Reply to SC-1

From the perspective of a modeller with an interest in *Trichodesmium*, this is a useful paper. One reference you may have missed is Oliver et al. (2012). It would be interesting to see a comparison of the results in your manuscript with the results you'd expect given the energetic cost of nitrogen fixation versus nitrate uptake, and the energetic cost of chlorophyll production. Oliver et al. (2012) would be a useful resource to help make that comparison.

Author response:

Thanks for your appreciation and it is a good suggestion to do such comparison. In this study, we did not perform nitrate assimilation and chlorophyll dynamic monitor measurements, so it is hard to fulfill this target. However, in this informative paper, we got many helpful cellular metabolism physiology behaviors on energy allocation between different processes and added that information in our discussion part (Page 12 line 21-25 and Page 13 line 1-2). Regarding to the energetic cost of nitrogen fixation versus nitrate uptake, Eichner et al. (2014) had given a detailed study. Hope our founding could help the modellers.

Eichner, M., Kranz, S. A., and Rost, B.: Combined effects of different CO_2 levels and N sources on the diazotrophic cyanobacterium *Trichodesmium*, Physiologia plantarum, 152, 316-330, 2014.

Reply to RC-1

Besides DDN release, what new in our paper are 1) this is the first data report of N_2-fixation irradiance curve with precise I_k for *Trichodesmium*, particularly, in the field, 2) simultaneous measurements of C/N_2 fixation reveals light effect on *Trichodesmium*'s metabolism, 3) we applied the most advanced $^{15}N_2$ pre-enriched seawater method for N_2-fixation and DDN release

Referee #1, major comment #1

Diazotroph derived nitrogen (DDN) release increases with increase in light intensity at S0320 (Fig. 5 a) but there was no variation at the other two stations (Fig. 5b,c). However, % of the total NF release always decreases. On the other hand, N_2 fixation increases with increase in light intensity and saturates at some

point (Fig. 4a). Put all these pieces of information together, it appears that it becomes difficult to say what role light play in DDN release. Diazotrophs would release N anyways, so what is the role light (they would release even if put them in absolute dark). Therefore, the discussion provided in the section 4.3 is not convincing

Author response:

Reviewer is correct about the light does not regulate the absolute amount of release and diazotrophs would release N anyway. However, the % release is an indication of budget or balance of N in cell. To discuss the physiological status for DDN redistribution, % release is a proper indicator. Stand on this point, light regulate the percent retention of fixed N in *Trichodesmium*.

According to this comment, we added two sentences to address the properness of using % release instead of absolute amount of release to represent the physiology status of *Trichodesmium* (Page13 line 9-13).

Referee #1, major comment #2

Were all the incubations samples at each station taken from the same Niskin Bottle? I believe not because of bottle capacity. As we know the sample (especially when the cell abundance is high) in different Niskin bottles could not be homogeneous although taken from the same depth and the same CTD. If the cell abundances were different in different light incubations to start with, then the rates would be different because of cells and not because of light. So it would be helpful if authors provide the biogeochemical data (at least in supp info) for each Niskin that is used for different light incubations

Author response:

All the incubations samples were taken from the same cast (same depth) but not same bottle. Six samples for *Trichodesmium* abundance may come from different Niskin bottles, however, results in Table 1 showed that the *Trichodesmium* abundance varied within 25% revealing consistency. On the other hand, NF rates varied from 400 to 2000% under different light conditions (n=3 for each light level). Although samples came from different Niskin bottles, such highly variable NF rates over irradiance should reflect mainly the physiological response of *Trichodesmium* to light. The heterogeneity was included in the error bar of NF measurements.

Referee #1, major comment #3

How does the "average" intensity of light estimated. Were the light measurements continuous or

monitored n times during the day?

Author response:

The light measurements were continuously monitored at ten second interval over the entire cruise. The "average" intensity of light was the averaged value of measured PAR larger than 1μE m^{-2} s^{-1} during the incubation period. In this version we added more information regarding the light monitoring and estimation of "average" light intensity.

