

Ocean warming alleviates iron limitation of marine nitrogen fixation

Hai-Bo Jiang¹, Fei-Xue Fu², Sara Rivero-Calle², Naomi M. Levine², Sergio A. Sañudo-Wilhelmy², Ping-Ping Qu², Xin-Wei Wang³, Paulina Pinedo-Gonzalez², Zhu Zhu⁴ and David A. Hutchins^{2*}

The cyanobacterium *Trichodesmium* fixes as much as half of the nitrogen (N₂) that supports tropical open-ocean biomes, but its growth is frequently limited by iron (Fe) availability^{1,2}. How future ocean warming may interact with this globally widespread Fe limitation of *Trichodesmium* N₂ fixation is unclear³. Here, we show that the optimum growth temperature of Fe-limited *Trichodesmium* is ~5°C higher than for Fe-replete cells, which results in large increases in growth and N₂ fixation under the projected warmer Fe-deplete sea surface conditions. Concurrently, the cellular Fe content decreases as temperature rises. Together, these two trends result in thermally driven increases of ~470% in Fe-limited cellular iron use efficiencies (IUEs), defined as the molar quantity of N₂ fixed by *Trichodesmium* per unit time per mole of cellular Fe (mol N₂ fixed h⁻¹ mol Fe⁻¹), which enables *Trichodesmium* to much more efficiently leverage the scarce available Fe supplies to support N₂ fixation. Modelling these results in the context of the IPCC representative concentration pathway (RCP) 8.5 global warming scenario⁴ predicts that IUEs of N₂ fixers could increase by ~76% by 2100, and largely alleviate the prevailing Fe limitation across broad expanses of the tropical Pacific and Indian Oceans. Thermally enhanced cyanobacterial IUEs could increase future global marine N₂ fixation by ~22% over the next century, and thus profoundly alter the biology and biogeochemistry of open-ocean ecosystems.

Atmospheric N₂ fixed by marine cyanobacteria such as the colonial genus *Trichodesmium* largely furnishes the new N₂ that supports food webs across the vast subtropical central gyres of the oceans. Iron limitation of *Trichodesmium* growth is, however, common and widespread, imposing a major constraint on new nitrogen supplies to the marine biota from N₂ fixation^{2,3}. This prevailing limitation by Fe has often been neglected when predicting future *Trichodesmium* physiological and biogeographical responses to ocean warming^{3,5,6}, which have implicitly assumed that the effects of temperature and nutrient limitation are simply additive. Interactive effects seem likely, however, since warming directly stimulates N₂ fixation⁶, respiration⁷ and photosynthetic CO₂ fixation⁸, which all require large investments of cellular Fe for enzymatic catalysis or electron transport⁹. Some eukaryotic phytoplankton can vary their biological Fe demand and IUE (the growth or production rate per unit of cellular Fe) to acclimate to environmental changes^{3,9,10}, but potential interactions between Fe limitation and warming in N₂-fixing cyanobacteria have not been explored.

Our laboratory experiments used Fe-limited and Fe-replete *Trichodesmium erythraeum* cultures (strain IMS101) grown using semicontinuous culturing methods (Supplementary Methods) at

four temperatures (22, 27, 32 and 35°C) that span the thermal range across which this species is typically found^{2,6}. Specific growth rates of *T. erythraeum* in Fe-replete cultures were higher than those in Fe-deficient cultures at each experimental temperature ($P < 0.05$), which verifies that low Fe concentrations limited growth (Fig. 1). The optimal growth temperature within our experimental thermal range of 22–32°C was 27°C for Fe-replete cultures, but this increased to 32°C for Fe-limited cultures ($P < 0.05$ (Fig. 1)). At 27°C, *T. erythraeum* growth rates in the Fe-limited treatment were ~83% of those in the Fe-replete treatment ($P > 0.05$ (Fig. 1)), which suggests warming nearly alleviated Fe limitation at this temperature. In addition, a 5°C warming from 22°C to 27°C increased the growth rate of Fe-limited cells to the same extent as fully relieving Fe limitation at 22°C (Fig. 1). The maximum thermal limits of Fe-limited cells are the same as those reported here and elsewhere^{5,6} for Fe-replete *Trichodesmium* (Fig. 1). Cells in neither Fe treatment survived at 35°C, so in contrast to the optimal growth range, the upper thermal limit of *T. erythraeum* growth was unaffected by Fe limitation (Fig. 1).

