochemistry (Fq'/Fm');

\[
\frac{F_q'}{F_m'} = \left[ \frac{F_m' - F'}{F_m'} \right]
\]

where \(F_m'\) is the maximum fluorescence in the light-adapted state and \(F'\) is the steady-state fluorescence at any point.

Photosystem II (PSII) electron transport rates were normalised to a Chl a (mol e⁻ (g Chl a)⁻¹ h⁻¹) and total carbon (mol e⁻ (g C)⁻¹ h⁻¹) basis;

\[
ETR^{Chl(C)} = \frac{F_q'}{F_m'} \cdot E \cdot (a^{Chl(C)} \cdot FAQ_{PII}) \cdot 3600 \cdot SCF
\]

where \(F_q'/F_m'\) is the operating efficiency of PSII photochemistry; E is the light intensity (mol photons m⁻² s⁻¹), a^{Chl(C)} is the Chl-a-specific (C-specific) effective light absorption (m² g⁻¹ Chl a and m² g⁻¹ C, respectively), FAQ_{PII} is the fraction of absorbed photons directed to PSII, which was set to 0.5 [39], with the assumption that the quantum yield of electron transport of one trapped photon within a reaction centre is equal to 1 [40]; 3600 converts seconds to hours and SCF is a spectral correction factor of 1.194, which converts electron transport rates to the culturing LED spectrum (S1 Fig).

ETR curves were modelled using a P-E equation [37], performed on each individual replicate using a Marquardt–Levenberg least squares algorithm to generate the best fit (R² > 0.993);

\[
ETR = ETR^{m'} \cdot \left[ 1 - e^{\left(\frac{-2ETR}{ETR^{m'}} \cdot E\right)} \right] e^{\left(\frac{-\beta_{ETR} \cdot E}{ETR^{m'}} \right)}
\]

where \(ETR^{m'}\) is the hypothetical Chl(a(C)-specific maximum electron transport rate that would be achieved if there was no photoinhibition (mol e⁻ (g Chl(a(C))⁻¹ h⁻¹); \(a_{ETR}\) is the initial slope of the Chl(a(C)-specific ETR-light curve (mol e⁻ (g Chl(a(C))⁻¹ h⁻¹ (µmol photons m⁻² s⁻¹)⁻¹); \(\beta_{ETR}\) is the parameter that accounts for downregulation and/or photoinhibition at supra-optimal light intensities (mol e⁻ (g Chl(a(C))⁻¹ h⁻¹ (µmol photons m⁻² s⁻¹)⁻¹); and E is the light intensity (µmol photons m⁻² s⁻¹).

The realised maximum PSII electron transport rate in the presence of photoinhibition (ETR_m), light intensity at which ETR is maximal (E_{opt}), the light-saturation parameter (E_k)
and the light inhibition parameter ($E_p$) were calculated from the fitted parameters as follows:

$$ETR_m = ETR_m^0 \cdot \left( \frac{\alpha_{ETR}}{\alpha_{ETR} + \beta_{ETR}} \right) \cdot \left( \frac{\beta_{ETR}}{\alpha_{ETR} + \beta_{ETR}} \right)$$  \hspace{1cm} (17)

$$E_{opt} = ETR_m^0 \cdot \ln \left( \frac{\alpha_{ETR} + \beta_{ETR}}{\beta_{ETR}} \right)$$  \hspace{1cm} (18)

$$E_a = \frac{ETR_m}{\alpha_{ETR}}$$  \hspace{1cm} (19)

$$E_p = \frac{ETR_m}{\beta_{ETR}}$$  \hspace{1cm} (20)

The ratio of PSII electron transport to gross O$_2$ evolution ($E_o$) under light-limitation ($\Phi_{ec}$) and light-saturation ($\Phi_{em}$) were calculated as follow;

$$\Phi_{ec} = \frac{\alpha_{ETR}}{\alpha_g}$$  \hspace{1cm} (21)

$$\Phi_{em} = \frac{ETR_m}{E_{oom}}$$  \hspace{1cm} (22)

**Cellular elemental composition and light absorption**

Samples for determining particulate organic carbon (POC), nitrogen (PN) and phosphorus (PP) (S5 File), chlorophyll $a$ (S6 File) and in vivo light absorption (S7 File) were collected with each MIMS measurement, with each sample being a biological replicate.

**Modelling the in vivo light absorption from pigment absorption spectra**

In vivo light absorption was reconstructed using the light absorption spectra of Chla and photoprotective carotenoids (PPC) taken from Woźniak et al. [41] and the light absorption spectra of phycourobilin (PUB1, PUB2, PUBx, PUB4, PUB5a, PUBb, PUB5d, PUB5g and PUB5j), phycoerythrin (PE1, PE2a, PE2b and PE3b), alloplastocyanin (APC) and plastocyanin (PC1 and PC2) taken from Küpper et al. [42] (S2 Fig).

The Chla-specific light absorption coefficient was modelled as the sum of the contribution of all pigments:

$$a_{mod}^{Chla}(\lambda) = \sum \beta_i \cdot a_i(\lambda)$$  \hspace{1cm} (23)

where $a_{mod}^{Chla}$ is the modelled in vivo light absorption at a specific wavelength ($\lambda = 400–700$ nm); $\beta_i$ is the contribution of each pigment to $a_{mod}^{Chla}$ and $a_i$ is the pigment-specific spectral absorption coefficient of pigment $i$, in m$^2$ (g pigment $i$)$^{-1}$.

The modelled in vivo light absorption spectra ($a_{mod}^{Chla}(\lambda)$) was optimised to the measured spectra between 400 and 700 nm using a reduced sum of squares method (Sigmaplot 11.0). If a zero value was returned for a $\beta_i$ parameter, that pigment was removed from the model and the curve fit reapplied.
Results

Inorganic C-chemistry, growth rate and cell composition

Balanced growth of *T. erythraeum* IMS101 was 0.34 d\(^{-1}\) when grown on N\(_2\), increasing by 10% and 13% when grown in the presence of NH\(_4^+\) and NO\(_3^-\), respectively (Table 1). Particulate C: N, C:P and N:P ratios were all influenced by the presence of additional N-sources. When compared to the N\(_2\) treatment, C:N decreased by 36% and 43% for the NH\(_4^+\) and NO\(_3^-\) treatments, respectively. Ratios of C:P and N:P were comparable between NH\(_4^+\) and NO\(_3^-\) treatments, but were significantly lower (~ 60% and 35%, respectively) compared to the N\(_2\) treatment (Table 1). Ratios of Chl\(_a\):C were 80% and 67% higher for the NH\(_4^+\) and NO\(_3^-\) treatments than for the N\(_2\) treatment, while Chl\(_a\):N was not significantly different between treatments (Table 1). Carbon and Chl\(_a\)-specific N\(_2\) fixation rates were highest for the N\(_2\) treatment, decreasing significantly by 84% and 80% (Chl\(_a\)-specific) and 73% and 68% (C-specific) for the NH\(_4^+\) and NO\(_3^-\) treatments, respectively (Table 1).

