Supporting Information

Shi et al. 10.1073/pnas.1216012109

SI Materials and Methods

Cultures and Carbonate Chemistry Manipulations. The Gulf Stream seawater was enriched with chelexed 10 μM NaH$_2$PO$_4$, filter-sterilized vitamins, trace metals (8 nM Cu, 20 nM Zn, 8 nM Co, 20 nM Ni, 10 nM Se, and 18 nM Mn) buffered with 20 μM EDTA, and various concentrations of Fe. Concentrations of inorganic Fe, Fe′, were calculated according to Sunda et al. (1). Both long-term experiments with cultures growing at steady exponential rate and short-term experiments were conducted. For the long-term experiments, cultures were preacclimated to experimental conditions for more than 15 d, and only cells in mid-exponential phase were used for further experiments. Partial pressure of CO$_2$ (pCO$_2$)/pH in media was manipulated either by bubbling with humidified CO$_2$-air mixes (experiments in Table 1 and Figs. 4 and 5) or by the addition of ultrapure HCl/NaOH (experiments in Figs. 1 *A* and *B* and 2). For the short-term ^{59}Fe-uptake experiment (Fig. 1*C*), carbonate chemistry was changed by the addition of ultrapure HCl/NaOH. For the short-term (2-h) acetylene-reduction and H$_2$-evolution experiments (Fig. 3), carbonate chemistry was manipulated by both bubbling and the addition of HCl/NaOH to vary pCO$_2$ and pH independently. Carbonate chemistry for the different experiments is shown in Tables S1 and S2. pH was measured spectrophotometrically (2), and dissolved inorganic carbon (DIC) and alkalinity (Alk) were analyzed by membrane inlet mass spectrometry and Gran titration, respectively.

Chlorophyll *a* Measurement. *Trichodesmium* IMS101 was gently filtered onto 5-μm polycarbonate membrane filters (Millipore). Chlorophyll *a* (Chl *a*) was extracted by heating the sample at 70 °C for 6 min in 90% (vol/vol) methanol. After extraction the filter was removed from the vial, and cell debris was spun down via centrifugation (5 min at 20,000 × *g*) before spectrophotometric analysis according to Tandeau De Marsac and Houmard (3).

Short-Term Fe Uptake. The uptake medium was prepared from 0.22 μm filtered Gulf Stream seawater containing 50 nM Fe and 20 μM EDTA. ^{59}Fe was pre-equilibrated overnight with EDTA at a ratio of 1:1.1 before being introduced into the uptake medium. The medium pCO$_2$/pH was adjusted by the addition of ultrapure HCl/NaOH and equilibrated overnight before the uptake experiment conducted at midday. Fe-limited exponentially growing cells were filtered, rinsed three times with filtered Gulf Stream seawater, and resuspended in fresh seawater. Aliquots then were dispensed to polycarbonate bottles (Nalgene) containing the uptake medium. Intracellular Fe was analyzed by filtering the cells through a 5-μm polycarbonate membrane filter with a subsequent washing step with an oxalate-EDTA solution (4). ^{59}Fe retained on the membrane was measured via liquid scintillation counting.

^{13}C and ^{15}N Fixation. Serum vials (60 mL) were overfilled with exponentially growing cultures, and NaH^{13}CO$_3$ (Cambridge Isotope Laboratories) was added to a final isotope enrichment of 5% before the vial was sealed with Teflon-coated septa (West Pharmaceutical Services). One milliliter of 98% pure ^{15}N$_2$ gas (Cambridge Isotope Laboratories) then was injected into the inverted serum vial. After the 4-h incubation cells were collected onto 25-mm precombusted GF/F filters and stored at −80 °C. The filters were dried at 60 °C, acidified to remove inorganic C, dried at 60 °C again, and then packed in tin cups (Costech Analytical Technologies) before analysis on an elemental analyzer (Eurovector) connected to a continuous-flow isotope ratio mass spectrometer (IsoPrime; GVI) at Rutgers Institute of Marine and Coastal Sciences. Rates were corrected for pressure, salinity, and temperature on the solubility of ^{15}N$_2$ gas in seawater and the time dependency of the equilibrium of ^{15}N$_2$ gas with surrounding seawater (5).