As with growth rates, the temperatures at which the maximum nitrogen (N)-specific N₂-fixation rates and carbon (C)-specific CO₂-fixation rates were observed increased from 27°C under Fe-replete conditions to 32°C in Fe-limited treatments (Fig. 2a,b). As expected, N₂-fixation rates in Fe-deficient cultures were much lower than those in Fe-replete cultures (~11, ~9 and ~5-fold lower at 22, 27 and 32°C, respectively) ($P < 0.05$). Nevertheless, warming the Fe-limited *T. erythraeum* cell lines from 22°C to 32°C linearly increased the N₂-fixation rates by 308% (Fig. 2a). CO₂-fixation rates exhibited similar trends to N₂ fixation, but were somewhat less sensitive to Fe deficiency (Fig. 2b). Warming alone thus increases the N₂- and CO₂-fixation capabilities of *T. erythraeum* even under Fe-limiting conditions, consistent with the growth rate results.

Significant changes were also seen in the cellular major elemental ratios of *T. erythraeum* in response to Fe deficiency and/or increasing temperature. At 22°C, there were no significant differences between the C:N ratios in the two Fe treatments ($P > 0.05$ (Supplementary Table 1)). At 27°C and 32°C, however, Fe-limited C:N ratios were 48% and 34% higher than under the Fe-replete conditions, respectively ($P < 0.05$ (Supplementary Table 1)). The cellular phosphorus (P) content decreased even more under Fe-deplete conditions, as evidenced by higher C:P ratios (177–378%) and N:P ratios (133–370%) compared to Fe-replete conditions ($P < 0.05$ (Supplementary Table 1)). Higher temperature also reduced the cellular P content under Fe-replete conditions (Supplementary Table 1). These major changes in *Trichodesmium* elemental uptake rates and ratios as a function of Fe availability and temperature point to

¹Central China Normal University, Wuhan, Hubei, China. ²University of Southern California, Los Angeles, CA, USA. ³Xiamen University, Xiamen, Fujian, China. ⁴Shanghai Jiao Tong University, Minhang District, Shanghai, China. *e-mail: dahutch@usc.edu

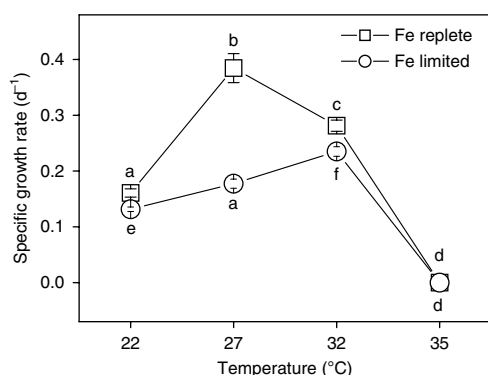


Fig. 1 | *Trichodesmium* growth responses to Fe availability and warming interactions. C-specific growth rates of *T. erythraeum* IMS101 in Fe-replete and Fe-limited cultures at four temperatures (22, 27, 32 and 35 °C). Values represent the means and error bars for the standard deviations of biological replicates ($n=3-4$); bars marked with different letters are significantly different from each other ($P<0.05$).

potentially large excursions from the current biological stoichiometry in the future ocean.

Radiotracer-derived Fe uptake rates of Fe-replete *T. erythraeum* were much higher than those in Fe-limited cultures ($P<0.05$), as expected from the 25-fold greater total dissolved Fe concentrations in the replete cultures (Supplementary Methods). As for the other rate measurements, the thermal optimum for Fe uptake rate increased from 27 °C in Fe-replete cultures to 32 °C under Fe-limited conditions (Supplementary Fig. 1). In contrast to Fe uptake rates, however, cellular Fe content measured as Fe:C ratios ($\mu\text{mol}:\text{mol}$) was lower at 32 °C than at either 27 °C or 22 °C in both Fe-replete and Fe-limited cells ($P<0.05$ (Supplementary Table 1)).