The inorganic carbon concentration, pH and alkalinity (A\(_T\)) did not vary significantly amongst N-source treatments. Overall, CO\(_2\) drawdown ranged between 78 to 92 μatm from the target concentration (i.e. 380 μatm) for all N-source treatments (Table 2) and exhibited little variability over a diurnal cycle (S3 Fig). Inorganic N concentrations were > 1 μM for the N\(_2\) treatment and were ~ 8 μM for the NH\(_4^+\) and NO\(_3^-\) treatments at the point of dilution (Table 2).

Light absorption

The effective light absorption coefficients were not significantly different between N-source treatments, nor were the modelled absorption coefficients significantly different to the measured coefficients; with modelled coefficients being only 1 to 3% higher across all N-source treatments (Table 3). 

*In vivo* light absorption spectra (Fig 1) exhibited peaks at ~ 440 nm (Chl\(_a\)), ~ 490–500 nm (phycourobilin; PUB), ~ 540 and 568 nm (phycoerythrin; PE), ~ 620 nm (phycocyanin; PC), ~ 640 nm (allophycocyanin; APC) and ~ 675 nm (Chl\(_a\)) (Table 3). Chlorophyll \(\alpha\) and photo-protective carotenoids (PPC) dominated light absorption, together accounting for ~ 65% of

<table>
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<th>Variables</th>
<th>Units</th>
<th>Growth rate</th>
<th>NH(_4^+)</th>
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| N\(_2\)    | d\(^{-1}\) | 0.340 (0.038)
| C:N        | mol: mol | 6.9 (0.7)  | 4.4 (0.9)  | 3.9 (0.7)  |
| C:P        | mol: mol | 122.6 (7.0)
| N:P        | mol: mol | 18.1 (1.3)
| Chl\(_a\):C| mg: mol  | 134 (8)  | 239 (4)   | 222 (2)   |
| Chl\(_a\):N| mg: mol  | 906 (43) | 1041 (209) | 855 (154) |

Abbreviations: C:N, C:P and N:P ratios are mol:mol, Chl\(_a\):C and Chl\(_a\):N ratios are mg:mol (n = 3). Letters in parenthesis indicate significant differences between N-source treatments (One Way ANOVA, Tukey post hoc test; P < .05); where [B] is significantly greater than [A].

https://doi.org/10.1371/journal.pone.0195638.t001

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Table 1. The median (± S.E.) balanced growth rates and mean elemental stoichiometry and N\(_2\) fixation rates for *T. erythraeum* IMS101 when acclimated to three N-source conditions (N\(_2\), NH\(_4^+\) and NO\(_3^-\)), at a target CO\(_2\) concentration (380 μatm), saturating light intensity (400 μmol photons m\(^{-2}\) s\(^{-1}\)) and optimal temperature (26 °C).
the total. PUB1 and PUB2 were the only pigments to exhibit significant differences, where relative to the N\textsubscript{2} treatment, the contribution of PUB1 to the total light absorption increased by 7.4% whereas PUB2 decreased by 1.3% in the presence of NO\textsubscript{3} (Table 3).

**Light-dependence of O\textsubscript{2} exchange**

The C-specific maximum rate (E\textsubscript{om} \textsuperscript{C}) and initial slope (α\textsubscript{g} \textsuperscript{C}) of light-dependent gross photosynthesis increased with additional N-sources (i.e. NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}) and was highest for the NH\textsubscript{4}\textsuperscript{+} treatment relative to the N\textsubscript{2} treatment (Table 4). There were also significant effects of additional N-sources on the Chl\textsubscript{a}-specific maximum rate (E\textsubscript{om} \textsuperscript{Chl\textsubscript{a}}) and initial slope of light-dependent gross photosynthesis (α\textsubscript{g} \textsuperscript{Chl\textsubscript{a}}) (S1 Table), however the effects were more pronounced when expressed as a C-specific rate, where E\textsubscript{om} \textsuperscript{C} increased by 143% from the N\textsubscript{2} to the NH\textsubscript{4}\textsuperscript{+} treatment, while E\textsubscript{om} \textsuperscript{Chl\textsubscript{a}} increased by only 36%.

The light saturation parameter (E\textsubscript{k} = E\textsubscript{om} \textsuperscript{C}/α\textsubscript{g} \textsuperscript{C}) of gross O\textsubscript{2} evolution did not vary significantly between N-source treatments (Table 4) and was due to covariation of α\textsubscript{g} \textsuperscript{C} and E\textsubscript{om} \textsuperscript{C}. The maximum quantum efficiency of gross O\textsubscript{2} evolution (ϕ\textsubscript{mgross} = α\textsubscript{g} \textsuperscript{C}/a\textsubscript{eff} \textsuperscript{C}) increased significantly by 76% from the N\textsubscript{2} to NH\textsubscript{4}\textsuperscript{+} treatment (Table 4) and was due to the relatively constant a\textsubscript{eff} \textsuperscript{C} and the significant increase in α\textsubscript{g} \textsuperscript{C}.

Carbon-specific dark respiration rates (R\textsubscript{d} \textsuperscript{C}) varied by ~ 24% and were slightly higher for the N\textsubscript{2} and NH\textsubscript{4}\textsuperscript{+} treatments than the NO\textsubscript{3}\textsuperscript{-} treatment (Table 4). Light-saturated net O\textsubscript{2} evolution rates (P\textsubscript{netm} \textsuperscript{C}) approximately trebled and more than doubled from the N\textsubscript{2} treatment to the NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} treatments respectively (Table 4); with the initial slope (α\textsubscript{n} \textsuperscript{C}) showing a similar pattern to P\textsubscript{netm} \textsuperscript{C}. This increase in α\textsubscript{n} \textsuperscript{C} for the NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} treatments resulted in the maximum quantum efficiency of net O\textsubscript{2} evolution (ϕ\textsubscript{mnet} = α\textsubscript{n} \textsuperscript{C}/a\textsubscript{eff} \textsuperscript{C}) increasing significantly by 86% and 100% respectively, relative to the N\textsubscript{2} treatment (Table 4). The light saturation parameter (E\textsubscript{k} = P\textsubscript{netm} \textsuperscript{C}/α\textsubscript{n} \textsuperscript{C}) for net O\textsubscript{2} evolution did not vary significantly between N-source treatments (Table 4).

