

LETTER

Nickel superoxide dismutase protects nitrogen fixation in *Trichodesmium*Chih-Chang Chen,¹ Irene B. Rodriguez,^{1,2} Yuh-ling Lee Chen ,³ Jonathan P. Zehr,⁴ Yun-Ru Chen,⁵ Shang-Te Danny Hsu,^{5,6}
Shun-Chung Yang,¹ Tung-Yuan Ho ^{1,7*}¹Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan; ²Marine Science Institute, University of the Philippines Diliman, Quezon City, Philippines; ³Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan; ⁴Ocean Sciences Department, University of California, Santa Cruz, California, USA; ⁵Academia Sinica Protein Clinic, Department of Academic Affairs and Instrument Service, Academia Sinica, Taipei, Taiwan; ⁶Institute of Biochemistry, Academia Sinica, Taipei, Taiwan; ⁷Institute of Oceanography, National Taiwan University, Taipei, Taiwan**Scientific Significance Statement**

Nitrogen fixation is a key process influencing CO₂ uptake in the ocean since bioavailable nitrogen is a major limiting factor for marine phytoplankton growth. Nitrogen fixation is catalyzed by nitrogenase, an enzyme which is vulnerable to O₂ and oxidative stress generated in photosynthesis. *Trichodesmium*, a major N₂ fixation cyanobacterium in the tropical and subtropical oceans, carries out nitrogen fixation and photosynthesis simultaneously and possesses the capability to bloom in the surface water. Our previous studies demonstrated that sufficient nickel supply is essential for *Trichodesmium* to fix nitrogen under high light (HL) conditions. This study demonstrates that nickel superoxide dismutase reduces oxidative stress generated during photosynthesis in *Trichodesmium*, and thus protects its nitrogen fixation process under HL conditions.

Abstract

Trichodesmium, a major diazotroph in tropical and subtropical oceans, provides a significant amount of bioavailable nitrogen in oligotrophic oceans. Remarkably, the cyanobacteria carry out nitrogen fixation and photosynthesis simultaneously under high light (HL) conditions. Here, we hypothesize that nickel superoxide dismutase (NiSOD) protects the nitrogen fixation process from oxidative stress generated in photosynthesis under high solar radiation conditions. We found that the variations in nitrogen fixation rates closely corresponded to Ni availability under HL conditions. The expression of NiSOD and two representative photosynthetic proteins also corresponded to variations in both nitrogen fixation rates and Ni availability under HL conditions. *Trichodesmium* collected in the field had a high Ni quota and elevated SOD activity. The evidence supports the conclusion that NiSOD protects nitrogen fixation in *Trichodesmium* from oxidative stress. The ability to utilize NiSOD may be a major advantage for *Trichodesmium* under high solar radiation conditions.

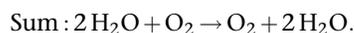
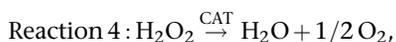
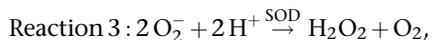
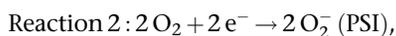
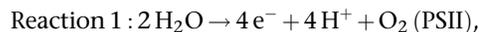
*Correspondence: tyho@gate.sinica.edu.tw**Associate editor:** Angelique White**Author Contribution Statement:** TYH, CCC, IBR, and JPZ conceived and designed the study. CCC and IBR carried out the laboratory culture experiments. CCC, YRC, and STDH produced and purified NiSOD protein. CCC, YLLC, and SCY conducted the field studies. CCC and TYH analyzed all data. TYH and CCC wrote the paper.**Data Availability Statement:** Data and metadata are available in the Dryad data repository (<https://datadryad.org/stash/share/UGPDQh6lBpD0BuQf6K4IUAtOxpsM6qx4xptd4BJMB68>).

Additional Supporting Information may be found in the online version of this article.

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Nitrogen fixation influences the biological uptake of carbon dioxide in the ocean since bioavailable nitrogen is a major limiting factor for marine phytoplankton growth (Capone et al. 1997; Zehr and Kudela 2011). *Trichodesmium* is a major diazotrophic cyanobacteria in tropical and subtropical oceans and contributes a significant amount of new production in the oceans (Karl et al. 2002; Capone et al. 2005; Mulholland et al. 2006). Nitrogenase is the key enzyme for the nitrogen fixation reaction and is vulnerable to elevated O_2 and reactive oxygen species (ROS) generated in photosynthesis (Gallon 1981; Fay 1992). Almost all cyanobacterial diazotrophs have developed strategies to separate nitrogen fixation from photosynthesis either spatially or temporally (Bergman et al. 2013). However, *Trichodesmium* is unusual as it carries out nitrogen fixation and photosynthesis simultaneously (Carpenter and Capone 1992; Bergman et al. 1997), and some studies have reported that nitrogenase is present in most of its cells (Ohki 2008). *Trichodesmium* can survive or even bloom in oligotrophic surface waters (Karl et al. 2002; Chen et al. 2014). The mechanism by which *Trichodesmium* mediates oxidative stress generated in photosynthesis under high light (HL) conditions has become the focus of our recent research (Ho 2013; Ho et al. 2013; Rodriguez and Ho 2014; Rodriguez and Ho 2017).

