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Effect of light on N_2 fixation and net nitrogen release of *Trichodesmium* in a field study

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Abstract. Dinitrogen fixation (NF) by marine cyanobacteria is an important pathway to replenish the oceanic bioavailable nitrogen inventory. Light is the key to modulating NF; however, field studies investigating the light response curve (NF-I curve) of NF rate and the effect of light on diazotroph-derived nitrogen (DDN) net release are relatively sparse in the literature, hampering prediction using models. A dissolution method was applied using uncontaminated $^{15}N_2$ gas to examine how the light changes may influence the NF intensity and DDN net release in the oligotrophic ocean. Experiments were conducted at stations with diazotrophs dominated by filamentous cyanobacterium *Trichodesmium* spp. in the western Pacific and the South China Sea. The effect of light on carbon fixation (CF) was measured in parallel using the ^{13}C tracer method specifically for a station characterized by *Trichodesmium* bloom. Both NF-I and CF-I curves showed a I_k (light saturation coefficient) range of 193 to 315 $\mu E\,m^{-2}\,s^{-1}$, with light saturation at around 400 $\mu E\,m^{-2}\,s^{-1}$. The proportion of DDN net release ranged from ∼ 6 to ∼ 50 %, suggesting an increasing trend as the light intensity decreased. At the *Trichodesmium* bloom station, we found that the CF / NF ratio was light-dependent and the ratio started to increase as light was lower than the carbon compensation point of 200 $\mu E\,m^{-2}\,s^{-1}$. Under low-light stress, *Trichodesmium* physiologically preferred to allocate more energy for CF to alleviate the intensive carbon consumption by respiration; thus, there is a metabolism tradeoff between CF and NF pathways. Results showed that short-term (< 24 h) light change modulates the physiological state, which subsequently determined the C / N metabolism and DDN net release by *Trichodesmium*. Reallocation of energy associated with the variation in light intensity would be helpful for prediction of the global biogeochemical cycle of N by models involving *Trichodesmium* blooms.

1 Introduction

The bioavailable nitrogen introduced via nitrogen fixation (NF) by cyanobacteria is important to fertilize the tropical and subtropical oligotrophic surface ocean (Karl et al., 1997). In such environments, nitrate supplied from the subsurface is generally limited by thermostructure-induced stratification and NF can directly input bioavailable nitrogen to the euphotic zone (Capone et al., 2005). Among the variety of diazotrophs, the filamentous non-heterocystous cyanobacterium *Trichodesmium* is recognized as a major player, contributing up to 80–110 Tg N annually, i.e. ∼ 50 % of global marine NF (Capone et al., 1997). It often forms colonies or aggregates and under appropriate circumstances it forms large surface blooms (Zehr, 2011).

Light is the primary energy source for the photoautotrophic diazotrophs, and the energy-exhausting NF process is tightly linked with photosynthesis (LaRoche and Breitbarth, 2005, and reference therein). Regarding the light response of *Trichodesmium*, several previous field studies put efforts into carbon fixation (CF) and oxygen production in response to irradiance (PI curve) and showed that photosynthetic rates of *Trichodesmium* were proportional to light intensities, and a relatively high irradiance requirement and a high respiration rate were needed to protect the nitrogenase enzyme from O_2 deactivation (Lewis et al., 1988; Carpenter and Roenneberg, 1995). By using the C_2H_2 reduction method, Carpenter et al. (1993) investigated the light response of nitrogenase activity for the field-towed *Trichodesmium*, which showed a response pattern as a function of irradiance and resembling the PI curve. Similarly, by using ^{13}C / ^{15}N isotope labeling techniques, Holl et al. (2007) found that NF and CF rates of field-towed *Trichodesmium* were attenuated as light intensity decreased. In controlled laboratory experiments, Breitbarth et al. (2008) suggested that both nitrogenase activity and growth rates of *Trichodesmium* (IMS-101) are light-dependent (15 to 1100 $\mu E\,m^{-2}\,s^{-1}$), and Bell and Fu (2005) observed increasing NF rates with the increase in light intensity (PAR 10–160 $\mu E\,m^{-2}\,s^{-1}$), and the cellular concentrations of Chl *a* and phycobiliproteins (PBPs) increased under low-light conditions.

Meanwhile, statistical analysis performed on the global dataset of field NF suggests that light is an important environmental factor explaining most of the spatial variance in NF on the global scale (Luo et al., 2014). However, it has to be noted that some of the NF rate measurements available in this global database might be questionable due to previously unrealized technical problems, e.g., incomplete $^{15}N_2$ dissolution in the $^{15}N_2$ bubble tracer method (Mohr et al., 2010), bioavailable ^{15}N forms contamination in some commercial $^{15}N_2$ gas (Dabundo et al., 2014) and inconsideration of diazotroph-derived N (DDN) release in the filtrate fraction (Konno et al., 2010). Nevertheless, the abovementioned experiments and global analysis support the idea of a light control on NF activity, CF and oxygen evolution of *Trichodesmium*; however, limited field experiments have been conducted on studying the effect of light on CF and NF of bulk seawater, particularly during naturally occurring *Trichodesmium* blooms. Moreover, to our knowledge, no study using the improved $^{15}N_2$ dissolution tracer method (Mohr et al., 2010) has been implemented yet.