The relationship between net and gross O\textsubscript{2} evolution was linear (Fig 2D–2F), with the slope increasing by approximately 40% when cultured in the presence of an additional N-source (Table 4). This linear relationship suggests that light-dependent O\textsubscript{2} consumption (U\textsubscript{0} \textsuperscript{C}) was a

### Table 2. The growth conditions (± S.E.) for *T. erythraeum* IMS101 when cultured under three N-source conditions (N\textsubscript{2}, NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}), at a target CO\textsubscript{2} concentration (380 μatm), saturating light intensity (400 μmol photons m\textsuperscript{-2} s\textsuperscript{-1}) and optimal temperature (26 °C).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>N\textsubscript{2}</th>
<th>NH\textsubscript{4}\textsuperscript{+}</th>
<th>NO\textsubscript{3}\textsuperscript{-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>8.18</td>
<td>8.18</td>
<td>8.19</td>
</tr>
<tr>
<td>H\textsuperscript{+}</td>
<td>nM</td>
<td>6.6 (0.1)</td>
<td>6.6 (0.1)</td>
<td>6.4 (0.2)</td>
</tr>
<tr>
<td>A\textsubscript{C}</td>
<td>μM</td>
<td>2483 (47)</td>
<td>2427 (59)</td>
<td>2482 (56)</td>
</tr>
<tr>
<td>TCO\textsubscript{2}</td>
<td>μM</td>
<td>2066 (41)</td>
<td>2019 (51)</td>
<td>2056 (44)</td>
</tr>
<tr>
<td>HCO\textsubscript{3}\textsuperscript{-}</td>
<td>μM</td>
<td>1762 (35)</td>
<td>1723 (44)</td>
<td>1746 (33)</td>
</tr>
<tr>
<td>CO\textsubscript{3}\textsuperscript{2-}</td>
<td>μM</td>
<td>296 (7)</td>
<td>288 (9)</td>
<td>302 (12)</td>
</tr>
<tr>
<td>CO\textsubscript{2}</td>
<td>μM</td>
<td>8.3 (0.2)</td>
<td>8.2 (0.3)</td>
<td>8.9 (0.4)</td>
</tr>
<tr>
<td>pCO\textsubscript{2}</td>
<td>μatm</td>
<td>300 (8)</td>
<td>289 (9)</td>
<td>289 (7)</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}</td>
<td>μM</td>
<td>0.76 (0.13)</td>
<td>8.33 (0.45)</td>
<td>0.59 (0.07)</td>
</tr>
<tr>
<td>NO\textsubscript{3}\textsuperscript{-}</td>
<td>μM</td>
<td>0.07 (0.07)</td>
<td>0.46 (0.07)</td>
<td>8.24 (1.31)</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>35</td>
<td>34</td>
<td>10</td>
</tr>
</tbody>
</table>

Individual pH values were converted to a H\textsuperscript{+} concentration, allowing a mean pH value to be calculated.

https://doi.org/10.1371/journal.pone.0195638.t002
constituent proportion of gross $\text{O}_2$ evolution ($E_0^C$) and was independent of light intensity for all N-source treatments. Subtracting the slope from unity gave the ratio of light-driven $U_0^C$ to $E_0^C$, which was significantly lower for the $\text{N}_2$ treatment.

The ratio of gross photosynthesis ($E_0$) to $\text{N}_2$ fixation increased 9-fold and 6-fold for the $\text{NH}_4^+$ and $\text{NO}_3^-$ treatments relative to the $\text{N}_2$ treatment. In addition, the ratio of net photosynthesis ($P_{\text{net}}$) to $\text{N}_2$ fixation was 12-fold and 7-fold higher for the $\text{NH}_4^+$ and $\text{NO}_3^-$ treatments relative to the $\text{N}_2$ treatment (Table 4).

**Light-dependence of PSII electron transport**

The operating efficiency of PSII photochemistry ($F_q/F_m$) increased at low light intensities, reaching a maximum at ~ 110 to 130 $\mu$mol photons m$^{-2}$ s$^{-1}$, before decreasing significantly with increasing light intensity (Fig 3). The light saturation parameter ($E_k$) and the light at which ETR was maximal ($E_{\text{opt}}$) were significantly higher for the $\text{N}_2$ treatment than the $\text{NH}_4^+$ treatment. Conversely, the light inhibition parameter ($E_p$), absorption cross-section of PSII

### Table 3. The mean (± S.E.) measured and modelled effective light absorption coefficients and the relative contribution of each photosynthetic pigment to the total light absorption under the culturing LEDs within *T. erythraeum* IMS101, when acclimated to three N-sources ($\text{N}_2$, $\text{NH}_4^+$ and $\text{NO}_3^-$), at a target $\text{CO}_2$ concentration (380 $\mu$atm), saturating light intensity (400 $\mu$mol photons m$^{-2}$ s$^{-1}$) and optimal temperature (26 °C).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>$\text{N}_2$</th>
<th>$\text{NH}_4^+$</th>
<th>$\text{NO}_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{\text{eff}}^{\text{Chl}}$</td>
<td>m$^2$ (g Chl$^{-1}$)</td>
<td>9.9 (0.6)</td>
<td>7.7 (0.9)</td>
<td>8.1 (0.3)</td>
</tr>
<tr>
<td>$a_{\text{eff}}^{\text{C}}$</td>
<td>m$^2$ (g C$^{-1}$)</td>
<td>0.111 (0.013)</td>
<td>0.154 (0.020)</td>
<td>0.149 (0.006)</td>
</tr>
<tr>
<td>$a_{\text{mod}}^{\text{Chl}}$</td>
<td>m$^2$ (g Chl$^{-1}$)</td>
<td>10.0 (0.6)</td>
<td>7.8 (0.9)</td>
<td>8.3 (0.3)</td>
</tr>
<tr>
<td>$a_{\text{mod}}^{\text{C}}$</td>
<td>m$^2$ (g C$^{-1}$)</td>
<td>0.112 (0.013)</td>
<td>0.156 (0.019)</td>
<td>0.154 (0.005)</td>
</tr>
<tr>
<td>Chl$\text{a}$</td>
<td>%</td>
<td>35.66 (0.41)</td>
<td>36.74 (0.40)</td>
<td>39.18 (2.14)</td>
</tr>
<tr>
<td>PPC</td>
<td>%</td>
<td>30.64 (3.19)</td>
<td>27.48 (3.05)</td>
<td>27.44 (2.87)</td>
</tr>
<tr>
<td>PUB1</td>
<td>%</td>
<td>2.67 (1.36)$^\text{[A]}$</td>
<td>5.04 (4.67)</td>
<td>10.11 (2.13)$^\text{[B]}$</td>
</tr>
<tr>
<td>PUB2</td>
<td>%</td>
<td>1.34 (0.28)$^\text{[B]}$</td>
<td>1.63 (1.04)</td>
<td>0.06 (0.06)$^\text{[A]}$</td>
</tr>
<tr>
<td>PUBx</td>
<td>%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB4</td>
<td>%</td>
<td>0.02 (0.02)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB5a</td>
<td>%</td>
<td>0.42 (0.21)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB5b</td>
<td>%</td>
<td>0.24 (0.23)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB5d</td>
<td>%</td>
<td>0.05 (0.03)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB5g</td>
<td>%</td>
<td>0.18 (0.18)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUB5j</td>
<td>%</td>
<td>0.19 (0.19)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PE1</td>
<td>%</td>
<td>8.10 (3.73)</td>
<td>7.51 (3.70)</td>
<td>3.19 (2.01)</td>
</tr>
<tr>
<td>PE2a</td>
<td>%</td>
<td>1.17 (0.75)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PE2b</td>
<td>%</td>
<td>1.02 (0.72)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PE3b</td>
<td>%</td>
<td>10.93 (2.28)</td>
<td>13.14 (3.60)</td>
<td>13.03 (0.92)</td>
</tr>
<tr>
<td>APC</td>
<td>%</td>
<td>5.45 (1.30)</td>
<td>5.17 (1.34)</td>
<td>5.97 (1.17)</td>
</tr>
<tr>
<td>PC1</td>
<td>%</td>
<td>0.94 (0.57)</td>
<td>0.19 (0.19)</td>
<td>0</td>
</tr>
<tr>
<td>PC2</td>
<td>%</td>
<td>2.22 (1.15)</td>
<td>3.08 (1.61)</td>
<td>1.02 (0.52)</td>
</tr>
</tbody>
</table>