In photosynthesis, solar radiation energy splits and oxidizes H_2O into O_2 , electrons, and protons in photosystem II (PSII). High solar radiation thus accelerates electron generation via PSII and elevates the gross production rates of O_2 and ROS. On the other hand, superoxide, a major ROS generated in photosystem I (PSI), serves as the primary intermediate in the photoreduction of O_2 to transport electrons to the Mehler reaction or also known as the Water–Water cycle (Asada 1999). Originating from the reduction of two O_2 molecules, the two superoxide molecules are spontaneously scavenged by superoxide dismutase (SOD) and disproportionately reduced and oxidized to one H_2O_2 and one O_2 . H_2O_2 , another ROS intermediate, is oxidized to water by catalase (CAT) to complete the electron transfer chain and reduce the ROS accumulation or oxidative stress (Asada 1999).



It is worth noting that reactions 2–4 in PSI not only comprise the electron transfer chain but may also serve as steps in the O_2 consumption process. Theoretically, the one oxygen atom from 2 O_2 shown in reaction 2 would be spontaneously transformed to H_2O in reaction 4 if sufficient SOD and CAT are

present. Indeed, early studies have found that *Trichodesmium* can reduce intracellular oxygen levels through the Mehler reaction (Kana 1993; Berman-Frank et al. 2001; Milligan et al. 2007). Kana (1993) and Milligan et al. (2007) observed extremely high O_2 consumption rates in *Trichodesmium*, whereas nitrogen fixation rates were relatively high in the middle of the light period. To consume O_2 and ROS, SOD expression must be sufficient to unblock reactions 2–4. An early study proposed that elevated SOD expression is associated with nitrogenase protection (Puppo and Rigaud 1986). Inactivating intracellular SOD in a model cyanobacterium impairs nitrogen fixation (Zhao et al. 2007). Many studies have shown that ROS accumulation damages the photosystems and suppresses their activity (Tjus et al. 2001; Krieger-Liszka et al. 2011). In summary, sufficient SOD expression is essential for reducing oxidative stress in photoautotrophic diazotrophs.

FeSOD, MnSOD, and Cu/ZnSOD are the most common SODs utilized by marine phytoplankton (Fridovich 1995; Pilon et al. 2011). Not only may individual phytoplankton possess more than one of these SODs, but they may also use these metals as cofactors for numerous enzymes in many other key metabolic reactions. Thus, it is challenging to distinguish the functions of these SODs specifically for nitrogen fixation or photosynthesis. In *Trichodesmium*, there are only three possible Ni-containing enzymes: NiSOD, NiFe uptake hydrogenases, and urease (Palenik et al. 2003). Since NiSOD is the only Ni enzyme involved in both nitrogen fixation and photosynthesis in *Trichodesmium*, it serves as an ideal model to study the role of SOD in protecting nitrogen fixation and photosystems from the oxidative stress.

Ho (2013) first reported that an increase in Ni concentrations elevated cellular SOD activity and nitrogen fixation rates in *Trichodesmium* (Ho 2013). Further studies have observed that nitrogen fixation rates corresponded with the Ni availability, light intensity, and spectral quality (Ho 2013; Rodriguez and Ho 2014; Rodriguez and Ho 2017). We thus hypothesized that NiSOD is essential for protecting nitrogen fixation from oxidative stress generated during photosynthesis in *Trichodesmium* under HL conditions (Ho 2013; Rodriguez and Ho 2014). In this study, we validated this hypothesis by measuring NiSOD concentrations and key parameters in *Trichodesmium* grown under varying light intensities and Ni availabilities. The parameters included specific growth rates, trace metal quotas, nitrogen fixation rates, SOD activities, NiSOD concentration, and the concentrations of two representative photosynthetic proteins (PsbA and PsaC). We also compared the cellular Ni quota and SOD activity in *Trichodesmium* collected from the field to evaluate the importance of Ni. These results provide crucial information for understanding the unique capability of *Trichodesmium* and may give important insight on how marine phytoplankton mediate oxidative stress under high solar conditions.

Materials and methods

Trichodesmium erythraeum IMS101 was obtained from the National Center for Marine Algae and Microbiota (NCMA) and grown in 1 L acid-washed polycarbonate bottles. Trace metal defined culture media were modified from YBC II artificial seawater prepared for growing *Trichodesmium* (Chen et al. 1996; Ho 2013). Without using trace metal clean culture medium, Ni background concentrations in the culture medium may be high and sufficient for the growth of *Trichodesmium* under HL conditions (Breitbarth et al. 2008). The background concentration of Ni in the chelated culture medium was only 0.025 nM (Ho et al. 2010; Wang et al. 2014). The background concentrations of nitrate were below the detection limit, 0.1 μM . The total dissolved Ni concentrations in the low, medium, and

high conditions in the media were set to be 10, 40, and 100 nM, respectively (Fig. 1). The cellular biomass of two other low Ni treatments (2 and 5 nM) in our preliminary tests were also measured and presented. An artificial organic chelator, ethylenediaminetetraacetic acid (EDTA), at a final concentration of 20 μM , was used to buffer trace metals in the medium. Estimated inorganic Ni concentrations were 0.11, 0.42, and 1.1 pM, respectively, as calculated by Visual MINTEQ 3.1 (Gustafsson 2011). The total dissolved concentrations of the other trace metals, including Fe, Mn, Co, Cu, Zn, Cd, and Mo, were 400, 10, 10, 10, 10, 10, and 100 nM, respectively.