During the NF process, *Trichodesmium* release 10 to 50 % of the DD^{15}N in the dissolved pool (Glibert and Bronk, 1994; Konno et al., 2010), primarily as dissolved organic N (DON, such as dissolved free amino acid) and NH_4^+ (Capone et al., 1994; Mulholland et al., 2004). High DON and NH_4^+ concentrations are often measured within *Trichodesmium* blooms (Karl et al., 1992; Lenes et al., 2001), being supportive of DDN release. As most NF rate measurements were via incorporation of $^{15}N_2$ into particulate organic N (PON), the ^{15}N enrichment in the dissolved pool had not been taken into account, resulting in the aforementioned potential underestimation of NF rates. Conversely, diatom and dinoflagellate blooms have often been observed following *Trichodesmium* blooms, suggesting that DDN potentially supported non-diazotrophic phytoplankton growth (Devassy et al., 1978; Lenes et al., 2001). By using nanometer-scale secondary ion mass spectrometry, Bonnet et al. (2016a) recently showed that the DDN is quickly (1–3 days) transferred to surrounding plankton, predominantly diatoms and bacteria, during *Trichodesmium* blooms. A mesocosm experiment performed in the western tropical South Pacific (VAHINE) revealed an incommensurately high contribution of NF to export production (> 50 %; Knapp et al., 2016) during a bloom of UCYN-C bloom. The contribution of NF to export can be up to 92 % in some studies (Kumar et al., 2017). However, the effect of NF on export was largely indirect, i.e., attributable to quick recycling processes of DDN transfer to non-diazotrophs that were subsequently exported (Bonnet et al., 2016b, c; Knapp et al., 2016). In spite of the importance of DDN release in C and N cycles, the factors controlling *Trichodesmium* DDN release remained unclear. In particular, the effect of light on DDN release has been poorly studied. To date, only one *Trichodesmium* culture study has reported a significant release of DDN and DOC after a rapid shift from low-light to high-light regimes to protect the photosynthetic apparatus (Wannicke et al., 2009).

Here we investigated the effect of light on DDN release and C / N fixation stoichiometry of *Trichodesmium* in the field under contrasting situations, i.e. during a *Trichodesmium* bloom in the western equatorial Pacific and in a non-bloom area in the South China Sea.

2 Material and methods

This study was performed onboard the R/V *Dong Fang Hong* 2 during two cruises to the western equatorial Pacific Ocean (6 December 2015 to 12 January 2016) and the South China Sea (15 May to 7 June 2016). Experiments were conducted at three stations (Supplement Fig. S1), among which one of them was characterized by the presence of a *Trichodesmium* bloom (western equatorial Pacific Ocean station S0320); the other two were located in the South China Sea (A3, D5).

2.1 Seawater sampling and experimental procedures

Water samples were collected from 3 to 5 m depth using 10 L Go-Flo bottles that were attached to a CTD rosette (Sea-Bird 911 CTD). In our experiments, the same 4.5 L surface water samples were collected in the polycarbonate (PC) bottles and then put in six on-deck incubators with different light intensities for NF rate incubations. The light source was natural solar irradiance. Light intensity gradients (92, 54, 28, 14, 8

and 1 % of surface irradiance) were created by using neutral density and blue (061 Mist Blue; 172 Lagoon Blue) filters to adjust the light level (Fernandez et al., 2013; Rijkenberg et al., 2011; Mourino-Carballido et al., 2011). During the incubation period, the light intensity was monitored on deck with a flat 2π photosynthetically available radiation (PAR) sensor (PQS 1 PAR Quantum sensor, Kipp & Zonen) at 1 min intervals. We took the average light intensity of the incubation light period ($> 1\,\mu E\,m^{-2}\,s^{-1}$) as the surface irradiance to calculate light intensities of the six light gradients.

2.2 Nutrients, Chl *a* and abundance of *Trichodesmium*

Nutrient samples were collected in 100 mL high-density polyethylene (HDPE) bottles and kept frozen at −20 °C until analysis. Nanomolar levels of soluble reactive phosphorous (SRP) were determined according to Ma et al. (2008) with a detection limit of 1.4 nM and relative precision of ±2.5 %. Nanomolar levels of nitrate were analyzed using the chemiluminescent method (Garside, 1982) with a detection limit of 2 nM.

For Chl *a* concentration determination, 1 L of seawater was filtered on GF/F filters, wrapped in aluminum foil and stored at −20 °C until analysis onshore. Chl *a* was extracted in 90 % acetone, refrozen for 24 h and analyzed fluorometrically according to the method described by Welschmeyer (1994).

For *Trichodesmium* abundance determination, 1 L of seawater was sampled in HDPE bottles and immediately fixed with 10 mL Lugol's solution. Onshore, subsamples were settled for 48 h, the supernatant was removed and *Trichodesmium* filaments (trichomes) were counted on a Nikon Eclipse 50i optical microscope.

2.3 Molecular assessment of diazotrophs

For DNA analysis, 4 L of seawater was filtered through 0.2 µm pore-sized membrane filters (Supor 200, Pall Gelman, NY, USA), which were stored in liquid nitrogen until analysis. DNA was extracted according to Massana et al. (1997) with some modifications. Briefly, each filter was cut into pieces and placed into a 2 mL sterile screw cap micro-tube containing 0.2 g autoclaved glass beads and 0.8 mL GTE buffer (100 mM EDTA, 50 mM tris, 0.75 M sucrose). The tubes were agitated three times for 40 s in a homogenizer (FastPrep-24, MP Bio, USA) at $4.5\,m\,s^{-1}$, and they were then frozen and thawed three times in liquid nitrogen. The next steps followed the protocol of Massana et al. (1997).

Four published quantitative polymerase chain reaction (qPCR) probe–primer sets (Church et al., 2005a, b) were used for qPCR analysis. Relevantly, the *nif*H genes of four photoautotrophic diazotroph groups were targeted: *Trichodesmium* spp., *Richelia* spp. associated with *Rhizosolenia* spp. (het-1), and the unicellular groups A (UCYN-A) and B (UCYN-B). We used the thermal cycling conditions and reaction mixtures as described previously by Zhang et al. (2011) with slight modifications. Triplicate 20 µL qPCR mixtures were used for each sample and standard, reaction mixes contained 10 µL Premix Ex Taq (probe qPCR) (RR390A, Takara Bio Inc., Dalian, China), 400 nM each of forward and reverse primer, 400 nM of fluorogenic probe, and 1 µL of environmental DNA or plasmid standards. We used dilution series of four linearized plasmids as standards, which contained inserts matching four primer–probe sets. The real-time quantitative PCR was performed on a CFX96 real-time system (Bio-Rad Laboratories, USA) with the following thermal cycling conditions: 50 °C for 2 min, 95 °C for 2 min and 45 cycles of 95 °C for 15 s, followed by 60 °C for 1 min. The quantification limit was determined empirically to be one copy per reaction. The amplification efficiency varied between 90 and 100 %. The negative controls contained complete reaction ingredients except environmental DNA or standards; no amplification was found in the negative controls.