Quantification of Total Protein and Western Blotting. Sampling was done at 0, 3.5, 7, 10.5, 14, and 16 h after the onset of light with the last time point being 2 h into the dark period. Proteins were extracted and denatured in an extraction buffer (2% SDS, 10% glycerol, and 50 mM Tris at pH 6.8; 5% β-mercaptoethanol was added after protein quantification) with heating at 95 °C for 5 min. The extract was centrifuged at 20,000 × *g* for 10 min to pellet insoluble material. Total protein in the extract was quantified using the bicinchoninic acid (BCA) assay (Pierce, Thermo Scientific). Equivalent amounts of total protein (5 μg for NifH and PsaC; 2 μg for PsbA and RbcL) were separated on a 12% poly-acrylamide gel for 25–40 min at 200 V in 1× SDS running buffer. After electrophoresis, proteins were transferred to PVDF membrane in ice-cold transfer buffer [25 mM Tris, 192 mM glycine, and 2.5% (vol/vol) methanol] for 2 h at 200 mA. The membrane was blocked with 5% milk powder in TBST buffer [Tris-buffered saline containing 0.25% (vol/vol) Tween-20, pH 7.5] for 1 h at room temperature. The blocked membrane was incubated for 1 h with primary antibodies (Agrisera: NifH, Art no. AS01 021A; PsaC, Art no. AS10 939; PsbA, Art no. AS05 084; and RbcL, Art no. AS03 037), followed by five 5-min washes with TBST buffer. The membrane then was probed with alkaline phosphatase-linked, goat anti-rabbit IgG for 1 h, followed by five washes with TBST buffer. After washing, the membrane was rinsed three times with PhoA buffer (20 mM Tris, 100 mM NaCl, and 10 mM MgCl$_2$, pH 9.5), and protein bands were visualized with NBT/BCIP (Roche, Indianapolis) for NifH, RbcL and PsbA and with chemiluminescence for PsaC. Bands were quantified by densiometry, and protein levels were calculated from standard (Agrisera) curves.

SI Cellular Nitrogenase and Fe Concentrations

I. Cellular Fe Concentration in Nitrogenase at pCO$_2$ = 380 ppm. This experiment is shown in Fig. 4*A*, low-Fe conditions.

Assumptions:

(*i*) Stoichiometry of Fe-protein dimer and MoFe-protein tetramer in nitrogenase complex: 2:1.
(*ii*) Four Fe per Fe-protein dimer and 30 Fe per MoFe-protein tetramer for a total of 38 Fe per nitrogenase complex.
(*iii*) Proteins account for 30% of dry cell mass and C for 50% of dry cell mass.

Concentration of Fe in nitrogenase complex:

$$\text{Time 0 hour: NifH} = 0.05 \text{ pmol (μg protein)}^{-1} \times 1/2$$
$$= 0.025 \text{ μmol dimer (g protein)}^{-1} \times 1/2$$
$$= 0.0125 \text{ μmol nitrogenase (g protein)}^{-1}$$
$$\times 38 \text{ Fe (nitrogenase)}^{-1}$$
$$= 0.48 \text{ μmol Fe (g protein)}^{-1}$$

Time 14 hour: NifH $= 0.30$ pmol (μg protein)$^{-1} \times 1/2$

$\qquad = 0.15$ μmol dimer (g protein)$^{-1} \times 1/2$

$\qquad = 0.075$ μmol nitrogenase (g protein)$^{-1}$

$\qquad \times 38$ Fe (nitrogenase)$^{-1}$

$\qquad = 2.9$ μmol Fe (g protein)$^{-1}$

Cellular Fe concentration in nitrogenase:

Time 0 hour: 0.48 μmol Fe (g protein)$^{-1} \times (0.3$ g protein/g cell)

$\qquad \times (2$ g cell/g C) $\times (12$ g C/mol C) $= 3.5$ μmol Fe (mol C)$^{-1}$

Time 14 hour: 2.9 μmol Fe (g protein)$^{-1} \times (0.3$ g protein/g cell)

$\qquad \times (2$ g cell/g C) $\times (12$ g C/mol C) $= 21$ μmol Fe (mol C)$^{-1}$

II. Cellular Fe Concentration in Photosystem I and Associated Proteins at pCO$_2$ = 380 ppm. This experiment is shown in Fig. 4C, low-Fe conditions.

Assumptions:

(i) Stoichiometry of Photosystem I (PSI) (= PsaC), cytochrome b$_6$f complex (cyt b$_6$f), and ferredoxin: 1:1:1 (6, 7).
(ii) Twelve Fe per PSI complex, six Fe per cyt b$_6$f, and two Fe per ferredoxin for a total of 20 Fe per PSI complex and associated proteins.

(iii) Proteins account for 30% of dry cell mass and C for 50% of dry cell mass.
Cellular Fe concentration in PSI and associated proteins: For concentrations of 0.05 and 0.02 pmol PsaC (μg protein)$^{-1}$ at times 0 and 10 h, respectively, calculations as above yield 7 and 3 μmol Fe (mol C)$^{-1}$.

III. Cellular Concentration of Nitrogenase Complex at pCO$_2$ = 380 ppm. This experiment is shown in Fig. 4B, high-Fe conditions.