Trichodesmium was cultivated under 80 and 550 $\mu\text{E m}^{-2} \text{s}^{-1}$, representing low light (LL) and HL conditions, respectively. Cultures were grown in a temperature-controlled growth chamber at 26°C under the LL and HL conditions with

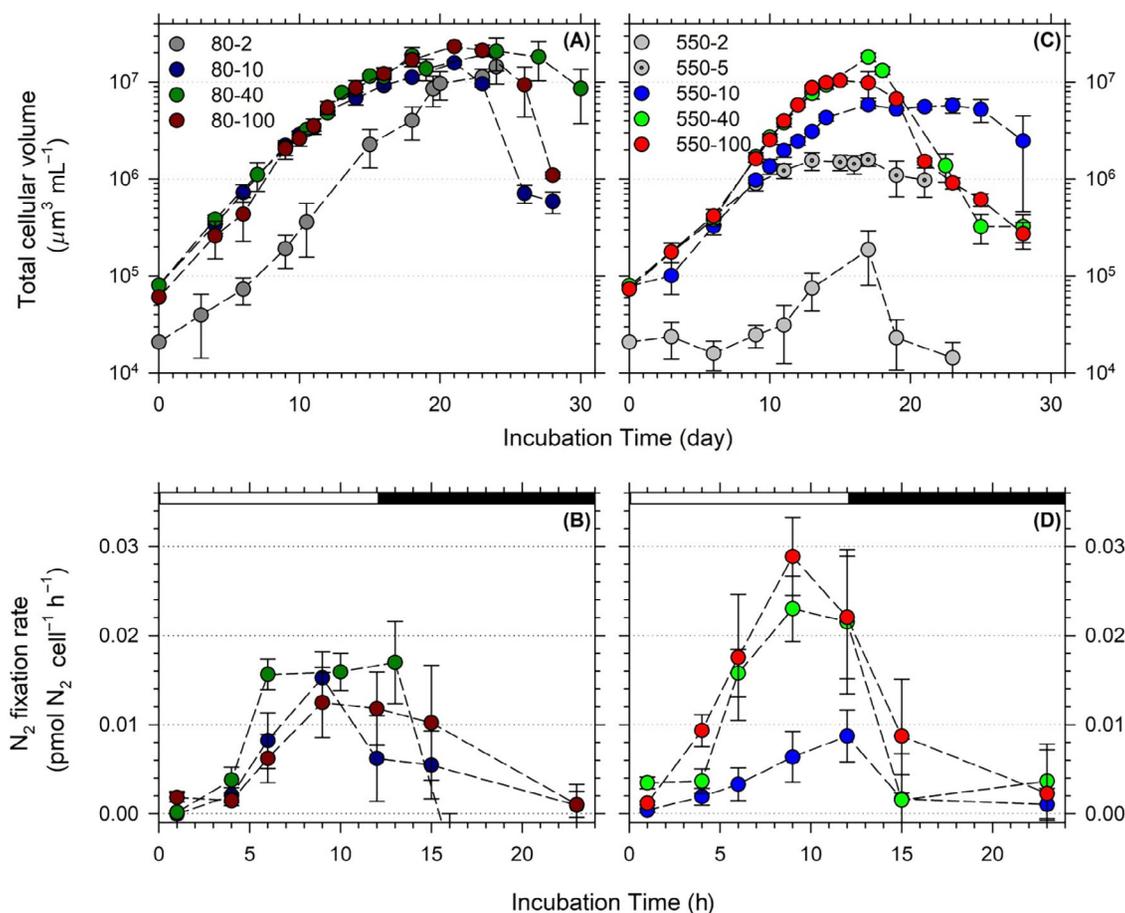


Fig. 1. The comparison of growth curves (A,C) and nitrogen fixation rates (B,D) of *Trichodesmium* among various Ni and light treatments. The left and right panels show LL and HL, respectively. The two numbers shown next to the symbols are the light intensities ($\mu\text{E m}^{-2} \text{s}^{-1}$) and total dissolved Ni concentrations (nM), respectively. The open and solid horizontal bars shown on the top of (B) and (D) indicate light and dark phases, respectively. The equivalent concentrations of the detection limit (DL, 3σ of the blank) of the acetylene reduction method were 0.054, 0.104, 0.054, 0.043, 0.056, and 0.042 ppm for the 6 treatments (80–10, 40, 100 and 550–10, 40, 100), respectively. Among all of the data by excluding the 1st time point in the 7-point time course, 97% of the data were 1 order of magnitude higher than the DL and 70% of the data were 2 orders of magnitude higher than the DL. The data obtained from the 2 and 5 nM HL treatments was obtained from preliminary experiments in which only the parameter of cellular volume was measured. Nitrogen fixation rates were calculated by the difference between the accumulated nitrogen fixed of two adjacent time points. The average rates are shown at the end of each time interval. Error bars stand for 1 SD of three replicate culture bottles.