Referee #1, major comment #4

While changing the light conditions, some density filters were used. Was the wavelength, which these filters block, was also estimated? Do they block the same fraction wavelength for all wavelength?

Author response:

This question was raised due to our unclear description. We followed published papers (Fernandez et al., 2013; Rijkenberg et al., 2011; Mourino-Carballido et al., 2011) to simulate lights by using Lee neutral density and blue (061 Mist blue; 172 Lagoon blue) filters. The neutral density filter blocked the same fraction wavelength while the blue filters prefer to block long wavelength and transmit more blue light. We added more descriptions in this version for light manipulation Material and Methods.

Referee #1, major comment #5

POC:PON ratio could be close to the Redfield ratio but the Carbon upatake:N_2 fixation ratios (Fig. 4b) are surprising. CF:NF ratios can be up to three order magnitude higher even in tricho bloom conditions, where highest N_2 fixation rates were measured (Gandhi et al., 2011). In not very active N fixation regions, this ratio could be even higher. This is simply because most of photoautotroph fix C but not all can N_2. So C fixation to N ($NO_3^+NH_4^+$Urea N_2 fixation) uptake ratios would be close to Redfield. I would suggest the authors to look for the hypotheses presented on page 11 (lines 18-23) and explanations at several other places.

Author response:

As mentioned by previous studies (Mulholland, 2007), *Trichodesmium* are known to exhibit a higher C:N_2 fixation ratios. Possible reasons were presented in the "Discussions 4.2" including the non-diazotrophs carbon fixation and other bioavailable N uptake mentioned by the reviewer. Indeed, in Gandhi et al. paper, most of observed CF:NF ratios were much higher than the Redfield ratio, however, in the surface bloom

condition (Station NF6), the NF rate in surface water was 1125 nM N h^{-1} and CF rate was 4594 nM C h^{-1}, with a CF: NF ratio of ~4, even lower than the Redfield ratio.

In fact, in pure culture experiments of various diazotrophs including *Trichodesmium* (Berthelot et al., 2015), CF:NF ratios (1.8-5.6) were low and quite close to the POC:PON ratio (3.8-5.5) of cultured biomass. In our field study, *Trichodesmium* abundance was up to 4227 trichomes. L^{-1}, the measured CF:NF ratios (9.3) at *in situ* light also matched with the initial POC:PON ratio (6.4). Consistency among aforementioned studies suggested that CF/NF should not be particularly high.

According to this comment, we added more discussions (page 12 Lines 8-13).

Referee #1, major comment #6

Provide an estimate of fraction of released DON and released inorganic N (ammonium) uptake by non-diazotrophs.

Author response:

Actually, the Fig.6 was a simply flux estimation of released DDN (both DON and DIN) uptake by non-diazotrophs at surface condition. The basic procedures and principle were present in "Material and Methods 2.7". Unfortunately, the method for isotopic composition of low level NH_4^+ was not established in our laboratory. In this study, the fractions of released DDN (both DON and DIN) uptake by non-diazotrophs were only around 5% for at all three stations under *in situ* light. To separate DDN into DON and DIN fractions is an interesting idea indeed, but, impossible for our laboratory at current stage.

Referee #1, minor comments:

Page 1: Title should be revised as "field *Trichodesmium*" reads a bit awkward. I would suggest: "The effect of light on N_2 fixation and net nitrogen release in a field study".

Author response:

We would like to keep *Trichodesmium* in the title to highlight its importance. The new tile is "Light effect on N_2 fixation and net nitrogen release of *Trichodesmium* in the field".

Page 3, line 8: Light is an ultimate source of energy for everything not only to photoautotrophs. Revised this sentence.

Author response:

We changed to “light is the primary energy source”.