The combination of minimum cellular Fe content and maximum N_2 and CO_2 -fixation rates at the warmest temperature of 32 °C (Fig. 2a,b and Supplementary Table 1) led to large increases in

IUEs, defined as the molar quantity of N_2 or CO_2 fixed by *Trichodesmium* per unit time per mol of cellular Fe (refs ^{9,11}). These IUEs quantify how efficiently *Trichodesmium* uses this key micronutrient to support growth and N_2 fixation under the three temperature treatments. In Fe-replete treatments, IUEs for both N_2 fixation (Fig. 2c) and CO_2 fixation (Fig. 2d) increased stepwise from 22 to 32 °C by 293 and 318%, respectively ($P<0.05$). Fe-limited IUEs were lower than those of Fe-replete cultures, and were not significantly different from 22 to 27 °C ($P>0.05$). However, relative to the two lower temperatures, at the experimental Fe-limited optimal growth temperature of 32 °C, IUEs were drastically elevated by 464–473% (N_2 fixation (Fig. 2c)) and 333–369% (CO_2 fixation (Fig. 2d)) ($P<0.05$). The IUE for CO_2 fixation in the Fe-limited cultures at 32 °C was not significantly different from Fe-replete IUE values at 27 °C ($P>0.05$ (Fig. 2d)).

We also similarly calculated the phosphorus use efficiencies (PUEs), as P often limits *Trichodesmium* in areas with higher Fe inputs, such as the North Atlantic^{2,12}, and P and Fe colimitation is also probably commonplace¹³. As for IUEs, PUEs for N_2 fixation and CO_2 fixation increased stepwise with temperature from 22 °C to 32 °C in both Fe-replete cultures (340–370%) and Fe-limited cultures (240–300%) (Supplementary Fig. 2) ($P<0.05$). As with Fe, *Trichodesmium* also required less P to support growth and N_2 fixation as temperature increased. Future vertically advected P supplies to N_2 fixers are expected to decline due to increased ocean stratification³. Like Fe and IUE, a higher PUE in a warmer ocean may have significant biogeochemical consequences by partly mitigating intensified P limitation. Decreased cellular P content has been previously observed in eukaryotic phytoplankton with warming^{10,14}, and attributed to increased protein synthesis rates but a decreased abundance of P-rich ribosomes¹⁵. A similar response may underlie the thermal effect on IUE observed here, in that *Trichodesmium* cells may require less catalytic Fe as the turnover rates of Fe-containing enzymes like nitrogenase increase with warming.

It has typically been assumed that changes in nutrient limitation and temperature have an additive effect on growth rates^{16,17}, but Fe-limited *Trichodesmium* grow and fix N_2 much faster at 32 °C