a 12 : 12 h light : dark cycle. Light intensities were verified by measuring the penetration of photosynthetic active radiation in a seawater-filled polycarbonate bottle using a submersible radiometer. Each treated culture was acclimated for approximately 20 generations under individual Ni concentrations for approximately 2 months before performing the formal experiments. All treatments were performed in triplicate ($n = 3$). The specific growth rates were determined using the cellular volume approach at four to six consecutive sampling points during the exponential phase (Fig. 1).

By measuring the total cellular volume per mL and average volume per cell with a particle counter and microscope, respectively, the cellular volume approach may provide accurate and quantitative biomass data for *Trichodesmium* (Ho 2013). The uncertainty of this method is less than 10% (Ho 2013). The total cellular volume was measured between the 2nd and 3rd h of the light cycle using a Coulter particle counter. The values of average volume per cell ranged from 188 to 215 μm^3 per cell for almost all treatments except the HL high Ni treatment (Table 1). We used 200 μm^3 per cell for the conversion from total cell volume to total cell numbers for all treatments except the HL high Ni treatment, for which we used 250 μm^3 per cell. The cells were harvested during the mid-exponential growth phase to measure the parameters. Nitrogen fixation rates were measured and quantified using the acetylene reduction method (Capone and Montoya 2001). One hour before the light period started, an aliquot of the culture was transferred into a 20-mL acid-washed glass vial for measurement. The vial was sealed with a Teflon-coated septum and an aluminum cap, and 2 mL of air

was removed and replaced with 2 mL of acetylene. The vials were then incubated under similar conditions corresponding to the source treatment for 24 h under a 12 : 12 h light : dark cycle. After incubation, the concentrations of ethylene in the headspace gas in the vials were measured at different time points (1, 4, 6, 9, 12, 15, and 23 h) and quantified using ethylene standards. Using a conversion ratio ($\text{C}_2\text{H}_2 : \text{N}_2$) of 4 : 1 (Punshon and Moore 2008), nitrogen fixation rates were calculated by the difference in the accumulated nitrogen fixed between two adjacent time points (Supporting Information Fig. S1). The average rates are shown at the end of each time interval (Fig. 1).

The harvest time for SOD activities, elemental quotas, and protein extraction was 6 h after light exposure. Cells (50 mL) were harvested using 5- μm polycarbonate filters before the SOD assay. The filter-collected cells were washed and resuspended in 1 mL of ultrapure Milli-Q water. The concentrated cells were broken by sonication (Q125, QSonica) coupled with flash freezing in liquid nitrogen twice. The broken cell samples were centrifuged and the supernatant was used for SOD analysis. Intracellular SOD activity was measured using the xanthine-xanthine oxidase method (Flohe and Otting 1984). For trace metal quota analysis, cultured cells were harvested onto an acid-washed 5- μm polycarbonate filter in a class-10 bench, and the filtered cells were rinsed with ultrapure Milli-Q water thrice, and then decomposed using a mixed ultrapure acid solution (50% HNO_3 and 10% HF, J. T. Baker Inc.) before inductively coupled plasma mass spectrometer (ICPMS) analysis (Ho 2013).

Table 1. The data for specific growth rates, maximum cellular volume, nitrogen fixation rates, SOD activities, and the averaged cellular volume of *Trichodesmium* grown under the various Ni and light treatments. Error bars stand for 1 SD of three replicate culture bottles.

Light intensity	Ni _{conc} (nM)	Growth rate* (d ⁻¹)	Max vol _{total} (10 ⁶ $\mu\text{m}^3 \text{ mL}^{-1}$)	N ₂ fixed (pmol cell ⁻¹ d ⁻¹)	SOD activity ($\mu\text{U cell}^{-1}$)	Cell volume† ($\mu\text{m}^3 \text{ cell}^{-1}$)
80	2	0.33 ± 0.01	14	N/A	N/A	N/A
	10	0.29 ± 0.01	16	0.13 ± 0.02	0.84 ± 0.11	188 ± 42 ($n = 32$)
	20	0.31‡	13‡	N/A	0.80‡	191 ± 37 ($n = 4$)
	40	0.34 ± 0.05	21	0.16 ± 0.01	0.98 ± 0.17	204 ± 37 ($n = 20$)
	100	0.31 ± 0.03	23	0.16 ± 0.04	1.01 ± 0.14	190 ± 39 ($n = 28$)
550	2	0.21 ± 0.01	0.19	N/A	N/A	N/A
	5	0.22 ± 0.03	1.6	N/A	N/A	N/A
	10	0.30 ± 0.01	5.8	0.08 ± 0.02	1.68 ± 0.18	205 ± 63 ($n = 30$)
	20	0.38‡	20‡	N/A	2.03‡	192 ± 36 ($n = 4$)
	40	0.38 ± 0.01	18	0.20 ± 0.03	1.99 ± 0.18	215 ± 45 ($n = 41$)
	100	0.42 ± 0.02	11	0.28 ± 0.06	3.04 ± 0.15	253 ± 67 ($n = 47$)

*The growth curves shown in Fig. 1A,C are expressed as the change in total cellular volume per mL with time.