2.4 N_2 and carbon fixation rate measurements

NF rates were determined according to the dissolution method: the $^{15}N_2$-enriched seawater was prepared following the same device and procedure as described in Shiozaki et al. (2015) and 200 mL $^{15}N_2$-enriched seawater was added into each 4.5 L PC incubation bottle (at bloom station S0320, 1.2 L PC bottles were used) triplicated. The $^{15}N_2$ gas (98.9 %) from Cambridge Isotope Laboratories was used. We conducted a blank check for $^{15}N_2$ gas (contamination of bioavailable non-N_2 ^{15}N) as mentioned in Dabundo et al. (2014). Briefly, triplicate 2 mL $^{15}N_2$ gas and 10 mL natural seawater were injected into 20 mL headspace vials, sealed with a septum stopper and then shaken overnight. The $\delta^{15}N$ of total dissolved nitrogen (TDN) was measured and compared with the $\delta^{15}N$ of natural seawater samples. Values of $\delta^{15}N$ TDN of the blank seawater and test seawater groups were 4.7 and 5.0 ‰, respectively, suggesting no contamination of the $^{15}N_2$ gas.

At station S0320, the *Trichodesmium* bloom station, ^{13}C-labeled sodium bicarbonate (99 atom% ^{13}C; Cambridge Isotope Laboratories) was added in parallel with $^{15}N_2$ to each bottle at a final tracer concentration of $70\,\mu mol\,L^{-1}$ to simultaneously measure the CF and NF rates. At each irradiance level, triplicate water samples (4.5 L or 1.2 L PC bottle) were incubated on deck in incubators with surface seawater flow through. The surface-cooling seawater was connected with incubators in parallel to keep the temperature variations synchronous in six incubators. Thus, temperature was not a variable parameter that could influence the variability of final rates.

After 24 h incubation, water samples were gently filtered (< 200 mm Hg) onto precombusted (450 °C, 4 h) 25 mm Whatman GF/F (0.7 µm) filters, preserved at −20 °C and then dried in an oven overnight (50 °C). The particulate or-

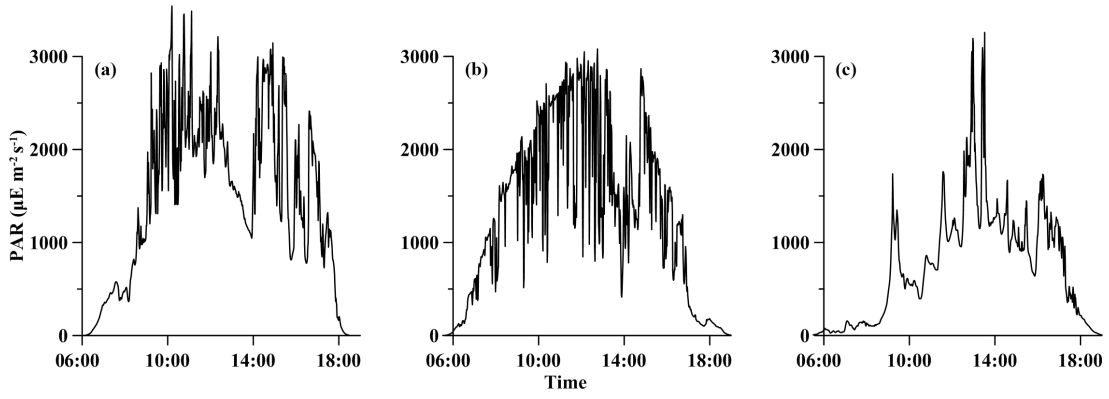


Figure 1. Temporal variations in photosynthetically active radiation (PAR $\mu E\,m^{-2}\,s^{-1}$) obtained on deck during the experiment periods, **(a)** for station S0320, **(b)** for station A3 and **(c)** for station D5.

ganic C (POC) / PON concentrations and isotopic values were analyzed on a Flash EA (Thermo Fisher Flash HT 2000) IRMS (Thermo Fisher Delta V plus). International reference material (USGS40) with a different amount of C / N and certified $\delta^{15}N$ and $\delta^{13}C$ values of -4.5 and $-26.2\,‰$, respectively, was inserted every eight samples to check the drift and ensure the accuracy of the measurements. The reproducibility for $\delta^{15}N$ and $\delta^{13}C$ measurements were both better than $0.3\,‰$. The NF and CF rates were calculated by using similar equations proposed by Montoya et al. (1996) and Hama et al. (1983), respectively.

2.5 Light response curves for N_2 fixation and carbon fixation

We follow the photosynthetic model by Webb et al. (1974):

$$N = N_m(1 - \exp(-\alpha I/N_m)) + N_d, \quad (1)$$

where N_m is the maximum rate of NF at light-saturating irradiance, N_d is the rate measured in darkness, I is the natural irradiance and α is the light affinity coefficient for NF rate, to construct the irradiance curves for NF. Similarly, the light response curve of CF was obtained. The light saturation coefficient I_k was defined as N_m/α.

2.6 DDN net release to the dissolved pool

To determine the TDN concentration and $\delta^{15}N$ TDN according to Knapp et al. (2005), 40 mL of the filtrate (passed through precombusted GF/F filters) of each NF incubation bottle was collected and preserved at $-20\,°C$. Briefly, TDN was oxidized to nitrate by a persulphate oxidation reagent (purified by recrystallization three to four times) and the concentration was measured using the chemiluminescent method (Garside, 1982). The $\delta^{15}N$ TDN-derived nitrate was analyzed by using the denitrifier method (Sigman et al., 2001). The reproducibility for $\delta^{15}N$ TDN measurements was better than $0.5\,‰$. The DDN released to the dissolved pool was calculated following the equation proposed by Bonnet et al. (2016a).

2.7 Transfer of DDN into non-diazotrophic plankton

To evaluate the short time (24 h) DDN transfer to non-diazotrophic plankton, we followed the method by Adam et al. (2015). Briefly, for the control group, a 10 µm sieve was used to remove most *Trichodesmium* colonies and the remaining community was incubated for 24 h with $^{15}N_2$-enriched seawater. In another group, the whole community was incubated for 24 h and *Trichodesmium* colonies were removed after incubation was terminated. Each experiment was performed in triplicate. The $\delta^{15}N$ difference between the two treatments was considered to be a proxy of the DDN transfer to non-diazotrophic plankton.