Page 4, line 9: Not most but only some NF rates have used ^{15}N techniques, most have used Acetylene reduction assay, see Table 3 in (Singh et al., 2013), Table 5 in (Capone et al., 2005) and Table 4 in (Benavides and Voss, 2015).

Line 10: “The ^{15}N. . .. . ..into account”. ^{15}N enrichment is taken into account as can be seen the equation (6) in (Montoya et al., 1996): the N_2 takes care of the enrichment. I think authors mean the released ^{15}N-TDN during the incubation is not taken into the account and hence the underestimation.

Author response:

Yes. The description is now “In most NF rates measurements that 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 aforementioned potential underestimation of NF rates”

Line 16-17: Contribution of N_2 fixation to export production can be up to 92% during Trichodesmium bloom (Gandhi et al., 2011; Kumar et al., 2017)

Author response:

We added reference to highlight the importance of NF in export production (Page 4 Lines 18) in Introduction

Line 21: “reports” should be replaced by “has reported”

Author response:

Corrected

Page 5: Line 5: Could 4000 cells/L be called bloom?

Author response:

In station S0320, the abundance of *Trichodesmium* was up to 4227 trichomes/filaments. L^{-1}. Comparing with the previous study by Bonnet et al. (2016), it was under bloom condition.

Line 16: Were the nutrient samples filtered through 0.2 μm filter? Were these measured at both the start and end of the incubations?

Author response:

The nutrient samples were filtered through 0.45 μm cellulose acetate fiber and only measured at the initial condition.

Line 22 and elsewhere: Reference format should be same throughout
Page 6, line 1: were should be replaced by was
Line 2: delete a
Line 5: put space after 40
Line 11: micron symbol throughout should be used rather than u

Author response:

Corrected

Line 22: (Mohr et al., 2010) is the original reference

Author response:

Yes. We gave the credit to Mohr et al. (2010) in proper places. As for the $^{15}N_2$-enriched seawater preparation in this study we adapted the same device and procedure described in Shiozaki et al. (2015).

Line 22: Were incubation done single, duplicates or triplicates?

Author response:

All the incubations were triplicated. We added "triplicates" in proper places.

Page 7, line 4: Were ^{13}C and $^{15}N_2$ added in the same bottles?

Author response:

Yes. The ^{13}C and $^{15}N_2$ tracers were added in the same bottles.

Line 7: Perhaps 0.7 μm pore size should be mentioned.

Author response:

We added 0.7 μm into parenthesis following GF/F.

Line 8: It would be a surprise if the authors were able to filter 4.5 L water on single 25 mm GFF filter?
Line 19: There is no I_k in equation (1)

Author response:

Reviewer is an expert indeed. At station D5 and A3, 4.5L PC bottles were used for incubations. While at S0320 with *Trichodesmium* bloom, 1.2L PC bottles were applied. We added more information in Material and Methods. We eliminated the description regarding I_k in old sentence. A new sentence "The light saturation coefficient I_k was defined as N_m/α." was added showing the derivation of I_k.

Page 7, line 12: replace classical by typical

Author response:

Changed.

Line 14: How the average value of PAR calculated?

Author response:

See reply in the major comment #2.

Line 18: 34.6 salinity is not really high. It is normal in open oceans

Author response:

We added "relatively".

Line 23: "thus . . .. in all the experiments" can be deleted as preceding part of the sentence implicitly

states the same.

Author response:

Deleted.

Page 9, line 2: how many samples were taken to obtain the standard deviation and mean Line 3: "two order of magnitude" is not quite true.

Author response:

Triplicated samples results were used. The description is now "The NF rate at the blooming station was 30-40 times higher than that of the two non-bloom stations.".

Line 4: "Detail" should be replaced by "detailed"

Author response:

Corrected

Line 6: biomass should be replaced by abundance or cells

Author response:

We changed "biomass" to "trichome".

Line 18: If at t=0, POC was same in all the light experiments, then how does POC decrease with light within 24 hrs so rapidly. With this logic, POC concentration will be drastically different during the evening and in the morning in the ocean.