Assumptions:

(i) 2:1 stoichiometry of Fe-protein dimer and MoFe-protein tetramer in nitrogenase complex.
(ii) Molecular mass of 60 kDa for the protein dimer and 240 kDa for the MoFe-protein tetramer for a total of 360 kDa per nitrogenase complex.

A final NifH concentration of 0.8 pmol NifH (μg protein)$^{-1}$ yields:

0.8 pmol NifH (μg protein)$^{-1} \times 1/2$

$\qquad = 0.4$ μmol dimer (g protein)$^{-1} \times 1/2$

$\qquad = 0.2$ μmol nitrogenase (g protein)$^{-1}$

0.2 μmol nitrogenase (g protein)$^{-1} \times 3.6$

$\qquad \times 10^5$ g nitrogenase/mol $= 72$ mg nitrogenase (g protein)$^{-1}$

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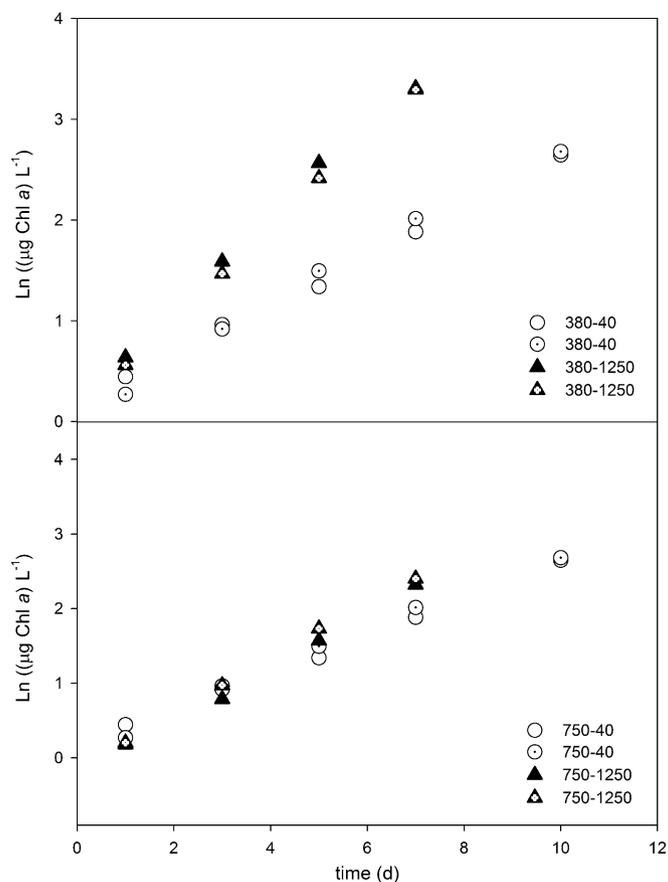
Fig. S1. Growth curves of *Trichodesmium* at different *p*CO$_2$ and Fe concentrations in the experiment shown in Fig. 2.

Fig. S2. Growth curves of *Trichodesmium* at different pCO_2 and Fe concentrations in the experiment shown in Table 1. Numbers following legend symbols are ppm pCO_2 and pM Fe′, respectively.

Table S1. Parameters of the seawater carbonate system from the exponentially growing cultures of Table 1

Parameters (unit)	pH [total scale] (mol kg⁻¹ sol) Measured	Alkalinity (µmol kg⁻¹) Measured	DIC (µmol kg⁻¹)		pCO_2 (ppm) Calculated
			Measured	Calculated	
380 ppm	8.08	2,470	2,093	2,119	381
750 ppm	7.88	2,768	2,494	2,515	748

Salinity 35 psu; temperature 27 °C. The medium carbonate chemistry was controlled by bubbling CO_2-enriched air. Calculations of pCO_2 were based on measurements of pH and Alk of the medium using the carbonic acid dissociation constants of Mehrbach et al. (1) that were refitted in different functional forms by Dickson and Millero (2).

1. Mehrbach C, Culberso CH, Hawley JE, Pytkowic RM (1973) Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography* 18(6):897–907.
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Table S2. Parameters of the seawater carbonate system from the exponentially growing cultures of Figs. 1 *A* and *B* and 2 and the short-term Fe uptake experiment of Fig. 1*C*

Parameters (unit)	pH [total scale] (mol kg^{-1}-sol) Measured	DIC (μmol kg^{-1}) Measured	Alk (μmol kg^{-1})		pCO$_2$ (ppm) Calculated
			Measured	Calculated	
pH 7.80	7.80	2,060	2,260	2,240	747
pH 8.10	8.10	2,060	2,450	2,418	352
pH 8.35	8.35	2,060	2,680	2,637	181

Salinity 35 psu; temperature 27 °C. The medium carbonate chemistry was controlled by acid/base addition. Calculations of pCO$_2$ were based on measurements of pH and DIC.