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Supporting Information for

Effects of CO_2 on the Nitrogen Isotopic Composition of Marine Diazotrophic Cyanobacteria

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Supporting Text

We proposed a quasi-steady-state cellular model, adapted from Silverman et al. (2019), to understand the organismal isotope effects of nitrogen fixation for *Trichodesmium* and *Crocosphaera* in this study (Fig. S4).

In brief, the rate of N_2 fixation by nitrogenase in the cells (Φ_{fix}) balances with the difference between the cellular N_2 inflow (Φ_{in}) and outflow (Φ_{out}). The Φ_{in} and Φ_{out} are proportional to the extracellular ($C_{N2\cdot ext}$) and intracellular ($C_{N2\cdot int}$) concentrations of N_2, respectively. Thus, we obtain the following set of equations:

$$\Phi_{fix} = \Phi_{in} - \Phi_{out}$$
$$\Phi_{in} = K_1 \times C_{N2\cdot ext}$$
$$\Phi_{out} = K_2 \times C_{N2\cdot int} \quad \text{(S1)}$$

where K_1 and K_2 are the extracellularly and intracellularly diffusion rate constants of N_2, respectively (Laws et al., 1995).

Furthermore, the corresponding isotope balances are formulated as follows:

$$\Phi_{fix} \times \delta_{\Phi_{fix}} = \Phi_{in} \times \delta_{\Phi_{in}} - \Phi_{out} \times \delta_{\Phi_{out}}$$
$$\delta_{N_{Biomass}} = \delta_{\Phi_{fix}} = \delta_{N2_{int}} + \varepsilon_{fix}$$
$$\delta_{\Phi_{out}} = \delta_{N2_{int}} + \varepsilon_{diff}$$
$$\delta_{\Phi_{in}} = \delta_{N2_{gas}} + \varepsilon_{\frac{aq}{g}} + \varepsilon_{diff} \quad \text{(S2)}$$

where the isotopic composition of the respective step is reported as δ_{Φ_X}; $\delta_{N_{Biomass}}$, $\delta_{N2_{int}}$ and $\delta_{N2_{gas}}$ are the isotopic composition of the organic matter of diazotroph, the intracellular N_2, and the atmospheric N_2, respectively. $\varepsilon_{aq/g}$ is the equilibrium fractionation factor between the aqueous and gaseous phase of N_2; ε_{diff} is the kinetic isotope fractionation factor of N_2 diffusion through the cell membrane; and ε_{fix} is the intrinsic kinetic isotope fractionation factor of nitrogenase during the nitrogen fixation reaction.

Combining Eqs. S1 and S2, the following expression for the overall organismal fractionation factor between biomass and N_2 gas ($\varepsilon_{N.Biomass/N2.gas}$, which is defined here as $\delta^{15}N_{Biomass} - \delta^{15}N_{N2.gas}$, with $\delta^{15}N_{N2.gas} = 0‰$) can be derived:

$$\varepsilon_{\frac{N_{Biomass}}{N_{2.gas}}} = \delta_{N_{Biomass}} - \delta_{N_{2.gas}} = \varepsilon_{\frac{aq}{g}} + \varepsilon_{diff} + \left(\varepsilon_{fix} - \varepsilon_{diff}\right) \times \left(1 - \frac{\Phi_{fix}}{K_1 \times C_{N2\cdot ext}}\right) \quad \text{(S3)}$$

Given that the ε_{diff} is assumed to be negligible (~0‰) (Silverman et al. 2019), Eq. S3 can be simplified to the following final equation:

$$\varepsilon_{\frac{N_{Biomass}}{N_{2.gas}}} = \delta_{N_{Biomass}} = \varepsilon_{\frac{aq}{g}} + \varepsilon_{fix} - \frac{\varepsilon_{fix}}{K_1 \times C_{N2\cdot ext}} \times \Phi_{fix} \quad \text{(S4)}$$

where $\varepsilon_{aq/g}$, K_1, and $C_{N2.ext}$ were constant in the experimental system.