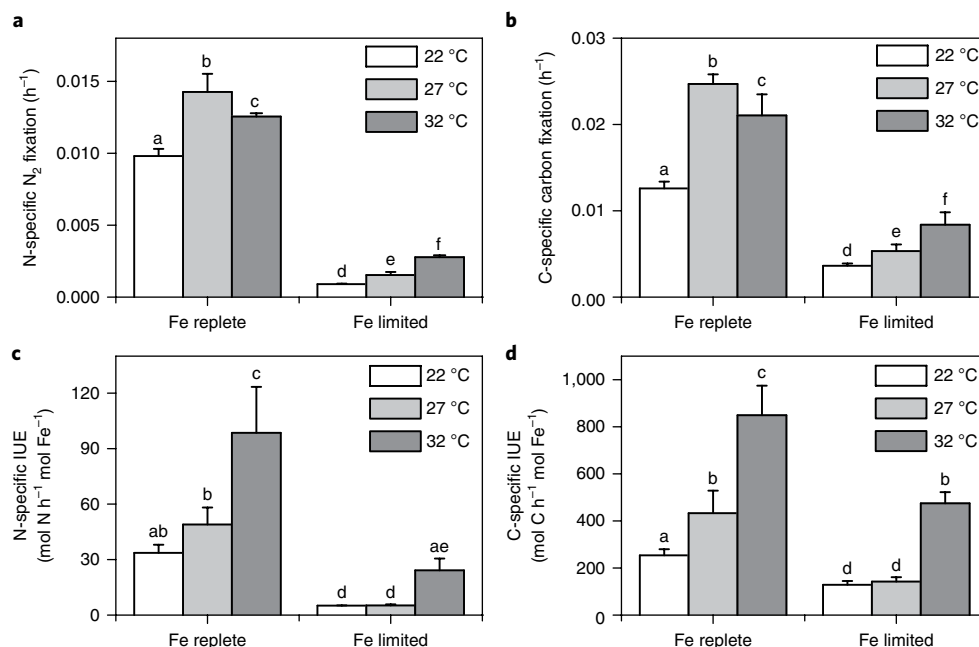


Fig. 2 | Responses of *Trichodesmium* N_2 fixation, CO_2 fixation and IUEs to Fe availability and warming. a–d, N-specific N_2 -fixation rates (a) and C-specific CO_2 -fixation rates (b), and N-specific (c) and C-specific (d) IUEs of Fe-replete and Fe-limited cultures of *T. erythraeum* IMS101 grown at three temperatures (22, 27 and 32 °C). Values represent the means and error bars are the standard deviations of biological replicates ($n=3$); bars marked with different letters are significantly different from each other ($P<0.05$).

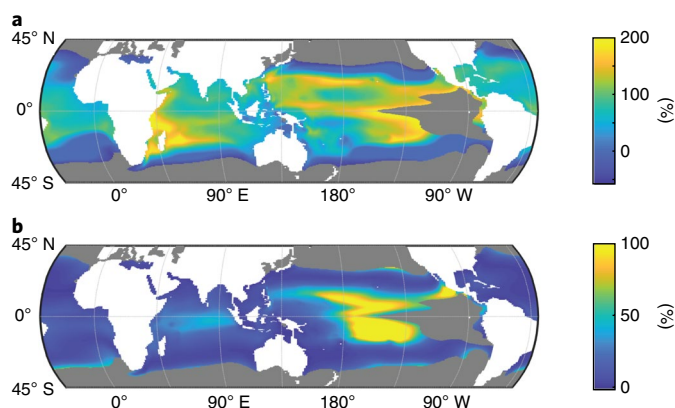


Fig. 3 | Modelled consequences of Fe and warming interactions for future changes in global IUE and N_2 fixation. **a**, Change in *Trichodesmium* IUE due to warming under the IPCC RCP 8.5 scenario for year 2100 relative to 2010. **b**, Change in future N_2 -fixation rates in year 2100 when comparing projections using the interactive model (global warming and variable IUE) versus the additive model (global warming and constant IUE), and assuming the IUE in all the N_2 fixers responds to warming in a manner similar to *Trichodesmium*. Grey regions mask areas in which nitrate concentrations are $>5 \mu\text{M}$ or the thermally determined growth rates are $<0.01 \text{ d}^{-1}$, and therefore assumed to have negligible N_2 fixation.

than would be predicted based on the simple sum of nutrient limitation and warming effects (Fig. 1 and Supplementary Fig. 6). Thus, interactions between Fe availability and warming are non-linear, which leads to synergistic effects of IUE on physiological rates. To examine the global biogeochemical implications of this interactive effect, we used our experimental results to parameterize growth rates, N_2 -fixation rates and IUE as a function of temperature and Fe concentrations to compare the two contrasting hypotheses of an additive versus an interactive synergistic response (Supplementary Fig. 6). We then estimated the consequences of these temperature-induced IUE changes for future global N_2 -fixation changes using the National Center for Atmospheric Research Global Climate Model¹⁸ (Supplementary Methods).