Author response:

The POC concentration ranged from 10.9 ± 1.0 μM to 17.3 ± 1.2 μM, which is 100 times higher than our EA-IRMS detection limit (1 μgC). The discrepancies among various light incubations were 0.5 -3.9μM, which is apparently measurable. From our triplicates, the discrepancy is significant statistically. Moreover, such change happened in both POC and PON. The detectable POC/PON change may be due to the high biomass in blooming condition. Nevertheless, changes in *Chl*-a within 24 hrs incubation were reported in many previous studies.

Page 10, line 8: replace “was decreasing” by “decreased”

Author response:

Replaced.

Line 12: Define this mentioned fraction. Is it the ratio of ^{15}N TDN uptake by nondiazotrophs and total production of ^{15}N TDN by diazotrophs. Or is it the ratio of ^{15}N TDN uptake by non-diazotrophs and total ($^{15}N + ^{14}N$) uptake by non-diazotrophs.

Author response:

This mentioned fraction is the ratio of ^{15}N TDN uptake by non-diazotrophs and total DDN flux. The description is now “After 24 h incubation, the DDN transfer rates (transferred to the non-diazotrophic plankton) were 18.6± 3.6, 0.5 ± 0.3 and 0.7 ± 0.5 nM N d^{-1} corresponding to 5% ± 1%, 4% ± 3% and 5% ± 4% of total NF (net plus dissolved), respectively, for Stas. S0320, D5 and A3 (Fig. 6).”

Line 19: “locates” does not read properly. Revise the sentence.

Author response:

Changed to “fell within”.

Line 21: Replace “strong of” by “strong”

Author response:

Corrected.

Line 24: (Gandhi et al., 2011; Kumar et al., 2017) could also be proper citations here.

Author response:

Added.

Page 11, line 2: 15-40 m is confusing here. Does it mean Trichos are more abundance in 15-40 m compared to that in 1-15 m?

Author response:

The sentence was changed to "By taking into account light extinction coefficient of seawater, the maximum depth for *Trichodesmium* to perform NF would be shallower than 15-40m."

Line 14: this section (including the hypothesis presented) should be revised as suggested in the major comments.

Author response:
We revised as suggested following the major comment #2.

Page 12, line 6: "sever" should be replaced by "severe"

Author response:

Corrected.

Lines 9-13: Not clear what the authors want to convey in this sentence

Author response:

The sentence was changed to "Since our experiments of short-term light manipulation in our experiments resembles the natural variation of irradiance, such metabolism tradeoff between carbon and nitrogen fixation under low light for *Trichodesmium* may happen frequently and widespread in the field, such as cloudy day and rainy day."

Page 13, line 16: (Montoya et al., 1996) is the original reference

Author response:

Added.

Line 16: Why do the used a different technique may lead to higher DDN release?

Author response:

This issue is thoroughly discussed in Berthelot et al. (2015). The main reason is in previous method DDN release were estimated by the comparison of the gross and net NF rate, the large uncertainty of gross NF

measured by acetylene reduction assays (ARA) and underestimation of net NF by the $^{15}N_2$ bubble method may overestimate the DDN release. While, the method used in this study was directly measure the recently fixed ^{15}N signal in dissolved pool.

Page 14, line 4: Replace "recently" by "recent"

Author response:

Corrected.

Table 1: Also provide P* values (as expressed by (Deutsch et al., 2007)) in a column

Author response:

In this study, three sampling stations are all oligotrophic surface ocean the bioavailable nitrogen concentrations only around 10 nM, and the SRP concentrations were also at nM level, except in bloom station S0320. We has listed all measurable data in table for readers to estimate P* values. We hope reviewer can accept our answer.

Table 2: Also provide the fraction of diazotrophic biomass to the total phytoplankton biomass

Author response:

This is a good idea; unfortunately, there is no feasible way to separate diazotrophic biomass from bulk biomass now.