As indicated by Eq. S3 and S4, if the intrinsic kinetic isotope fractionation factor of nitrogenase (ε_{fix}) is assumed to be stable in response to CO_2 perturbations, the overall $\varepsilon_{N.Biomass/N2.gas}$ only depends on the variable Φ_{fix}, that is the rate of N_2 fixation by nitrogenase in the cells, which can be constrained by measured nitrogen fixation rate in the experiments. Although the value of ε_{fix} is unknown for different diazotroph species, the positive correlations and negative intercepts (Figures 4 and S3) suggested that both ε_{fix} of *Trichodesmium* and

Crocosphaera are negative values, which were analogous to the estimated value of *A. cylindrica* (Silverman et al., 2019).

In addition, as the N_2 fixation rates of both *Trichodesmium* and *Crocosphaera* were found to be significantly linearly related to the growth rate (μ) and nitrogenase activity efficiency (E) (Fig. S5), the Φ_{fix} in Eq. S4 could further be replaced by μ or E:

$$\varepsilon_{\frac{N_{Biomass}}{N_{2.gas}}} = \delta_{N_{Biomass}} = -\frac{A \times \varepsilon_{fix}}{K_1 \times C_{N2 \cdot ext}} \times \mu + \varepsilon_{\frac{aq}{g}} + \left(1 - \frac{B}{K_1 \times C_{N2 \cdot ext}}\right) \varepsilon_{fix} \quad \text{(S5)}$$

$$\varepsilon_{\frac{N_{Biomass}}{N_{2.gas}}} = \delta_{N_{Biomass}} = -\frac{C \times \varepsilon_{fix}}{K_1 \times C_{N2 \cdot ext}} \times E + \varepsilon_{\frac{aq}{g}} + \left(1 - \frac{D}{K_1 \times C_{N2 \cdot ext}}\right) \varepsilon_{fix} \quad \text{(S6)}$$

where $\varepsilon_{aq/g}$, K_1 and $C_{N2 \cdot ext}$ are constants in our culturing experiments; A (or C) and B (or D) are the slope and intercept of the regression line between Φ_{fix} and μ (or E) (Figure S5). Thus, we proposed that the $\varepsilon_{N.Biomass/N2.gas}$ is linearly correlated with the growth rate (Eq S5) or nitrogenase activity efficiency (Eq S6) as found in Fig. S3 and Fig. 4. It should be noted that in the above discussion, ε_{fix} is assumed to be stable following the approach in Silverman et al. (2019). However, of note is that in our case, the intrinsic kinetic isotope fractionation factor of nitrogenase (ε_{fix}) may potentially be altered as the nitrogenase efficiency changed with varied $p$$CO_2$, though the varied range is unknown and mechanisms remained unclear.