†The total cellular volume can be accurately converted to cell number by measuring the average volume per cell using a microscope. Our previous studies found that the average cellular volume generally ranges from 200 to 250 μm^3 per cell (Ho 2013; Ho et al. 2013). In this study, the average volume per cell for each treatment was determined using a microscope. Although the relative standard deviation (RSD) of the volume per cell in one trichome is relatively high, generally ranging from 20% to 30%, as shown here, the RSD of average volume per cell among trichomes is approximately 5% or less.

‡Only one culture bottle was used for treatment.

Samples for protein analysis were processed by sonication for 10 s in protein extraction buffer for two cycles, then centrifuged at 13,000 rpm for 5 min. Total protein concentration was quantified using the Lowry assay (Lowry et al. 1951). For protein separation, 5 μ g of total protein sample was first denatured by heating at 95°C for 10 min and then was processed by electrophoresis at 200 V for 40 min on a 4–20% (w/v) sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) using a Bio-Rad TGX Stain-Free PreCast Acrylamide Kit. The gel was transferred to a polyvinylidene difluoride membrane for western blot analysis. The membrane was blocked with 5% nonfat dried milk in Tris-buffered saline (TBS) tween-20 (TBST) for 1 h at room temperature with agitation. The membranes were then incubated at 4°C overnight with primary antibodies, including PsbA, PsaC, and NiSOD antibodies. The NiSOD antibody was custom-designed using CFWAT KERDV TWYKA S sequences with a purity of 98% (Yao-Hong Biotechnology Inc.). After rinsing with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1.5 h at 25°C. The membranes were washed with TBST again to remove secondary antibodies. Chemiluminescent substrates were added to the membrane blots for 5 min and the resulting bands were quantified using a Biorad CCD camera-based imager.

To quantify NiSOD concentrations, the Tery_0891 gene (NCBI Accession No. ABG50292) of *Trichodesmium* IMS101 was cloned into a pET-21a (+) vector. *Escherichia coli* BL21 (DE3) competent cells (ECOSTM) transformed with pET-21a (-+)-Tery_0891 were grown in Luria-Bertani medium containing ampicillin (100 μ g mL⁻¹) at 37°C. When the OD600 reached approximately 0.6, isopropyl β -D-1-thiogalactopyranoside was added to a final concentration of 1 mM at 37°C for 5 h. The cells were harvested by centrifugation at 6000 rpm for 30 min at 4°C, resuspended in lysis buffer, and disrupted via sonication. The overexpressed NiSOD from the cell lysate was purified using Ni-NTA affinity chromatography and size-exclusion chromatography. The purity of the protein was confirmed and quantified using SDS-PAGE and nano-photometer. The NiSOD concentrations in the samples were then quantified using purified NiSOD.

The data and metadata of this study are available in the Dryad data repository (Chen et al. 2022).

Results and discussion

Specific growth rates and nitrogen fixation rates

We found that the cellular demand for Ni in *Trichodesmium* was mainly controlled by the light intensity (Fig. 1). Under LL, the specific growth rates (0.33, 0.29, 0.34, 0.31 d⁻¹) were not statistically different among the treatment groups with Ni concentrations at 2, 10, 40, and 100 nM, respectively (Fig. 1A; Table 1). The comparable growth rates demonstrate that Ni requirements under LL are relatively low, indicating that 2 nM Ni with 20 μ M EDTA may be sufficient to support maximum

growth and biomass under LL conditions (Table 1). However, under HL, the growth rates and maximum total cellular volume increased with increasing Ni availability, with specific growth rates of 0.21, 0.22, 0.30, 0.38, 0.38, and 0.42 for total Ni concentrations of 2, 5, 10, 20, 40, and 100 nM, respectively (Fig. 1C; Table 1). The growth curves obtained from the 2 and 5 nM Ni treatments also exhibited similar patterns between LL and HL, with extremely low growth rates under HL, but comparably high growth rates under LL (Fig. 1A,C).

The lowest nitrogen fixation rates were observed in the HL low Ni treatment and the highest rates were observed in the HL high Ni treatment, with a more than threefold difference (Fig. 1B,D; Table 1), indicating that *Trichodesmium* may fix more nitrogen under HL only when the Ni supply is sufficient. In terms of LL treatments, similar to the specific growth rates, nitrogen fixation rates were comparable among all three Ni concentrations, indicating that Ni availability did not affect nitrogen fixation rates in *Trichodesmium* under LL (Fig. 1B,D; Table 1). In addition, the maximum nitrogen fixation rate under HL high Ni condition was almost twice the rate observed for LL. All of these observations support the protection hypothesis.