3 Results

3.1 Environmental conditions

The temporal patterns of PAR were shown in Fig. 1. The sun rose at $\sim$06:00 and set at $\sim$18:00 (all measurements at the S0320 station were made in local time and all measurements at the other two stations were made in Beijing time). The value of PAR (sampling at 10 s intervals) varied rapidly in a wide range from 0 to 3000 $\mu E\,m^{-2}\,s^{-1}$, which is the typical range of values observed at low latitudes, yet much higher than those generally used in laboratory culture experiments (Bell and Fu, 2005; Wannicke et al., 2009). Although incubations were conducted for 24 h, average PAR during the incubation period (light intensity $> 1\,\mu E\,m^{-2}\,s^{-1}$) was applied for discussion. The average PAR values were 1464 ± 888 (61 %), 1293 ± 903 (70 %) and 743 ± 619, (83 %) $\mu E\,m^{-2}\,s^{-1}$ for stations S0320, A3 and D5, respectively.

The hydrographic and biogeochemical parameters are shown in Table 1. All three stations were characterized by

Table 1. Environmental conditions of the three stations' surface water. nd: not determined.

Station	Salinity	Temperature (°C)	Chl *a* ($\mu g\,L^{-1}$)	SRP (nM)	Nitrate (nM)	DON (μM)	*Trichodesmium* colonies (trichomes L^{-1})
S0320	34.5	29.7	1.2	100	6	9.8 (1.2)	4227 ± 679 ($n = 6$) *thiebautii*
A3	34.6	27.6	0.39	13	7	7.2 (0.5)	nd
D5	34.6	29.3	0.25	24	11	8.4 (0.3)	190 ± 50 ($n = 6$) *thiebautii*

Table 2. Synthesis of PON, POC, and DON concentrations, C / N, carbon consumption and the corresponding NF and CF rates, and NF / CF at station S0320. The "< 10 µm-a" represents a NF rate of < 10 µm community incubated with > 10 µm *Trichodesmium* colonies, "< 10 µm-b" represents the background NF rate of < 10 µm community, and carbon consumption was calculated using POC concentration variation from each irradiance point final concentration to initial POC concentration minus the carbon fixation rate at the corresponding irradiance point.

Irradiance ($\mu E\,m^{-2}\,s^{-1}$)	PON ($\mu M\,L^{-1}$)	Particulate NF rate ($nM\,L^{-1}\,d^{-1}$)	Dissolved NF rate ($nM\,L^{-1}\,d^{-1}$)	POC ($\mu M\,L^{-1}$)	Particulate CF rate ($\mu M\,L^{-1}\,d^{-1}$)	NF / CF	C / N	Carbon consumption ($\mu M\,L^{-1}$)
Initial condition	2.1 (0.0)	–	–	13.4 (0.1)	–	–	6.4 (0)	–
1349	2.5 (0.19)	391 (20)	32 (1.3)	17.3 (1.2)	3.6 (0.19)	9.3 (1.0)	7.0 (0.1)	−0.29 (1.0)
1349 (< 10 µm-a)	0.55 (0.05)	25.1 (3.2)	–	4.1 (0.2)	0.28 (0.03)	11.6 (1.0)	7.5 (0.1)	–
1349 (< 10 µm-b)	0.5 (0.1)	6.5 (1.6)	–	–	–	–	–	–
792	2.3 (0.07)	430 (39)	47 (6.2)	15.7 (0.13)	3.4 (0.1)	7.8 (0.7)	6.8 (0.2)	1.04 (0.12)
410	2.5 (0.09)	401 (40)	54 (7.8)	15.8 (0.87)	2.9 (0.08)	7.4 (0.6)	6.3 (0.1)	0.53 (0.79)
211	2.4 (0.25)	235 (10)	50 (15)	13.9 (0.93)	2.0 (0.07)	8.6 (0.5)	5.9 (0.3)	1.53 (0.90)
129	2.3 (0.09)	85 (23)	13 (3.3)	12.6 (0.28)	0.98 (0.26)	11.6 (0.5)	5.6 (0.4)	1.80 (0.53)
15	2.1 (0.22)	27 (8)	7.7 (0.35)	10.9 (1.0)	0.44 (0.08)	16.8 (3.2)	5.3 (0.1)	2.88 (0.97)

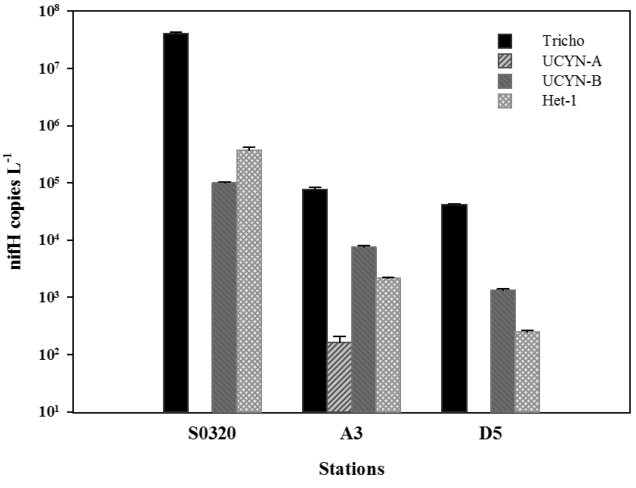


Figure 2. Cyanobacteria diazotroph *nif*H phylotype abundances (*nif*H gene copies L^{-1}). Tricho: *Trichodesmium* spp.; UCYN: unicellular N_2-fixing cyanobacteria from Group A, B; Het-1: heterocystous cyanobacteria from Group 1. Error bars represent the standard deviation for triplicate natural samples.

low nutrient concentrations (NO_3^- 6 to 11 nM, PO_4^{3-} 13 to 100 nM), relatively high salinity (34.5–34.6) and high sea surface temperature (27.6–29.7 °C). At the *Trichodesmium* bloom station (station S0320), Chl *a* concentrations were 1.2 mg m^{-3}, much higher than those measured at the other stations (0.25 and 0.39 mg m^{-3}). Results from the *nif*H phylotype abundances showed that *Trichodesmium* accounted for > 98.8, 88.6 and 96.4 % of the diazotrophic community at stations S0320, A3 and D5, respectively (Fig. 2). The dominant *Trichodesmium* species was *Trichodesmium thiebautii* for stations S0320 and D5, with an abundance of 4227 ± 679 ($n = 6$) and 190 ± 50 ($n = 6$) trichomes L^{-1}, respectively. POC / N concentration of the < 10 µm fraction represented < 25 % of the bulk POC / N (see Table 2 and below), supporting that *Trichodesmium* was the dominant phytoplankton community at the blooming station.