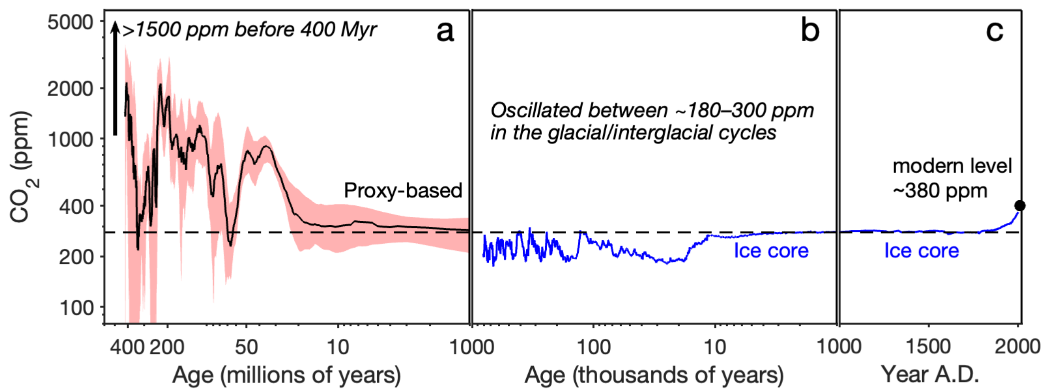

Figure S1. Atmospheric CO_2 fluctuations over the last 420 million years. Proxy-based atmospheric CO_2 on a log timescale and associated uncertainty envelope (red shadow) (Foster et al., 2017). (b and c) Ice core atmospheric CO_2 from Bereiter et al. (2015). The dashed lines in (a), (b) and (c) are pre-industrial CO_2 (278 ppm). Black dot in (c) is the modern CO_2 level (~380 ppm).

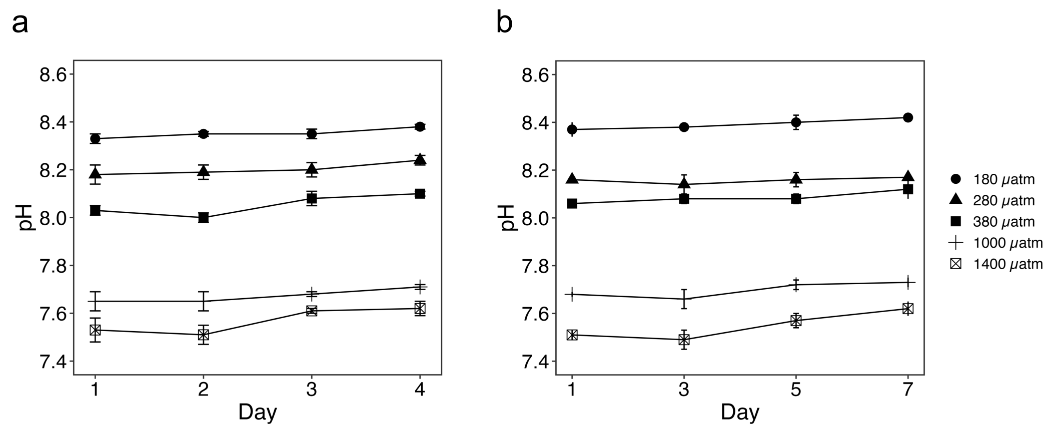

Figure S2. pH values throughout the experimental period. (a) *T. erythraeum* and (b) *C. watsonii*. Symbols represent the target CO_2 levels manipulated. The values are presented as mean ± SD (n = 3).