Comparison with key parameters

Figure 2A shows that Ni quotas for HL were positively correlated with Ni availability, increasing from 0.011, 0.035, to 0.10 fmol cell⁻¹ for 10, 40, and 100 nmol L⁻¹ Ni treatments, respectively. We found that Ni quotas in the HL 100 nM Ni treatment were 2.9- and 9.2-fold of the quotas observed in the HL 40 and 10 nM Ni treatments, respectively, which are close to the ratios of their corresponding bioavailable Ni concentrations. Moreover, the comparable quotas between HL and LL for low and medium Ni treatments suggest that Ni uptake may be limited by the coupling effects of relatively low bioavailable Ni concentrations (Ni') and the diffusion coefficient of Ni ions through the cell membranes (Hudson and Morel 1993). Overall, the increasing trend of Ni quotas for HL was similar to that observed for nitrogen fixation rates (Fig. 1).

Figure 2E shows that total SOD activity was also significantly elevated with increasing light intensity and Ni availability in *Trichodesmium*. Under LL, total SOD activities were comparable among the three Ni concentrations, only slightly varying from 0.84 to 1.01 μ U cell⁻¹ (Table 1). Under HL conditions, total SOD activities increased from 1.68 to 3.04 μ U cell⁻¹ with increasing Ni concentrations (Table 1). These variations of the total SOD activities between LL and HL were also consistent with variations in nitrogen fixation rates.

PsbA (or D1 protein) and PsaC, the two core subunits of PSII and PSI, are useful indicators of photosynthetic activity. We found that the concentrations of PsbA and PsaC were generally low and did not vary significantly among the three LL treatments. However, the concentrations of PsbA and PsaC were significantly higher in the HL high Ni treatment than in all other treatments (Fig. 2C,F). The variation patterns of PsbA

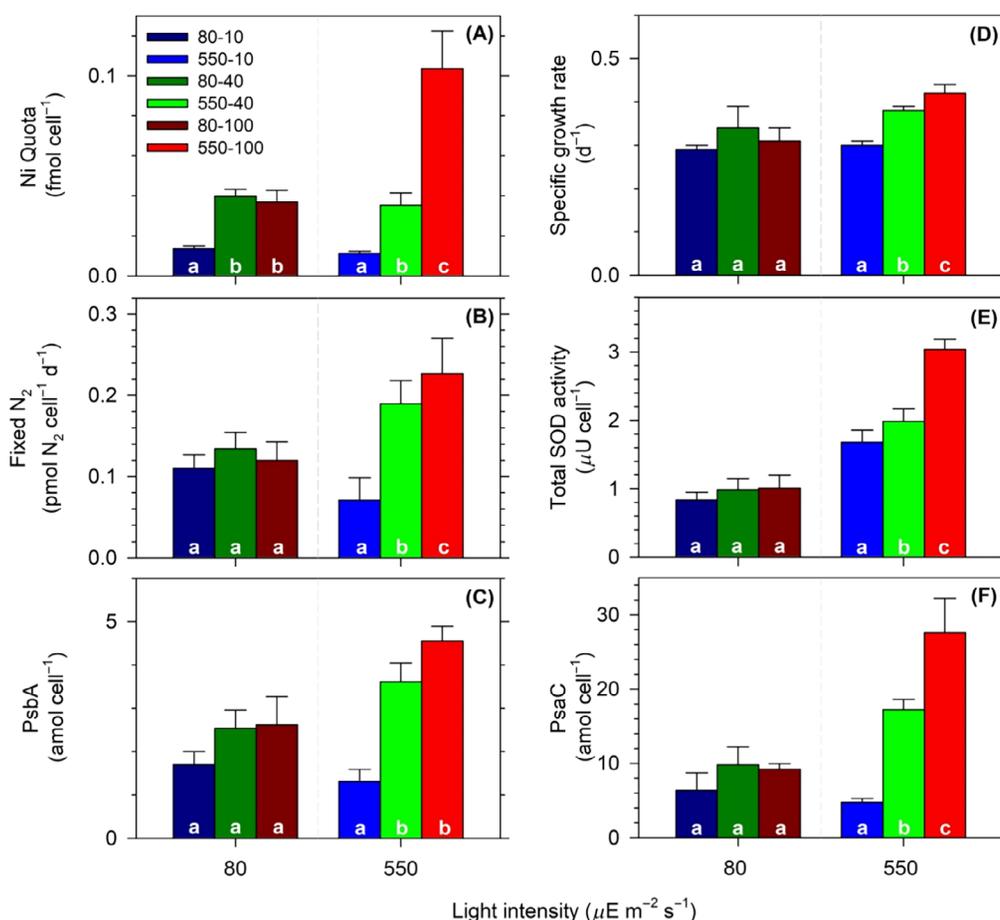


Fig. 2. The comparison of cellular Ni quotas (A), nitrogen fixation rates (B), the concentration of PsbA (C), specific growth rates (D), total SOD activities (E), and the concentration of PsaC (F) of *Trichodesmium* grown under various Ni and light conditions. As shown in Figs. 3 and 4, error bars represent 1 SD of three culture bottles; different letters shown at the bottom of each bar stand for significant difference ($p < 0.05$) among the three Ni treatments under same light condition.

and PsaC concentrations were also comparable to those of nitrogen fixation rates and SOD activities (Fig. 2B,E). The correspondence of NiSOD activities, PsbA, and PsaC concentrations to HL high Ni condition also supports the hypothesis that elevated NiSOD expression alleviates the oxidative stress and maintains high photosynthetic efficiency under HL conditions (Fig. 3).