Projected temperature and Fe concentration changes under the IPCC RCP 8.5 'business as usual' scenario ($\sim 3.7^\circ\text{C}$ mean global atmospheric temperature increase)⁴ yield an average 76.1% global increase in *Trichodesmium* IUE by 2100 (Fig. 3a). Although the highest absolute IUEs were found in several warm oligotrophic regions where Fe concentrations are relatively elevated (for example, the North Atlantic, western Pacific and Indian Oceans (Supplementary Fig. 15), by far the largest percentage increases were observed in Fe-limited regions (Fig. 3a). The central Pacific Ocean basin is among the mostly severely Fe-limited areas in the ocean¹⁹, and N_2 -fixation rates are consequently extremely low^{2,20}. Our model projects that, by 2100, *Trichodesmium* IUE will increase across much of this region by up to $\sim 230\%$ (Fig. 3a).

Under present-day conditions, the interactive IUE/warming response model shows an average 11.0% greater N_2 -fixation rate compared to predicted rates using the additive impact of temperature and Fe stress (Supplementary Fig. 14a,b), assuming that IUE in all N_2 fixers responds to warming in a manner similar to *Trichodesmium*. By 2100 under the IPCC RCP 8.5 scenario, this synergistic enhancement doubles, with an average 21.4% difference in N_2 -fixation rates between the interactive and additive predictions (Fig. 3b and Supplementary Fig. 14c,d). The effects of warming on IUE are particularly pronounced across the vast, highly Fe-limited Pacific basin (Fig. 3a), where N_2 -fixation rates increase by up to 814% in 2100 relative to 2010 (Fig. 3b and Supplementary Fig. 14f). N_2 fixation also increases by up to $\sim 40\%$ in the equatorial

Indian Ocean (Fig. 3b). In contrast, in the western Equatorial Pacific and parts of the Indian Ocean, where warming will exceed optimal growth temperatures⁵ (Fig. 1 and Supplementary Fig. 14e,f), we observe a 39–57% decrease in N_2 -fixation rates. Given both the positive interactive IUE response in some regions and the negative effects of excessive warming in others, we predict that the net integrated global marine N_2 -fixation rates will increase by an average of 21.5% by 2100 (range -51.9 to $+800.8\%$ (Supplementary Fig. 14e,f). A thermal IUE effect of this magnitude would increase the estimates of N_2 fixed annually by *Trichodesmium* alone from the current $60\text{--}80 \text{ Tg yr}^{-1}$ (ref. ²¹) to $73\text{--}97 \text{ Tg yr}^{-1}$ by the year 2100, in addition to any similar effects on the global contributions of other N_2 -fixing taxa.

These thermally mediated increases in IUE will probably also interact with other aspects of a complex changing ocean environment. For instance, N_2 -fixation rates of Fe-replete *Trichodesmium* and other marine cyanobacteria increase dramatically under elevated CO_2 concentrations^{22,23}, but this CO_2 stimulation effect is negated by a severe Fe limitation^{13,24}. Thus, if thermally elevated IUE largely relieves the Fe limitation of N_2 -fixing cyanobacteria in regions like the tropical Pacific, a concurrently rising CO_2 is likely to drive another incremental increase in global N_2 fixation. Current models also predict that the vertical advection of new N_2 supplied as nitrate from the deep ocean to the phytoplankton in surface waters will decrease due to an intensifying ocean stratification^{3,25}, which leads to a lower overall oceanic productivity^{26,27}. Enhanced new N_2 supplies as warming relieves the Fe limitation of N_2 fixation could largely offset this expected loss of nitrate inputs, and so enhance food-web productivity across broad expanses of the future tropical oceans.

Received: 30 December 2017; Accepted: 14 June 2018;

Published online: 16 July 2018

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Acknowledgements

This study was supported by US National Science Foundation grants OCE 1657757, OCE 1638804, OCE 1538525, OCE 1260233 and OCE 1260490 and National Natural Science Foundation of China grants 31470171 and 31770033.

Author contributions

H.B.J., D.A.H. and F.-X.F. contributed to conceiving and planning the experiments, H.B.J., F.-X.F., P.P.Q., X.-W.W. and Z.Z. performed the lab experiments, P.P.Q. and S.A.S.-W. contributed analytical work, S.-R.C. and N.M.L. contributed modelling work, H.B.J. and D.A.H. contributed to writing the paper and all of the authors contributed comments, revisions and editing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41558-018-0216-8>.

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