Light absorption coefficients were spectrally corrected to the culture LEDs and were normalised to a chlorophyll $a$ ($\text{m}^2 \text{g Chl}^{-1}$) and carbon ($\text{m}^2 \text{g C}^{-1}$) basis. Abbreviations; $a_{\text{eff}}^{\text{Chl}}$ and $a_{\text{eff}}^{\text{C}}$ are the measured Chl- and C-specific light absorption coefficients, while $a_{\text{mod}}^{\text{Chl}}$ and $a_{\text{mod}}^{\text{C}}$ are the modelled Chl- and C-specific light absorption coefficients. $a_{\text{mod}}^{\text{Chl}}$ and $a_{\text{mod}}^{\text{C}}$ were constructed from a range of pigment light absorption spectrums ($\lambda = 400–700$); comprising chlorophyll $a$ (Chl$\text{a}$), photoprotective carotenoids (PPC), phycourobilins (PUB1, PUB2, PUBx, PUB4, PUB5a, PUB5b, PUB5d, PUB5g and PUB5j), phycoerythrin (PE1, PE2a, PE2b and PE3b), alloplastocyanin (APC) and plastocyanin (PC1 and PC2). Letters in parenthesis indicate significant differences between N-source treatments (One Way ANOVA, Tukey post hoc test; $P < .05$); where $[B]$ is significantly greater than $[A]$. 

https://doi.org/10.1371/journal.pone.0195638.t003
photochemistry ($\sigma_{\text{PII}}$) and the time constant for the re-opening of a closed PSII reaction centre ($\tau_f$) in the dark-adapted state were not significantly different between N-source treatments. Furthermore, both $\sigma_{\text{PII}}$ and $\tau_f$ exhibited no light-dependency, remaining relatively constant across the entire range of actinic light intensities (Fig 3, Table 5).

The light intensity at which ETR was maximal ($E_{\text{opt}}$) was significantly lower (by $\sim 120 \, \mu$mol photons m$^{-2}$ s$^{-1}$) for the NH$_4^+$ treatment relative to the N$_2$ treatment (Fig 4). The Chl$_a$ and C-specific maximum electron transport rate and initial slope ($\alpha_{\text{ETR}}$) of the ETR-light curves were not significantly different between N-source treatments (Table 5, S2 Table). In contrast, the light-saturated photoinhibition slopes ($\beta_{\text{ETR}}$) were significantly different, with $\beta$ increasing by 5% and 10% for the NO$_3^-$ and NH$_4^+$ treatments, relative to the N$_2$ treatment (Table 5).

The ratio of PSII electron transport to gross O$_2$ evolution under light-limitation ($\Phi_{\text{eo}}$) was $\sim 4$ and did not vary significantly between N-source treatments. Light saturated ratios ($\Phi_{\text{em}}$) increased relative to $\Phi_{\text{eo}}$ for all N-source treatments, with the N$_2$ treatment being 46% and 35% higher than the NH$_4^+$ and NO$_3^-$ treatments, respectively (Table 5).
Table 4. The parameters (± S.E.) of the C-specific light-response curves for gross and net photosynthetic O₂ evolution of *T. erythraeum* IMS101 (*n* = 3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>N₂</th>
<th>NH₄⁺</th>
<th>NO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gross O₂ evolution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E</em>₀&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mmol O₂ (g C)&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>6.05 (0.37)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>14.71 (1.20)&lt;sup&gt;[C]&lt;/sup&gt;</td>
<td>10.98 (0.33)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Eₖ</em></td>
<td>μmol photons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>238 (55)</td>
<td>227 (44)</td>
<td>255 (55)</td>
</tr>
<tr>
<td><em>α</em>ₕ&lt;sup&gt;c&lt;/sup&gt;</td>
<td>μmol O₂ (g C)&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt; (μmol photons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>27.9 (5.3)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>67.7 (8.2)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>49.8 (15.1)</td>
</tr>
<tr>
<td><em>φ</em>₉&lt;sup&gt;mgross&lt;/sup&gt;</td>
<td>mol O₂ (mol photons)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.07 (0.01)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>0.12 (0.01)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>0.09 (0.03)</td>
</tr>
<tr>
<td><em>E</em>₂&lt;sup&gt;Nfix&lt;/sup&gt;</td>
<td>mol O₂ (mol N₂)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>31 (4)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>289 (32)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>185 (57)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Net Photosynthesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P</em>&lt;sub&gt;net&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mmol O₂ (g C)&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.75 (0.27)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>11.48 (1.56)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>9.59 (0.37)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Eₖ</em></td>
<td>μmol photons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>250 (69)</td>
<td>277 (8)</td>
<td>220 (37)</td>
</tr>
<tr>
<td><em>α</em>ₕ&lt;sup&gt;n&lt;/sup&gt;</td>
<td>μmol O₂ (g C)&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt; (μmol photons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>16.8 (3.4)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>41.5 (5.8)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>46.1 (8.0)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>R</em>ₚ&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mmol O₂ (g C)&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>-1.63 (0.19)</td>
<td>-1.53 (0.28)</td>
<td>-1.16 (0.76)</td>
</tr>
<tr>
<td><em>φ</em>ₙ&lt;sup&gt;net&lt;/sup&gt;</td>
<td>mol O₂ (mol photons)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.04 (0.01)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>0.08 (0.02)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>0.09 (0.01)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P</em>ₙ&lt;sup&gt;net&lt;/sup&gt;&lt;sup&gt;Nfix&lt;/sup&gt;</td>
<td>mol O₂ (mol N₂)&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>18 (1)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>207 (29)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>163 (36)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Gross (x) vs. Net (y)</strong></td>
<td>Dimensionless</td>
<td>0.60 (0.02)&lt;sup&gt;[A]&lt;/sup&gt;</td>
<td>0.82 (0.03)&lt;sup&gt;[B]&lt;/sup&gt;</td>
<td>0.83 (0.01)&lt;sup&gt;[B]&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: *E*₀<sup>c</sup>, the C-specific maximum gross O₂ evolution rate; *P*<sub>net</sub><sup>c</sup>, the C-specific maximum net O₂ evolution rate; *Eₖ*, the light saturation parameter; *α*ₕ<sup>c</sup> and *α*ₕ<sup>n</sup> are the C-specific initial slopes the light response curve for net and gross photosynthesis; *φ*₉<sup>mgross</sup> and *φ*ₙ<sup>net</sup> are the maximum quantum efficiencies of gross and net O₂ evolution; *R*ₚ<sup>c</sup>, the C-specific dark respiration rate; slope, the gradient of the regression between *P*<sub>net</sub><sup>c</sup> and *Eₖ*; *E*₂<sup>Nfix</sup> and *P*ₙ<sup>net</sup><sup>Nfix</sup>, the ratio of gross and net photosynthesis to N₂ fixation, where rates of *Eₖ* and *P*ₙ<sup>net</sup> were calculated at 400 μmol photons m<sup>-2</sup> s<sup>-1</sup>, matching to light intensity of the N₂ fixation incubations; slope, the gradient of the regression between *P*<sub>net</sub><sup>c</sup> and *Eₖ*.<sup>c</sup>. The *r*² values of all curve fits were > 0.982. Letters in parenthesis indicate significant differences between CO₂ 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].