The net NF rates in the surface light intensity were 390.6 ± 20.4, 12.2 ± 1.8 and 9.9 ± 0.4 nM N d^{-1} at stations S0320, A3 and D5, respectively. The NF rate at the blooming station was 30–40 times higher than that of the two non-bloom stations. Detailed experimental data, including concentrations and isotopic values, for initial and final time points are listed in Tables S1–S3 in the Supplement. However, trichome-normalized rates were 92 and 52 pM N trichomes^{-1} d^{-1}, respectively, for stations S0320 and D5, revealing a more consistent rate per trichome.

3.2 Light response of net (particulate) N_2 fixation

As shown in Fig. 3, these NF-I curves showed a general pattern indicating that net NF rates increased significantly with light intensity from 10 to 400 $\mu E\,m^{-2}\,s^{-1}$. The R^2 values

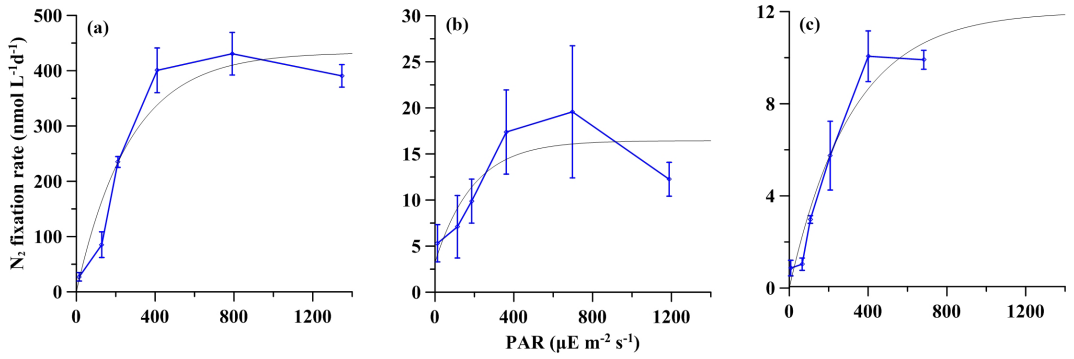


Figure 3. Net (particulate) NF versus irradiance. The gray curves represent the fitted NF-I curves. The error bar represents the standard deviation of triplicate incubations. **(a)** Station S0320; **(b)** station A3; **(c)** station D5.

of fitted NF-I curves were 0.92, 0.71 and 0.95 at stations S0320, A3 and D5, respectively (all p values < 0.0001) and then saturated at around 400 $\mu E\,m^{-2}\,s^{-1}$. The simulated I_k values for NF were 271, 193 and 315 $\mu E\,m^{-2}\,s^{-1}$, respectively, for stations S0320, A3 and D5, with an average value of $260 \pm 51\ \mu E\,m^{-2}\,s^{-1}$.

Results of CF for stations S0320 showed a traditional PI curve pattern without apparent light inhibition (solid curve in Fig. 4a). The fitted curve of CF showed a pattern consistent with those of NF (dashed curve in Fig. 4a), giving an I_k value of 292 $\mu E\,m^{-2}\,s^{-1}$ falling within the I_k range of the three NF-I curves, and the R^2 of the fitted CF-I curve was 0.90 (p value < 0.0001).

3.3 Particulate C / N metabolism of *Trichodesmium* bloom

The ratio of CF to NF was variable as light varied (Fig. 4b). The values of CF / NF ranged from 7.4 ± 0.6 to 9.3 ± 1.0 when light intensities were saturated, while the ratios increased significantly from 7.4 ± 0.6 to 16.8 ± 3.2 as light intensities decreased from 410 to 15 $\mu E\,m^{-2}\,s^{-1}$.

The initial concentrations ($n = 3$) of POC and PON were 13.4 ± 0.1 and $2.1 \pm 0.0\ \mu M$, respectively, with a mean C : N molar ratio of 6.4 (horizontal lines in Fig. 4c and d), which is almost identical to the Redfield C / N ratio of 6.6. After incubations under various light intensities, the final POC concentrations showed a decreasing trend (p value < 0.0001) ranging from 17.3 ± 1.2 to $10.9 \pm 1.0\ \mu M$ as the irradiance decreased. Below $\sim 200\ \mu E\,m^{-2}\,s^{-1}$, the final POC concentration was even lower than the initial POC concentration (red dashed line in Fig. 4c), suggesting that the light compensation point (I_c) is around 200 $\mu E\,m^{-2}\,s^{-1}$. A similar light-dependent pattern was found for PON. However, final PON concentrations, varying from 2.1 ± 0.2 to $2.5 \pm 0.2\ \mu M$, were always higher than the initial concentration (blue dashed line in Fig. 4c) without a compensation point.

The observed C : N ratio of bulk particulate matter (5.3–7.0; Fig. 4d) is consistent with previously reported ranges for *Trichodesmium* (LaRoche and Breitbarth, 2005; Mulholland, 2007). However, a strong light dependency was also observed for the final C / N after incubation. The saturated irradiance of $\sim 400\ \mu E\,m^{-2}\,s^{-1}$ was likely a threshold; below the saturation light the final C / N tended to be lower than the initial C / N of 6.4 (dashed horizontal line in Fig. 4d).