Fig. 1: Why is there so much fluctuations (variation) within minutes in PAR values? Were the conditions cloudy during incubations?

Author response:

Yes, it was caused by floating cloud and cloud cover.

Fig. 4: Either use CF/NF or PP/NF. Be consistent.

Author response:

We used CF/NF. It is consistent now.

Supp Table 1: Normally enrichment in ^{13}C is much more than ^{15}N. How much was ^{13}C added, and how much would the approximate theoretical ^{13}C enrichment at t=0?

Author response:

In this study, ^{13}C-labeled sodium bicarbonate (99 atom% ^{13}C; Cambridge Isotope Laboratories) was added to each bottle at a final tracer concentration of 70 μmol L^{-1}. The enrichment of ^{13}C finally up to 3.5% at t=0. We added this information in this revision.

Reference

Berthelot, H., Bonnet, S., Camps, M., Grosso, O., and Moutin, T.: Assessment of the dinitrogen released as ammonium and dissolved organic nitrogen by unicellular and filamentous marine diazotrophic cyanobacteria grown in culture, Front. Mar. Sci., 2, doi:10.3389/fmars.2015.00080, 2015.

Bonnet, S., Berthelot, H., Turk‐Kubo, K., Cornet‐Barthaux, V., Fawcett, S., Berman‐Frank, I., Barani, A., Grégori, G., Dekaezemacker, J., and Benavides, M.: Diazotroph derived nitrogen supports diatom growth in the South West Pacific: a quantitative study using nanoSIMS, Limnol. Oceanogr., doi:10.1002/lno.10300, 2016.

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Gandhi, N., Singh, A., Prakash, S., Ramesh, R., Raman, M., Sheshshayee, M., and Shetye, S.: First direct measurements of N_2 fixation during a *Trichodesmium* bloom in the eastern Arabian Sea, Global BIogeochem. Cy., 25, doi:10.1029/2010GB003970, 2011.

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Shiozaki, T., Nagata, T., Ijichi, M., and Furuya, K.: Nitrogen fixation and the diazotroph community in the temperate coastal region of the northwestern North Pacific, Biogeosciences, 12, 4751-4764,doi:10.5194/bg-12-4751-2015, 2015.

Reply to RC-3

Referee #3, major comment #1

This manuscript does not include statistical analysis of the data which makes it difficult (impossible) to draw conclusions about the some of the measurements, such as Figure 5 which shows diazotroph derived nitrogen release rates.

Author response:

In this version, we did statistical analyses and presented results in proper places to support our statements.

For example, the R squares of fitted NF-I curves were 0.92, 0.71 and 0.95 at station S0320, A3 and D5, respectively, in Fig.3. For Fig.4a, the R squares of fitted CF-I curve was 0.90 at station S0320. For Fig.4b, the linear regression of CF/NF versus PAR (<410 $\mu E\ m^{-2}\ s^{-1}$) showed that the slope was -0.023, R squares value was 0.72 and the P was 0.0005.

Referee #3, major comment #2

It is not clear how the authors are defining a bloom of *Trichodesmium* bloom. I am aware of *Trichodesmium* accumulations in the form of slicks which are visually observed, but a bloom to me is prolonged and active growth which should be validated

Author response:

Yes, we saw the form of slicks from naked eyes at station S0320 (0° N, 142° W), where the *Trichodesmium* abundance was up to 4227 trichomes/filaments. L^{-1}. The surface CF rate was up to 3.6 μM L^{-1} d^{-1} and the surface NF rate was 391 nM L^{-1} d^{-1}, resulting in a turnover time (C, N based) of ~4-5 days.