https://doi.org/10.1371/journal.pone.0195638.1004

**Discussion**

**Effect of acclimation to variation of N-sources on growth rates and elemental stoichiometry**

Growth rates achieved under diazotrophic conditions were similar to most previous studies [23, 43–45], as was the increase in growth rate observed under non-diazotrophic conditions [23, 43], which we attribute to the lowered demand of NADPH and ATP for nitrogenase activity, where NADPH and ATP could be re-directed to CO₂ fixation and/or biosynthesis. Our data shows that at saturating light intensity, the energetic cost of diazotrophy constrains *Trichodesmium* growth by ~ 13%. However, in a natural system, potential changes to inorganic carbon chemistry (influencing the activity of the carbon concentrating mechanism (CCM)), temperature (influencing enzyme activity), or other key nutrients (i.e. Fe, P), all of which were controlled in our experiments, will almost certainly influence this estimate.

The decrease in C:N, C:P and N:P under non-diazotrophic conditions is consistent with previous findings [43]. The high C:N under diazotrophic conditions may be due to accumulation of stored glycogen, whereas the decrease in C:N under non-diazotrophic conditions is likely due to high cellular N concentrations, likely due to the luxury uptake of NH₄⁺ and NO₃⁻, where surplus N is stored within cyanophycin granules [26]. Given the concurrent decrease in C:N, C:P and N:P under non-diazotrophic conditions, it is likely that utilising NH₄⁺ or NO₃⁻ as a N-source enables *Trichodesmium* cells of low carbon biomass to maintain a Chl a concentration comparable to diazotrophic conditions. This is supported by previous observation made by Eichner et al. [43] and is also reflected by the higher Chla:C yet comparable Chla:N ratios for NH₄⁺ or NO₃⁻ treatments.
Growing evidence points towards nitrogenase being expressed in subsets of cells within filaments, called diazocytes [21, 25]. To date, no translocation transport mechanisms for N compounds have been observed in *Trichodesmium*, leading to suggestions that diazocytes release N into the external medium for use by neighbouring cells [25, 46]. This is partially supported by observations of *Trichodesmium* exhibiting a high capacity for NH$_4^+$ uptake during active N$_2$ fixation [17, 18]. While such mechanisms may exist, we did not observe significant concentrations of dissolved inorganic NO$_3^-$ or NH$_4^+$ in the medium of our control treatment.

**Effect of acclimation to different N-sources on gross photosynthesis**

We show an effect of N-source on C-specific light saturated gross O$_2$ evolution rates. The more than two-fold increase in the maximum O$_2$ evolution rate and initial slope when *T. erythraeum* IMS101 was grown on NH$_4^+$ or NO$_3^-$ than when growing diazotrophically was largely due to differences in the ratio of Chl:a:C as chlorophyll *a*-specific photosynthetic parameters varied by only 36% between N$_2$ and NH$_4^+$ treatments.
The increase of C-specific gross O\textsubscript{2} evolution rates when *Trichodesmium* is supplied with NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-} may be due to an increase in the maximum rate of CO\textsubscript{2} fixation and/or to an increase in PSII concentration. Previous studies report high PSI:PSII ratios under diazotrophic conditions (ranging between 1.3 to 4) [47–51], which would allow cyclic photophosphorylation in diazocytes to provide most of the ATP required for N\textsubscript{2} fixation, with glycolysis and the Kreb’s cycle providing the required reducing equivalent. It may be that under non-
Table 5. The parameters (± S.E.) of the fluorescence light-response curves (FLCs) of T. erythraeum IMS101 (n = 3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>N\textsubscript{2}</th>
<th>NH\textsubscript{4}\textsuperscript{+}</th>
<th>NO\textsubscript{3}\textsuperscript{-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E\textsubscript{TR}m</td>
<td>mmol e\textsuperscript{-} (g C)\textsuperscript{-1} h\textsuperscript{-1}</td>
<td>62.5 (16.7)</td>
<td>70.3 (14.4)</td>
<td>91.0 (4.9)</td>
</tr>
<tr>
<td>E\textsubscript{k}</td>
<td>μmol photons m\textsuperscript{-2} s\textsuperscript{-1}</td>
<td>465 (8)\textsuperscript{[B]}</td>
<td>421 (3)\textsuperscript{[A]}</td>
<td>447 (20)</td>
</tr>
<tr>
<td>α\textsubscript{ETR}</td>
<td>mmol e\textsuperscript{-} (g C)\textsuperscript{-1} (μmol photons m\textsuperscript{-2} s\textsuperscript{-1})\textsuperscript{-1}</td>
<td>0.133 (0.033)</td>
<td>0.167 (0.033)</td>
<td>0.200 (0.003)</td>
</tr>
<tr>
<td>β\textsubscript{ETR}</td>
<td>mmol e\textsuperscript{-} (g C)\textsuperscript{-1} (μmol photons m\textsuperscript{-2} s\textsuperscript{-1})\textsuperscript{-1}</td>
<td>5081 (55)\textsuperscript{[A]}</td>
<td>5577 (55)\textsuperscript{[C]}</td>
<td>5332 (14)\textsuperscript{[B]}</td>
</tr>
<tr>
<td>E\textsubscript{opt}</td>
<td>μmol photons m\textsuperscript{-2} s\textsuperscript{-1}</td>
<td>1263 (22)\textsuperscript{[B]}</td>
<td>1144 (3)\textsuperscript{[A]}</td>
<td>1216 (54)</td>
</tr>
<tr>
<td>E\textsubscript{p}</td>
<td>μmol photons m\textsuperscript{-2} s\textsuperscript{-1}</td>
<td>0.99 (0.11)</td>
<td>0.67 (0.08)</td>
<td>0.83 (0.09)</td>
</tr>
<tr>
<td>F\textsubscript{v}/F\textsubscript{m}</td>
<td>Dimensionless</td>
<td>0.253 (0.009)</td>
<td>0.367 (0.003)</td>
<td>0.380 (0.032)</td>
</tr>
<tr>
<td>τ\textsubscript{f}</td>
<td>s\textsuperscript{-1}</td>
<td>489 (5)</td>
<td>494 (15)</td>
<td>631 (105)</td>
</tr>
<tr>
<td>Φ\textsubscript{em}</td>
<td>mol e\textsuperscript{-} (mol O\textsubscript{2})\textsuperscript{-1}</td>
<td>10.5 (1.1)\textsuperscript{[B]}</td>
<td>5.7 (1.1)\textsuperscript{[A]}</td>
<td>7.9 (0.5)</td>
</tr>
<tr>
<td>Φ\textsubscript{α}</td>
<td>mol e\textsuperscript{-} (mol O\textsubscript{2})\textsuperscript{-1}</td>
<td>2.5 (0.8)</td>
<td>2.8 (0.2)</td>
<td>4.5 (1.0)</td>
</tr>
</tbody>
</table>