3.4 DDN net release to the dissolved pool

The rate of $DD^{15}N$ net release in the TDN pool ranged from 7.7 ± 0.4 to $54.1 \pm 7.8\ nM\,N\,d^{-1}$ for station S0320, from 0.7 ± 0.2 to $1.0 \pm 0.1\ nM\,N\,d^{-1}$ for D5 and from 1.9 ± 1.5 to $5.0 \pm 1.6\ nM\,N\,d^{-1}$ for A3. The contribution of DDN net release to gross NF ranged from 8 ± 0 to 25 ± 6, 6 ± 6 to 45 ± 14 and 14 ± 11 to $50 \pm 5\,\%$ for stations S0320, D5 and A3, respectively (Fig. 5). The overall range agrees well with previous field studies (Glibert and Bronk, 1994; Mulholland et al., 2006; Bonnet et al., 2016a; Konno et al., 2010; Benavides et al., 2013; Berthelot et al., 2015). Our data revealed that the fraction of DDN release to gross NF increased as light decreased (all p values < 0.05).

3.5 DDN transfer to non-diazotroph biomass

After 24 h of incubation, the DDN transfer rates (transferred to the non-diazotrophic plankton) were 18.6 ± 3.6, 0.5 ± 0.3 and $0.7 \pm 0.5\ nM\,N\,d^{-1}$ corresponding to $5\,\% \pm 1\,\%$, $4\,\% \pm 3\,\%$ and $5\,\% \pm 4\,\%$ of total NF (net plus dissolved), respectively, for stations S0320, D5 and A3 (Fig. 6). Our fractions are consistent with previous reports by Bonnet et al. (2016a), in which $6\,\% \pm 1\,\%$ of $DD^{15}N$ was transferred to non-diazotrophic plankton in naturally occurring *Trichodesmium* blooms. Our fractions are slightly lower than the $DD^{15}N$ transfer ($\sim 12\,\%$) by Berthelot et al. (2016), who inoculated *Trichodesmium erythraeum* into natural surface oligotrophic seawater. Our results confirm that *Trichodesmium* could actively transfer newly fixed nitrogen to non-diazotrophs.

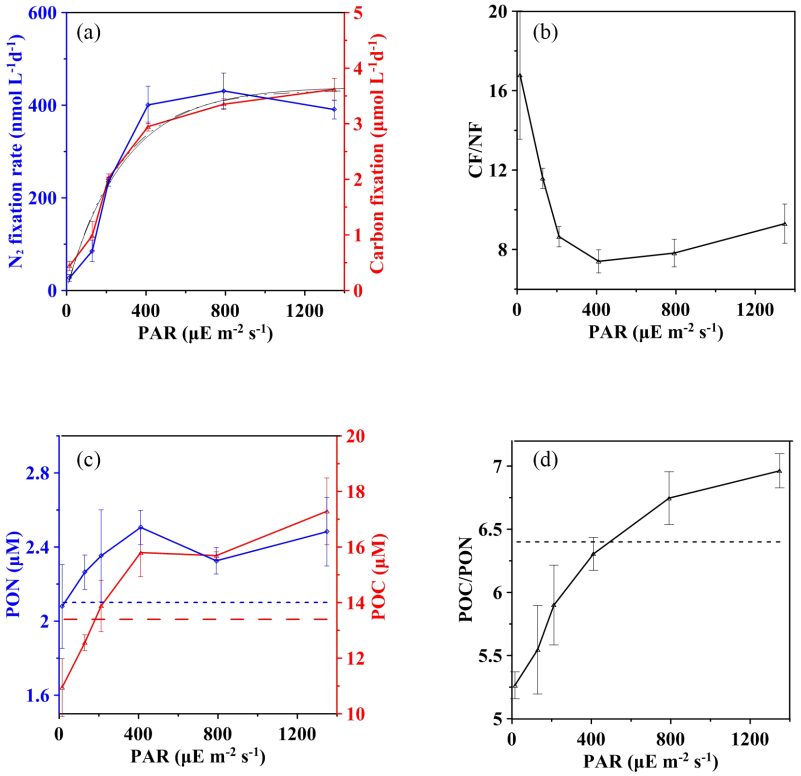


Figure 4. Effect of light on the carbon and nitrogen budget at station S0320 with a *Trichodesmium thiebautii* bloom. **(a)** Carbon (red solid line) and nitrogen (blue solid line) fixation at different light intensities with fitted light response curves (black solid line for CF and black dotted line for NF). **(b)** The CF / NF ratios at different light intensities. **(c)** The final concentrations of POC (red solid line) and PON (blue solid line) after incubations under different light intensities and initial values of POC (red dashed line) and PON (blue dashed line) concentration. **(d)** C / N ratios after incubation in different light conditions. Error bars represent the standard deviation of triplicates.

4 Discussions

4.1 High light demand for *Trichodesmium* N_2 fixation

The simulated I_k values in this field study for *Trichodesmium* fell within the high end of the reported I_k values for photosynthesis (LaRoche and Breitbarth, 2005). These values suggest a high light demand for *Trichodesmium* NF. The high energy requirement of *Trichodesmium* is not only for breaking the strong triple bond of the N_2 molecule but also for numerous strategies, such as high respiration rates and the Mehler reaction, to protect the sensitive nitrogenase against the oxygen evolved by photosynthesis during daytime (Kana, 1993). Thus, *Trichodesmium* generally dwells in the upper euphotic zone of the tropical and subtropical ocean to meet the high light demands (Capone et al., 1997; Gandhi et al., 2011).

Generally, in the tropical and subtropical regions, average surface light intensities are around 1000 $\mu E\,m^{-2}\,s^{-1}$ on sunny days. By taking into account the light extinction coefficient of seawater, the maximum depth for *Trichodesmium* to perform NF would be shallower than 15–40 m. This result matches well with many field observations that most NF occurs in the well-lit (0–45 m) region of the euphotic zone (Capone et al., 1997; Böttjer et al., 2016). This also agrees well with the observation that maximum *Trichodesmium* densities often appear at around 15 m depth and typically form blooms at the surface (Carpenter and Price 1977; Capone et al., 1997; Gandhi et al., 2011).