Quantifying NiSOD concentrations

To the best of our knowledge, this is the first study to report NiSOD concentrations in *Trichodesmium*. We found that NiSOD concentrations increased significantly with increasing Ni concentrations only under HL, with concentrations of 7.1 ± 0.2 , 14.2 ± 2.2 , and 21.9 ± 0.9 amol cell $^{-1}$ for the three Ni treatments, respectively (Fig. 3; Supporting Information Table S1). Under LL, NiSOD concentrations were relatively low and without significant differences between the medium and high Ni treatments, with concentrations of 4.3 ± 0.6 , 9.0 ± 1.3 , and 8.0 ± 1.1 amol cell $^{-1}$, for the three

Ni treatments, respectively (Fig. 3; Supporting Information Table S1). Under the same Ni treatment, NiSOD concentrations under HL were significantly higher than those observed under LL conditions. These NiSOD concentration patterns demonstrate that *Trichodesmium* elevates NiSOD levels to enhance the turnover rate of superoxides generated in photosynthesis under HL conditions.

In addition to NiSOD, Ni may be used to synthesize NiFe hydrogenase and urease in cyanobacteria (Dupont et al. 2008; Ragsdale 2009). Because *Trichodesmium* was grown in a fixed nitrogen free medium with active nitrogen fixation, it is reasonable to assume that most of the intracellular Ni taken up was allocated to NiSOD and NiFe hydrogenase. With the quantitative information on NiSOD, we estimated the percentage of cellular Ni used in NiSOD by dividing the amount of Ni in NiSOD (Ni_{NiSOD}) by the total amount of cellular Ni measured (Ni_t , Supporting Information Table S1). We found that significant amounts of cellular Ni were utilized to synthesize NiSOD, with percentages of 24%, 17%, and 16%; 47%,

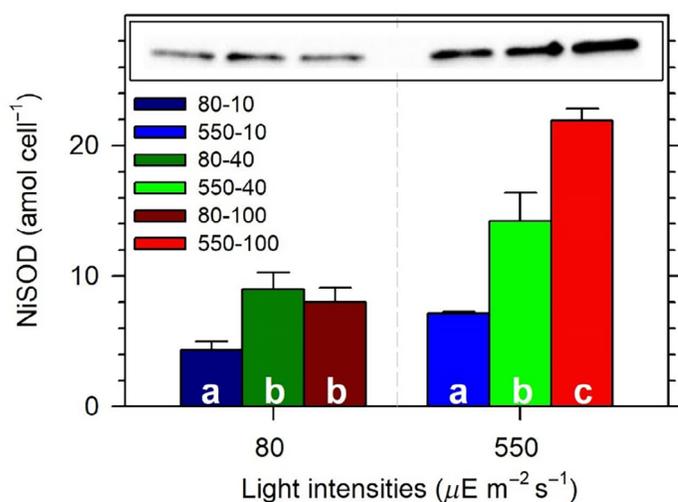


Fig. 3. The concentrations and expressions of NiSOD in *Trichodesmium*. NiSOD concentrations were quantified by a western-blotting approach using the purified NiSOD described in the “Materials and Methods” section.

30%, and 16% for low, medium, and high Ni treatments under LL and HL, respectively. The percentages increased significantly in the low and medium Ni treatments under HL (Supporting Information Table S1). The cellular quotas of Fe, Mn, and Mo and other trace metals are shown in Supporting Information (Table S2; Fig. S2). Although *Trichodesmium* is supposed to possess MnSOD, the consistent Mn quota among most of the LL and HL treatments suggests that Mn is not involved in the Water–Water cycle.

High Ni quotas in the field and the implications

Field studies also reported high Ni quotas in *Trichodesmium*, which were collected from the surface waters of the tropical and subtropical oceans (Supporting Information Table S2). The cellular P normalized Ni quota ranged from 5.9 to 6.3 mmol mol⁻¹ P in *Trichodesmium* collected in the oligotrophic surface waters of the South China Sea, the Western Philippine Sea, and the Kuroshio current (Fig. 4). The quotas observed in the other two studies in the surface water of the North Atlantic Ocean, NAO_1 and NAO_2, were 1.5 ± 0.5 and 7.1 ± 0.7 mmol mol⁻¹ P (Fig. 4). Since phytoplankton tends to have luxury uptake on phosphate, the quotas shown by this phosphate replete culture study and the NAO_1 study carried out in the region impacted by Amazon riverine input may be considered as the lower limit for the quota. In brief, all of these relatively high Ni quotas in the surface waters support that Ni demand and uptake in *Trichodesmium* are relatively high under HL intensity.