Abbreviations; E\textsubscript{TR}m\textsuperscript{C}, the C-specific maximum electron transport rate; α\textsubscript{ETR}\textsuperscript{C}, the C-specific initial slope of the electron transport rate light response curve; β\textsubscript{ETR}\textsuperscript{C}, the C-specific light saturated slope of the electron transport rate light response curve; E\textsubscript{k}, the light saturation parameter; E\textsubscript{opt}, the light at which ETR is maximal; E\textsubscript{p}, the light inhibition parameter; F\textsubscript{v}/F\textsubscript{m}, the maximum photochemical efficiency of PSII in the dark-adapted state; σ\textsubscript{PII}, the absorption cross-section of PSII photochemistry in the dark-adapted state; τ\textsubscript{f}, the average time constant for the re-opening of a closed PSII reaction centre in the dark-adapted state; Φ\textsubscript{em}, the light saturated ratio of PSII electron transport to gross O\textsubscript{2} evolution; Φ\textsubscript{α}, the light limited ratio of PSII electron transport to gross O\textsubscript{2} evolution. The r\textsuperscript{2} values of all curve fits were > 0.977. Letters in parenthesis indicate significant differences between N-source 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].

https://doi.org/10.1371/journal.pone.0195638.t005

Fig 4. Concurrent Chl\textsubscript{a} (a-c) and C-specific (d-f) gross O\textsubscript{2} evolution rates and PSII electron transport rates (ETR) for T. erythraeum IMS101 (n = 3). Cultures were acclimated to three N-sources (N\textsubscript{2}, NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}), at a target CO\textsubscript{2} concentration (380 μatm), saturating light intensity (400 μmol photons m\textsuperscript{-2} s\textsuperscript{-1}) and optimal temperature (26 °C).

https://doi.org/10.1371/journal.pone.0195638.g004
diazotrophic conditions and with lower nitrogenase activity, *Trichodesmium* enhances linear electron transport to increase NADPH production; a pathway that generates more evolved O$_2$.

**Effect of acclimation to different N-sources on N$_2$ fixation**

Nitrogenase activity declined significantly by 81–84% when *Trichodesmium* was cultured in the presence of an additional N-source. Despite being cultured under N-replete concentrations, *Trichodesmium* cells in the NH$_4^+$ and NO$_3^-$ treatments exhibited a baseline rate of N$_2$ fixation. Similarly, Milligan *et al.* [52] reported a ~ 85% decrease when *Trichodesmium* was cultured in 100 μM of NO$_3^-$ for 2 weeks and Holl and Montoya [44] reported a 66% decrease when cultured in 20 μM of NO$_3^-$, accrediting 8% of total N assimilation to diazotrophy despite the presence of additional N-sources. Maintaining the capability to perform N$_2$ fixation under non-diazotrophic conditions, albeit at a reduced rate, could reflect *Trichodesmium*’s natural environment and act a potential safeguard mechanism to variable light and nutrient regimes.

Noting that 16 moles of ATP are consumed per mole of N$_2$ fixed (Eq 1) and that 2.56 moles of ATP can be produced per mole of O$_2$ evolved by photophosphorylation linked LPET [53], we calculated that *T. erythraeum* IMS101 may use 20% of the ATP that could be generated from gross O$_2$ evolution to support the observed N$_2$ fixation rate during diazotrophic growth:

\[
\frac{N_{fix}}{E_0} = \frac{1\text{ mol N}_2}{31\text{ mol O}_2} \cdot \frac{1\text{ mol O}_2}{2.54\text{ mol ATP}} \cdot \frac{16\text{ mol ATP}}{1\text{ mol N}_2} = 0.2
\]

This proportion decreases to 2% and 4% for the NO$_3^-$ and NH$_4^+$ treatments, respectively, where the ratio of $E_0:C_{fix}$ increases to 289 for the NO$_3^-$ treatment and 185 in the NH$_4^+$ treatment, versus 31 in the N$_2$ treatment (Table 4).

Studies on natural populations of *Trichodesmium* spp. have shown that the addition of NO$_3^-$ (100 μM) in the morning can cause a gradual decrease of N$_2$ fixation over the photic period [22]. Further studies have also shown that addition of glutamine (10 μM) immediately decreases N$_2$ fixation rates, indicating a direct effect on enzyme activity as opposed to enzyme synthesis [54]. These observations have been accredited to accumulation of N-containing metabolites acting as potential inhibitors to the specific activity rather than abundance of nitrogenase [22, 54].

It is well known that intracellular nitrogen pools have a role in regulating nitrogenase activity in diazotrophs [55, 56]. Dinitrogenase reductase catalyses the reduction of N$_2$ to NH$_4^+$, which is assimilated into glutamine (gln) and then into glutamate (glu) via the glutamine synthetase (GS, EC 6.3.1.2)/glutamate synthase (GOGAT) pathway [54]. The intracellular pools of NH$_4^+$, glu and gln have been identified as important feedback regulators of N uptake and metabolism, with GS activity in *Trichodesmium* being sensitive to both intra- and extracellular N concentrations [55]. It could be hypothesised that the activity of nitrogenase is influenced by internally recycled N (e.g. NH$_4^+$ and gln), while the synthesis of nitrogenase is influenced by newly assimilated N (e.g. NO$_3^-$).

**Effect of acclimation to different N-sources on light-stimulated O$_2$ consumption and the relationship between net and gross O$_2$ evolution**

Net photosynthesis was significantly lower for the N$_2$ treatment than for the NH$_4^+$ and NO$_3^-$ treatments. Despite slight variations in $E'_0$, the difference in net photosynthesis was principally driven by O$_2$ consumption. Approximately 68%, 32% and 29% (N$_2$, NH$_4^+$ and NO$_3^-$, respectively) of $E'_0$ was consumed by O$_2$ consuming processes, which is comparable to previous observations [43, 52].
Several processes demand ATP in excess of the ATP:NADPH produced through linear photophosphorylation; two most notably being N₂ fixation and the operation of the CCM [57]. In this study, the carbon chemistry of all cultures was closely regulated to ensure that variation in O₂ consumption and net photosynthesis was due to the N-source treatments only. Linearity between gross O₂ evolution (E₀) and O₂ consumption was observed across all N-source treatments, suggesting that light-dependent O₂ consumption is linked to balancing ATP to NADPH production, as opposed to serving as a mechanism to dissipate excitation energy.