Our results also suggest that NF of *Trichodesmium* could respond to variable light intensity in the field within a short time period (24 h). Such results mean that light conditions during on-deck incubations should also be presented along with rate data if we want to compare field NF results among different studies. Unfortunately, the field NF rates had rarely been reported with consideration of in situ light conditions, although the light control on NF is well known to researchers.

Compared with laboratory strains acclimated to low light, field-observed NF-I curves are more representative of the real ocean with greater applicability. The parameter consistency among our three stations in the NF-I curves regardless of the wide range of trichome biomass and maximum NF rates, offers critical information on light-associated parameters in model predictions of global nitrogen fixation (Fennel et al., 2001; Hood et al., 2001).

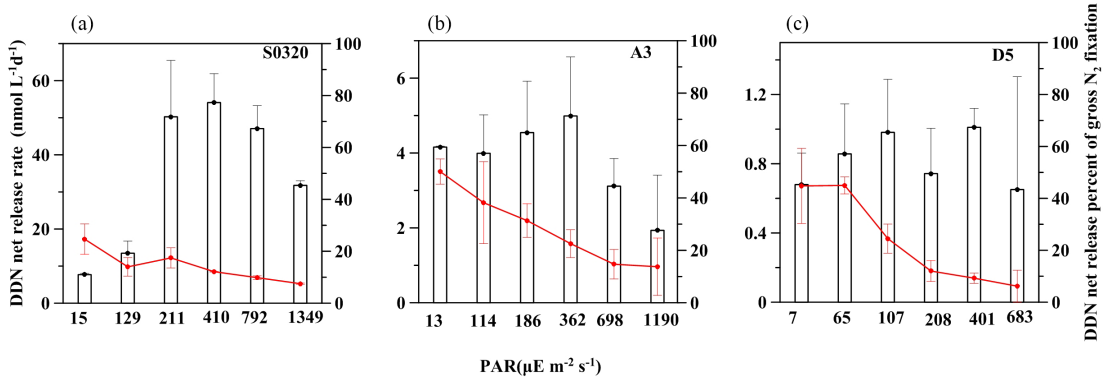


Figure 5. DDN net release rate (bar charters) and percentage of total NF (red lines) under different light intensities for stations S0320, A3 and D5. Error bars represent the standard deviation of triplicates.

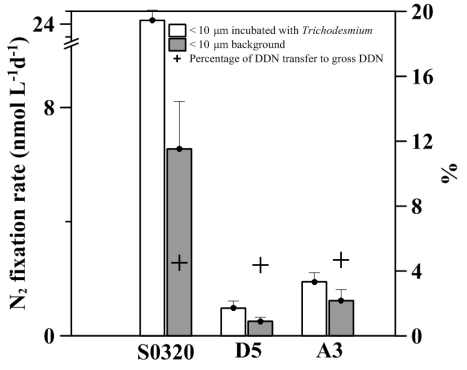


Figure 6. The NF and DDN transfer measured in two treatment groups for stations S0320, D5 and A3. The black bars represent a background NF rate of $< 10\,\mu m$ community. White bars represent a NF rate of $< 10\,\mu m$ community incubated with $> 10\,\mu m$ *Trichodesmium* colonies. The error bar represents the standard deviation of the triplicate. Crosses stand for the percentage of DDN transferred to the $< 10\,\mu m$ community to total N_2 fixation.

4.2 Metabolism tradeoff between carbon and nitrogen fixation under light stress

In our field incubations, bulk C / N molar ratios were always lower than the corresponding net CF : NF ratios at all light intensities (Fig. 4b, d). As reported in both culture and field studies, *Trichodesmium* usually exhibits a higher CF : NF ratio than the expected stoichiometric value of 6.6 (Mulholland, 2007). Several hypotheses have been proposed: (1) the underestimation of gross NF rates by overlooking the ^{15}N signal in the dissolved pool (Glibert and Bronk, 1994; Mulholland et al., 2004); (2) the underestimation of N assimilation rates if there is uptake of other N sources such as nitrate or ammonium (Mulholland et al., 1999); (3) high carbon requirements to synthesize carbohydrate as a ballast for vertical migration (Villareal and Carpenter, 1990); (4) the support of the high energy cost, high respiration and Mehler reaction pathways (Carpenter and Roenneberg, 1995); and (5) the CF by non-diazotrophic phytoplankton.

Here, the low DDN net release rate is not supportive of the first hypothesis. As the incubation experiments were used the same bulk water and only light intensity was manipulated, the initial bioavailable nitrogen concentration between different treatments was almost the same. Thus, no apparent evidence supports the second hypothesis. Meanwhile, the third and fourth hypotheses could not explain the increased CF : NF ratio trend with the decrease in light intensity over the low-light condition (p value was 0.0005). In fact, the contribution from non-diazotrophic phytoplankton to CF cannot be excluded from bulk water incubation; however, the contribution is limited even at low light after assessment (see Supplement). As aforementioned, *Trichodesmium* was the dominant phytoplankton species; thus, the variation pattern of CF rates and POC concentrations against different light intensities mainly reflects the carbon metabolism of *Trichodesmium*.

In fact, in unialgal culture experiments (Berthelot et al., 2015), CF : NF ratios (1.8–5.6) were quite close to the POC : PON ratio (3.8–5.5) of a variety of diazotrophs including *Trichodesmium*. In our field study, the abundance of *Trichodesmium* was up to 4227 trichomes L^{-1}, and the measured CF : NF ratios (9.3) at in situ light were close to the initial POC : PON ratio (6.4). Similarly, in a surface bloom of *Trichodesmium* in the Arabian Sea, Gandhi et al. (2011) also observed a low CF : NF ratio of ~ 4 (NF rate of 1125 nM N h^{-1} and CF rate of 4594 nM C h^{-1}), even lower than the Redfield ratio. Consistency among aforementioned laboratory and field studies suggested that CF : NF ratios of *Trichodesmium* should not be particularly high.

Under light limitation where *Trichodesmium* faced severe carbon consumption and energy shortage, energy was likely reallocated between CF and NF. We hypothesized that under low-light stress, *Trichodesmium* physiologically preferred to allocate more energy for CF to alleviate the intensive carbon consumption by respiration. This is analogous to the *Tri-*

chodesmium iron limitation metabolism, of which photosynthesis takes priority over NF to get iron (Shi et al., 2007). Since the short-term (< 24 h) light manipulation in our experiments resembles the natural variation in irradiance, such a metabolism tradeoff between CF and NF under low light for *Trichodesmium* may happen frequently and widespread in the field, for example, on cloudy days and rainy days.