The total cellular SOD activity of *Trichodesmium* collected in the Kuroshio waters was at least $5.5 \mu\text{U cell}^{-1}$ (Fig. 4). High Ni quota and SOD activity observed in the field support a high NiSOD requirement in *Trichodesmium*. The high cellular

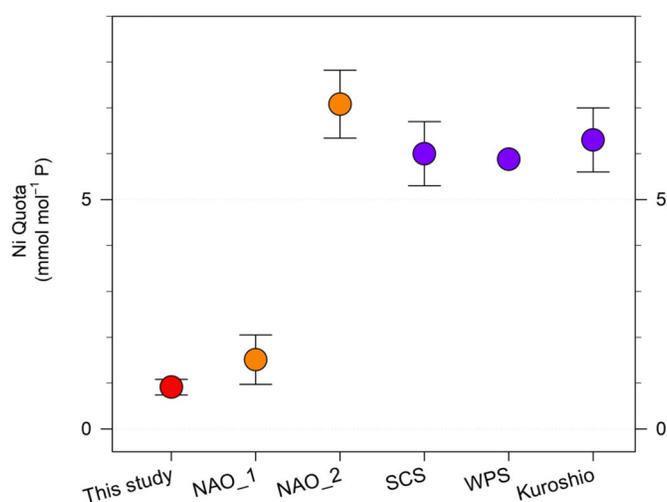


Fig. 4. The comparison of the Ni quota (mmol mol⁻¹ to P) in *Trichodesmium* observed in this culture study, the two previous studies in the North Atlantic Ocean (NAO_1 and NAO_2), and the samples collected in the South China Sea (SCS), the Western Philippine Sea (WPS), and the Kuroshio region from this study. The two NAO datasets, NAO_1 and NAO_2, are from the studies of Tovar-Sanchez et al. (2006) and Nuester et al. (2012), respectively. The sampling locations of the SCS and WPS datasets were at 18°N, 115.5°E and 23.5°N, 126°E, respectively. The Kuroshio samples were collected on the northern end of Lyudao in this study. Except for the WPS samples, all of the *Trichodesmium* samples were picked with an acid-washed polypropylene inoculating loop in a class-100 portable HEPA bench. The WPS sample was collected in July 2013 in a *Trichodesmium* bloom event using a 100-μm mesh plankton net tow at Sta. 7 (Liao et al. 2017). *Trichodesmium* biomass was filtered with 10-μm acid-washed polycarbonate filters in a class 100 HEPA bench for the WPS samples. The total average SOD activity in the Kuroshio sample was measured, which was $5.5 \mu\text{U cell}^{-1}$. For the activity measurement, we picked 300 tufted colonies for *Trichodesmium* by a loop. After examining the colony image that was picked, we used the averaged trichome number of 24 per tuft and the average cell number of 60 per trichome (Wu et al. 2018) to convert colony numbers to cell numbers for the activity calculation. As the Kuroshio samples were collected during day time and processed for a few hours in the laboratory, the activity obtained should be considered as a lower limit.

quota also suggests that dissolved Ni in the surface water may be bioavailable to *Trichodesmium*. It should be noted that the concentrations of total dissolved Ni is relatively high in the surface waters of the tropical and subtropical oceans globally, around 2 nM (Middag et al. 2020). The relatively high Ni availability in the surface water may explain the advantage of *Trichodesmium* in growing and fixing nitrogen in high solar radiation environments.

Our previous studies showed that Ni is essential for the growth of *Trichodesmium* and can elevate its SOD activities and nitrogen fixation rates (Ho 2013), with elevated high nitrogen fixation rates under HL, high Ni conditions (Ho et al. 2013; Rodriguez and Ho 2014). In this study, we observed that NiSOD, PsbA, and PsaC were expressed correspondingly with variations in Ni supply and nitrogen fixation rates under HL conditions, validating that NiSOD expression

is essential for protecting nitrogen fixation from oxidative stress generated during photosynthesis in *Trichodesmium* grown under HL conditions. These findings may also shed light on the mechanisms of photoinhibition for marine phytoplankton under high solar radiation conditions.

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Acknowledgments

We appreciate Associate Editor's and the reviewer's comments, which have improved the quality of this manuscript. We thank C.-C. Hsieh, M.-J. Ho, H.-T. Li, Y.-Y. Chen, and S. R. Null for providing technical support and useful comments. We thank the Academia Sinica Protein Clinic, funded by Academia Sinica Core Facility and Innovative Instrument Project AS-CFII-109-107 for supporting the recombinant protein production. This work was partially supported by the Simons Foundation, grants 724220 and 824082, JPZ. This work was mainly supported by grant MOST 108-2611-M-001-006-MY3 by Taiwan Ministry of Science and Technology and Investigator Award AS-IA-110-M03 to TYHO by Academia Sinica.

Submitted 18 December 2021

Revised 22 May 2022

Accepted 29 May 2022