Diazotrophic cells consume more O₂ per evolved O₂ across the entire range of actinic light intensities than the NH₄⁺ and NO₃⁻ treatments. This suggests a higher rate of water-water cycling due to either Mehler activity or operation of plastoquinone terminal oxidase when N₂ is being fixed. To maintain a sufficient supply of ATP relative to NADPH, *Trichodesmium* may utilise pseudocyclic photophosphorylation linked to the Mehler reaction to augment the ATP generated by linear electron transfer from water to NADP⁺ in addition to ATP produced by cyclic electron flow around PSI.

Measurements of O₂ evolution, ETR and N₂ fixation were all made at one time of day (4 to 6 hours into the photo-phase of a 12:12 L:D cycle) and as such cannot be extrapolated to a diel response given the reports of temporal separation of photosynthesis and N₂ fixation in *Trichodesmium* [21].

**Effect of acclimation to different N-sources on electron transport rates and photophysiology**

Like Eichner *et al.* [43], we observed a negligible effect of N-source on many photo-physiological parameters, including \( \frac{F_q}{F_m} \), \( \sigma_{PII} \) and \( \tau_f \). *Trichodesmium* exhibited a light response typical for most cyanobacteria, where the dark-adapted photochemical yield is significantly affected by respiratory electron flow [58]. This results from a proportion of PSI reaction centres remaining in a closed state despite being in the dark and is imposed by a reduction in the plastoquinone (PQ) pool, which prevents the oxidation of QA⁻. Moving from darkness to a low light intensity increases the electron flux through PSI, alleviates the bottleneck of electron transport through the Cyt b₆f complex, thereby increasing \( \frac{F_q}{F_m} \) and decreasing the re-oxidation time of QA⁻. Addition factors such as higher downregulation under dark-adapted conditions may also contribute to the increase in \( \frac{F_q}{F_m} \) under low light intensities.

**Ratio of electron transport to gross O₂ evolution**

Electrons are transferred from PSII (where O₂ is evolved) to an intermediate plastoquinone pool and eventually to ferredoxin to produce NADPH [59]. A minimum of four moles of electrons are transported through PSII for each mole of O₂ evolved at PSII. Most higher plants exhibit a linear correlation between gross O₂ evolution and electron transport rate [60]. In microalgae, this relationship is often ambiguous, especially at high light intensities where the relationship can become non-linear [61, 62].

Here we show that at low light intensities, the ratio of PSII electron transport to gross O₂ evolution (\( \Phi_{e\text{ld}} \)) is close to a 4:1 ratio for all N-source treatments. However, when light intensities exceed 150 µmol photons m⁻² s⁻¹, \( \Phi_e \) declines as ETR saturates at a higher light intensity (~ 900 µmol photons m⁻² s⁻¹) than \( E_0 \) (~ 400 µmol photons m⁻² s⁻¹). Similar responses have been reported for diatoms [63], microalgae [64] and the Baltic cyanobacteria, *Nostoc* [65]. Few studies have measured O₂ production rates in *Trichodesmium* [47, 66] and to our knowledge none have reported concurrent PSII electron transport rates.
Interestingly, we calculated a higher $\Phi_e$ for the $N_2$ cultures than for the $NH_4^+$ and $NO_3^-$ cultures, irrespective of using the light-limited or -saturated rates. This may be due to overestimating the proportion of light absorbed by PSII in the non-diazotrophic growth conditions (i.e. $NH_4^+$ and $NO_3^-$) relative to the diazotrophic condition. Here we assumed that 50% of absorbed light was directed to PSII reaction centres and 50% to PSI reaction centres (i.e. FAQP$_{PSII}$ of 0.5). It’s likely that FAQP$_{PSII}$ was overestimated for diazotrophic treatment (i.e. $N_2$) which may have had a higher ratio of PSI:PSII to support significant rates of cyclic photophosphorylation. In addition, non-diazotrophic cells may undergo more pronounced state transitions with phycobilin proteins being redistributed between PSII and PSI. Finally, a $\Phi_e > 4$ could be accredited to cyclic electron flow around PSII, which may act a mechanism to dissipate excess excitation energy under high light [67].

**Implications for future oligotrophic oceans**

In N-limited regions of the oligotrophic open ocean, diazotrophy provides a competitive advantage by allowing cells to access $N_2$ as an N-source against faster growing phytoplankton that rely on fixed N. Current ocean models predict a poleward shift in the 20˚C isotherm which could extend *Trichodesmium*’s niche into higher latitudes. On a global scale, this niche expansion is driven by increased SSTs; however, on regional scales persistence in an area may be dictated by *Trichodesmium*’s response to fluctuating nutrient regimes.

At the surface in oligotrophic waters, *Trichodesmium* is unlikely to encounter $NO_3^-$, $NO_2^-$ or $NH_4^+$ concentrations in excess of 0.1 $\mu$M [68], except during mixing events. While *Trichodesmium* is commonly observed in the upper meters of the water column [69], observations have been recorded down to 200 m depth [70]. Thus, *Trichodesmium* colonies and free trichomes are able to migrate to the nutricline [30, 31]. Such vertical migration has been suggested to allow luxury uptake of phosphates before colonies return to the surface. In addition to encountering phosphates, *Trichodesmium* will also encounter high concentrations of $NO_3^-$ in the nutricline. As such, $NO_3^-$ uptake is likely at these greater depths or at the surface after a mixing event.

Mulholland *et al.* [17] reported significant $NO_3^-$ uptake rates with the addition of 1 $\mu$M $NO_3^-$ to the growth media. Furthermore, Karl *et al.* [30] showed that concentrations of dissolved $NH_4^+$ reached 1.5 $\mu$M L$^{-1}$ and dissolved organic N (DON) reaching 13 $\mu$M L$^{-1}$ during a natural bloom of *Trichodesmium* spp. in the North Pacific gyre. These concentrations are far greater than typical oceanic N pools and could therefore be high enough to inhibit $N_2$ fixation rates [44]. It’s therefore possible that *Trichodesmium* colonies at depth may be utilising more combined N-sources than the blooms frequently measured on the surface. The energy and reductant conserved through utilising additional N-sources could significantly enhance *Trichodesmium*’s productivity and growth which could have major implications for biogeochemical cycles.