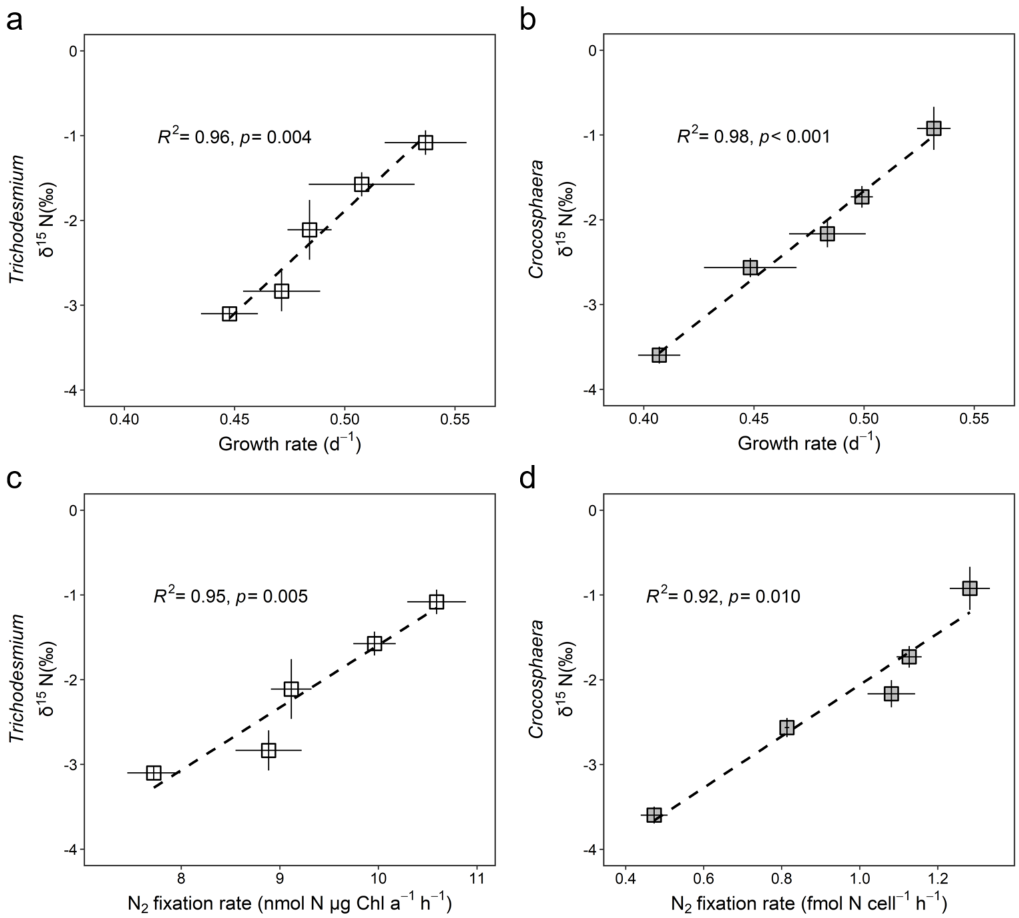

Figure S3. Relationships of biomass δ^{15}N against growth and N_2 fixation rates. Growth rate of *T. erythraeum* (a) and *C. watsonii* (b), N_2 fixation rate of *T. erythraeum* (c) and *C. watsonii* (d). Note that N_2 fixation rates of *T. erythraeum* and *C. watsonii* are measured in different units. The values are presented as mean ± SD (n = 3).

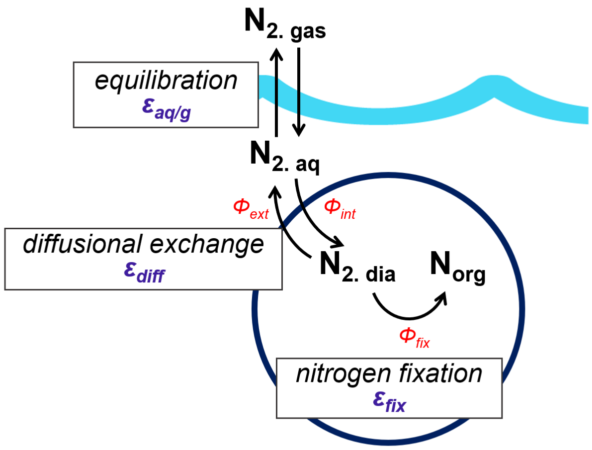

Figure S4. A quasi-steady-state cellular model of nitrogen isotope fractionation during nitrogen fixation for *Trichodesmium* and *Crocosphaera*.