The proper allocation and utilization of energy (ATP) and reductant (NADPH) among various cellular processes determines the growth rate of *Trichodesmium*. Light-dependent reactions of photosynthesis are the major pathways to produce these molecules. In cyanobacteria, both respiratory and photosynthetic electron transport occur in the thylakoid membrane and compete for the electron transport chain (Oliver et al., 2012). When light intensity decreases, the light-dependent reactions of photosynthetic activity decrease concurrently, resulting in reduced production of ATP and NADPH and increased respiration activity. The negative feedback of POC consumption lead to more ATP and NADPH being reallocated to CF process, and in turn, the NF process was down-regulated.

4.3 Light modulation of DDN net release fraction

A previous study found that *Trichodesmium* trichomes contain only 15–20 % of diazocyte cells capable of NF (Kranz et al., 2011, and references therein). The remaining non-diazocyte cells rely on the release of bioavailable N, mainly the form of ammonium or amino acid, from diazocytes (Mulholland et al., 2004; Kranz et al., 2011). This process is directly proved by ^{15}N labeling and the NanoSIMS method in which the ^{15}N signal is rapidly distributed into the majority cells of *Trichodesmium* trichomes and even the ^{15}N label signal is relatively lower in the center cells, which is probably a zone of diazocytes (Finzi-Hart et al., 2009; Bergman et al., 2013). Our results demonstrated that light does not directly regulate the absolute amount of DDN release. However, to discuss the physiological status for DDN distribution in the dissolved pool and particulate pool (mainly *Trichodesmium*), the proportion of DDN released into the dissolved pool is a proper indicator. In this study, the proportion of DDN in the dissolved pool increases with the light intensity decrease. This suggests that the physiology status of diazotrophs was modulated by light and could then take control of the DDN release process. At station A0320 high light intensities ($> 400\,\mu E\,m^{-2}\,s^{-1}$), the final POC and PON concentrations increased significantly, also implying an active physiology status of *Trichodesmium*, and the fraction of DD^{15}N release in the dissolved pool ranged from $6\,\% \pm 6\,\%$ to $23\,\% \pm 5\,\%$. Actually, several unialgal culture studies, including *Trichodesmium* and UCYN-B and UCYN-C, showed less than 2 % DD^{15}N release in the dissolved pool (Berthelot et al., 2015; Benavides et al., 2013). These low values were attributable to the exponential growth phase and optimal growth conditions and a lack of exogenous factor influence such as viral lysis (Hewson et al., 2004) and sloppy feeding (O'Neil et al., 1996). Nevertheless, our values at high light are congruent with the field study (7–17 %) by Berthelot et al. (2016). Similar to their findings, we suggested that the active cell status and exposure to the exogenous factor may only lead to a slightly higher proportion of DDN net release. Under the light limitation stress, the inactive physiology state condition of *Trichodesmium* was reflected by the decrease in POC concentrations and activity of the CF and NF; thus, the DDN fixed by diazocytes was likely not efficiently transferred to other cells along the trichomes, therefore accumulating in the dissolved pool. Furthermore, a number of cells could breakdown and directly release intracellular bioavailable nitrogen. The fraction of DD^{15}N release in the dissolved pool ranged from $17\,\% \pm 4\,\%$ to $50\,\% \pm 5\,\%$ at low-light conditions ($400\,\mu E\,m^{-2}\,s^{-1}$). This conclusion is also consistent with Bonnet et al. (2016a) for two natural *Trichodesmium* bloom studies that in the decaying bloom case, high ammonium concentration accumulation ($3.4\,\mu mol\,L^{-1}$) and a high proportion of DDN release (20 ± 5 to $48 \pm 5\,\%$) were observed, while in the exponentially growing bloom case, the proportion of DDN release only ranged from 13 ± 2 to $28 \pm 6\,\%$ and without apparent accumulation of ammonium.

As summarized in Berthelot et al. (2015), most of the higher end of reported DDN net release values were estimated using the difference between gross NF rates measured with acetylene reduction assays (ARAs) and the net NF measured using the $^{15}N_2$ bubble labeling technique (Montoya et al., 1996). The known uncertainty of the conversion factor for acetylene to N_2 for the ARA method (Montoya et al., 1996; Shiozaki et al., 2010) may bias the DDN release estimate, while potential underestimation of net NF from the $^{15}N_2$ bubble method may result in a higher DDN net release. In this study the direct measurement of the DD^{15}N in the dissolved pool using the improved dissolution $^{15}N_2$-enriched seawater method (Mohr et al., 2010) was applied to assess the DDN net release; thus, our data were quite reliable.

5 Conclusions

In this study, we provide quantitative information on the effect of light on NF and DDN net release of field *Trichodesmium* and found that the NF was a function of light intensity and biomass. The light requirement of *Trichodesmium* NF was higher relative to its photosynthesis light demand. The empirical I_k value suggests that the *Trichodesmium* population maxima should appear at < 15 m depth to obtain sufficient light energy. Furthermore, light intensity is a crucial factor to drive the physiological state of *Trichodesmium*, which subsequently determined the C / N metabolism and DDN net release. Thus, we suggest the necessity to provide field light data along with nitrogen fixation data for on-deck incubation for the future studies.

Recent studies suggested that unicellular cyanobacteria diazotrophs, inhabiting different niches, especially UCYN-A, are distributed more widely in the global ocean and may contribute equally to the NF flux with *Trichodesmium* (Zehr et al., 2016; Martínez-Pérez et al., 2016). More field studies are needed in future to explore the light response of those UCYN diazotrophs to further understand their light behavior and to optimize the role of diazotrophs in global NF models.

Data availability. The data associated with the paper are available from the corresponding author upon request.

Supplement. The supplement related to this article is available online at: https://doi.org/10.5194/bg-15-1-2018-supplement.

Competing interests. The authors declare that they have no conflict of interest.

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