Our results indicate the need to seek more information on the potential for natural populations of *Trichodesmium* to uptake fixed N-sources (e.g. $NO_3^-$, $NH_4^+$, labile dissolved organic nitrogen (DON)) at concentrations that migrating colonies or trichomes experience in the nutricline or that are encountered transiently after deep mixing events. The potential significance of *Trichodesmium* assimilating fixed N is indicated by a modelling study by McGillicuddy [33] which concluded that to obtain realistic simulations of biomass and export production *Trichodesmium* populations in the North Atlantic must utilise fixed N. Specifically, this study indicated that 15–20% of the N quota of *Trichodesmium* could be due to uptake of $NO_3^-$ and $NH_4^+$. Furthermore, although uptake of $NO_3^-$, $NH_4^+$ or DON will decrease $N_2$ fixation rates in the short-term, as these N-sources are depleted over longer time periods, the
increase in *Trichodesmium* biomass may lead to increased N$_2$ fixation and greater competition for other nutrients including Fe and P.

**Supporting information**

**S1 Fig.** The relative fluorescence excitation spectra of *T. erythraeum* IMS101 (Bold solid line) and the relative emission spectra of the Iso Light 400 LED (white) block used for O$_2$ evolution incubations (Solid line), FRRf LED (blue) used for the saturating flashlets (Long-Dashed line), FastAct LED (white) used for the actinic light source (Short-dashed line) and the culturing LED (white) (Dotted line). (A) The fluorescence excitation was measured on a 2 mL concentrated sample treated with 20 μM DCMU (final concentration) [71]. *Trichodesmium* cells were acclimated to 150 μmol photons m$^{-2}$ s$^{-1}$ on a 14:10 light:dark cycle, 26 °C and ambient CO$_2$. The sample was measured using a FluorWin fluorometer scanning between 400 to 715 nm at a 1 nm resolution, with the monochromator on the detector set to 730 nm emission [72]. Spectral correction factors were calculated using the FastPro8. (B) An example of an *in vivo* light absorption spectra of *T. erythraeum* IMS101 when spectrally corrected to the Culture, MIMS or FRRf LED spectra. (TIF)

**S2 Fig.** Reconstructed light absorption spectra of eighteen key photosynthetic pigments present within *T. erythraeum* IMS101. (A) The light absorption spectra of chlorophyll a (Chla), photoprotectant carotenoid (PPC), phycoerythrin (PE), plastocyanin (PC) and alloplastocyanin (APC) pigments. (B) The light absorption spectra of phycourobilin (PUB) pigments. Each pigment spectra was normalised to the maximum peak ($\lambda = 400–700$ nm). (TIF)

**S3 Fig.** Inorganic carbon chemistry (Ci) of *T. erythraeum* IMS101 cultures, measured at 2-hour intervals over the light period. The pH and TCO$_2$ was measured directly, while the pCO$_2$ concentrations were calculated via CO2SYS using the same constants as described in Boatman et al. [45] and S1 File. (TIF)

**S4 Fig.** Chla-specific light response curves for gross O$_2$ evolution, O$_2$ consumption, net photosynthesis ($n = 3$) (A-C) and the relationship between gross and net O$_2$ evolution (D-F) for *T. erythraeum* IMS101. Cultures were acclimated to three N-sources (N$_2$, NH$_4^+$ and NO$_3^-$), at a target CO$_2$ concentration (380 μatm), saturating light intensity (400 μmol photons m$^{-2}$ s$^{-1}$) and optimal temperature (26 °C). (TIF)

**S5 Fig.** Percentage of the modelled *in vivo* light absorption ($a_{mod}$) associated to each photosynthetic pigment ($\lambda = 400–700$) for *T. erythraeum* IMS101. Cultures were acclimated to three N-sources (N$_2$, NH$_4^+$ and NO$_3^-$), at a target CO$_2$ concentration (380 μatm), saturating light intensity (400 μmol photons m$^{-2}$ s$^{-1}$) and optimal temperature (26 °C). Pigments include chlorophyll a (Chla), photoprotectant carotenoid (PPC), phycourobilins (PUB1, PUB2, PUBx, PUB4, PUB5a, PUBb, PUB5d, PUB5g and PUB5j), phycoerythrin (PE1, PE2a, PE2b and PE3b), alloplastocyanin (APC) and plastocyanin (PC1 and PC2). (TIF)

**S6 Fig.** Chla and C-specific light response curves for gross O$_2$ evolution, O$_2$ consumption, net photosynthesis ($n = 3$) (A-C) and the relationship between gross and net O$_2$ evolution (D-F) for *T. erythraeum* IMS101. Cultures were acclimated to N$_2$-only, at a target CO$_2$...
concentration (380 μatm), saturating light intensity (400 μmol photons m⁻² s⁻¹) and optimal temperature (26 °C).

(TIF)

S7 Fig. Chla and C-specific light response curves for gross O₂ evolution, O₂ consumption, net photosynthesis (n = 3) (A-C) and the relationship between gross and net O₂ evolution (D-F) for T. erythraeum IMS101. Cultures were acclimated to a replete NH₄⁺ concentration, at a target CO₂ concentration (380 μatm), saturating light intensity (400 μmol photons m⁻² s⁻¹) and optimal temperature (26 °C).

(TIF)

S8 Fig. Chla and C-specific light response curves for gross O₂ evolution, O₂ consumption, net photosynthesis (n = 3) (A-C) and the relationship between gross and net O₂ evolution (D-F) for T. erythraeum IMS101. Cultures were acclimated to a replete NO₃⁻ concentration, at a target CO₂ concentration (380 μatm), saturating light intensity (400 μmol photons m⁻² s⁻¹) and optimal temperature (26 °C).

(TIF)

S1 Table. Physiological parameters (± S.E.) of the Chla-specific light-response curves for gross and net photosynthetic O₂ evolution of T. erythraeum IMS101 (n = 3). Abbreviations; E₀ₘ Chl, the Chl-a-specific maximum gross O₂ evolution rate; Pₘ Chl, the Chl-a-specific maximum net O₂ evolution rate; α₉ Chl and αₙ Chl are the Chl-a-specific initial slopes of the light response curve for net and gross photosynthesis; Rₐ Chl, the Chl-a-specific dark respiration rate. The r² values of all curve fits were > 0.982. Letters in parenthesis 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].

(PDF)

S2 Table. Physiological parameters (± S.E.) of the fluorescence light-response curves (FLCs) of T. erythraeum IMS101 (n = 3). Abbreviations; E₉ₘ Chl, the Chl-a-specific maximum electron transport rate; α₉ ETR Chl, the Chl-a-specific initial slope of the electron transport rate light response curve; β ETR Chl, the Chl-a-specific light saturated slope of the electron transport rate light response curve. The r² values of all curve fits were > 0.977. Letters in parenthesis indicate significant differences between N-source 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].

(PDF)

S1 File. Calculation of inorganic carbon speciation.

(PDF)

S2 File. Calculation of dissolved inorganic N concentration.

(PDF)

S3 File. Measuring O₂ production and consumption.

(PDF)

S4 File. MIMS sample preparation.

(PDF)

S5 File. Elemental stoichiometry.

(PDF)
S6 File. Spectrophotometric chlorophyll $a$ analysis.
(PDF)

(PDF)

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

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