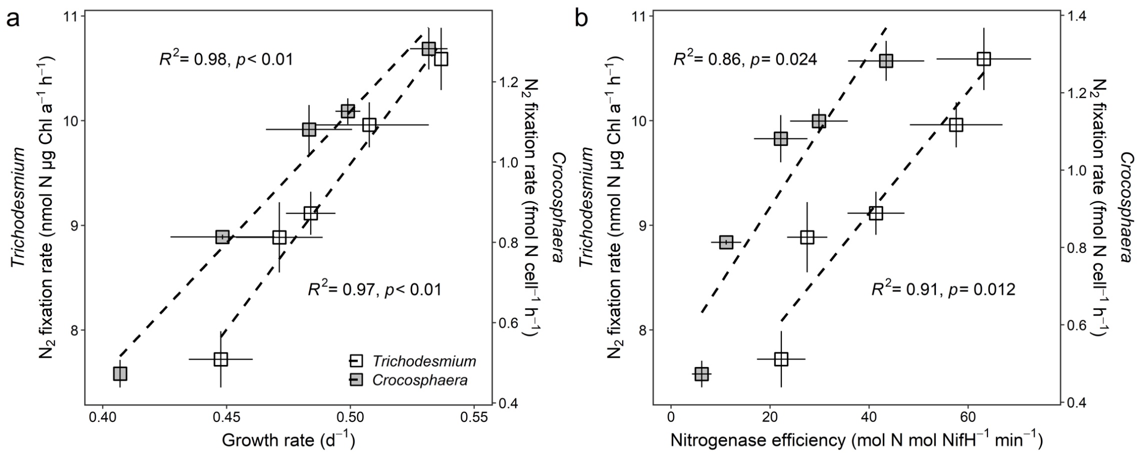

Figure S5. Relationships between nitrogen fixation rates and growth rate (a) or nitrogenase activity efficiency (b). Open squares, *T. erythraeum*; Grey squares, *C. watsonii*. Data shows the mean ± standard deviation of n = 3 biological replicates.

Table S1. Summary of data reported in this study. Data includes carbonate chemistry (pCO_2, µatm; pH; DIC, µmol kg^{-1}), biomass $\delta^{15}N$ (‰), growth rate (d^{-1}), N_2 fixation rate (*T. erythraeum*, nmol N µg Chla^{-1} h^{-1}; *C. watsonii*, fmol N cell^{-1} h^{-1}), *NifH* protein abundance (pmol µg protein^{-1}), and nitrogenase efficiency (mol N mol *NifH*$^{-1}$ min^{-1}). Values are presented as mean ± SD (n = 3).

Species	pCO_2	pH	DIC	Biomass $\delta^{15}N$	Growth rate	N_2 fixation rate	*NifH* abundance	Nitrogenase efficiency
T. erythraeum	174 ± 12	8.33 ± 0.02	1891 ± 19	-2.1 ± 0.4	0.48 ± 0.01	9.12 ± 0.21	0.44 ± 0.08	41.4 ± 5.8
	262 ± 30	8.18 ± 0.04	1889 ± 16	-1.6 ± 0.1	0.51 ± 0.02	9.96 ± 0.21	0.35 ± 0.05	57.6 ± 9.4
	373 ± 11	8.03 ± 0.02	1899 ± 11	-1.1 ± 0.1	0.54 ± 0.02	10.59 ± 0.30	0.34 ± 0.06	63.2 ± 9.5
	956 ± 22	7.65 ± 0.04	1873 ± 7	-2.8 ± 0.2	0.47 ± 0.02	8.89 ± 0.34	0.65 ± 0.10	27.5 ± 4.0
	1355 ± 21	7.53 ± 0.05	1869 ± 18	-3.1 ± 0.1	0.45 ± 0.01	7.72 ± 0.27	0.71 ± 0.17	22.2 ± 4.9
C. watsonii	157 ± 13	8.37 ± 0.03	1884 ± 8	-2.2 ± 0.2	0.48 ± 0.02	1.08 ± 0.06	0.78 ± 0.17	22.1 ± 5.4
	234 ± 12	8.16 ± 0.01	1890 ± 5	-1.7 ± 0.1	0.50 ± 0.01	1.13 ± 0.03	0.60 ± 0.11	29.9 ± 5.8
	374 ± 6	8.06 ± 0.01	1892 ± 7	-0.9 ± 0.3	0.53 ± 0.01	1.28 ± 0.05	0.47 ± 0.11	43.4 ± 7.7
	969 ± 22	7.68 ± 0.01	1872 ± 3	-2.6 ± 0.1	0.45 ± 0.02	0.81 ± 0.01	1.18 ± 0.28	11.1 ± 3.0
	1313 ± 16	7.51 ± 0.02	1867 ± 10	-3.6 ± 0.1	0.41 ± 0.01	0.47 ± 0.03	1.24 ± 0.29	6.2 ± 2.0

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