Comparing with the previous study by Bonnet et al. (2016), it was under bloom condition. Actually, the bloom area was not limited at S0320, the bloom covered >1x1 degree (0° N, 141° W). Unfortunately, we do not have proper remote sensing algorithm to identify the size of bloom specifically for *Trichodesmium.*

Referee #1, major comment #3

Elemental analysis of POC and PON is missing from the methods section. I suspect it derives from the ^{15}N-PON analysis but this should be discussed

Author response:

We added the detailed measurement method of POC and PON in this version manuscript (Page 7 line 10 - 12).

Referee #3, major comment #4

The uncertainty associated with the light levels should be provided, particularly since the irradiance experiments are a critical component of the manuscript The authors mention 92, 54, 28, 14, 8, 1% but there will be variability associated with all of these values and the authors should say whether it is plus/minus 5%, 10% etc.

Author response:

The variability of daily irradiance is 61% - 83% according to on-deck PAR record of a minute interval. We provided this uncertainty value in this version.

Referee #3, major comment #5

There is no mention of monitoring the temperature inside each of the incubators. If the incubators were plumbed with surface seawater then this can easily heat by >1°C and this will have an effect on the rates of carbon and nitrogen fixation.

Author response:

All bottles were incubated in on-deck incubator with rapid pumping surface water flow-through (~60 L min^{-1}). The total volume of 10 incubator tanks was ~540 L, so the water turnover time of every individual incubator was ~9 min.

In fact, the water temperature in incubation tank is slightly higher (<1 degree) than in situ surface sea water. Additionally, the forcing from temperature on the variability pattern can be ignored since all bottles were in the same temperature situation.

Referee #3, major comment #6

Its not clear to me why all of the rates are attributed to *Trichodesmium* when the experiments were conducted on natural assemblages of mixed diazotrophs.

Author response:

We did not attribute to *Trichodesmium* except for the Sta. S0320 with *nif*H gene of *Trichodesmium* >98.8%. For A3 and D5, we assumed the pattern of light effect is mainly driven by *Trichodesmium* according to their *nif*H gene abundance of >89% and >96%.

Referee #3, major comment #7:

Why does Figure 1 show PAR of 4000 μE? I was under the impression that maximum sunlight was approx. 2500 μE.

Author response:

We are really very grateful for review's this question. In this study, we have both spherical 4π photosynthetically available radiation (PAR) sensor (QSL-2100; Biospherical instruments Inc.) and flat 2π PAR sensor (PQS 1 PAR Quantum Sensor, Kipp & Zonen). According to this comment, we applied irradiance data from PQS for all plots. Results are more consistent with available reports.

Referee #1, minor comments:

Line 11 "NF pathway was likely preferentially blocked under low light to conserve energy for photosynthesis, thus, there is a metabolism tradeoff between carbon and nitrogen fixation pathways under light stress." I disagree with the wording of this statement. I think it is more likely that there is insuffiicent energy from photosynthesis at low light levels to support nitrogen fixation.

Author response:

In this version, we changed the wording and added one paragraph (Page 12, line 23-25 and Page 13, line 1-4) to elucidate the physiological mechanism of energy reallocation under light stress condition. "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 pathway 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 would decrease concurrently, resulting in reduced production of ATP and NADPH and increased activity of respiration. The negative feedback of POC consumption leads to more ATP and NADPH being reallocated to CF process, and in turn, the NF process would be down-regulated.".
We now rephrased our statement to "We hypothesized that under low light stress, *Trichodesmium* physiologically prefer to allocate more energy for CF to alleviate the intensive carbon consumption by respiration.".

Line 13 Define short-term light change. Is short-term <1 h or less than 1 day

Author response:

We added a parenthesis with less than 24h.

Page 3 Bell and Fu (2005) observed an increasing NF rates. remove 'an'

Author response:

Corrected.

Page 7, Line 1. It's not clear to me how you measured ^{15}N-TDN.

Author response:

The detailed information about ^{15}N-TDN measurement is now in Material and Methods 2.6.

Page 8 Section 2.7 How much confidence do you have in this filtration method to evaluate the transfer of DDN to no-diazotrophs

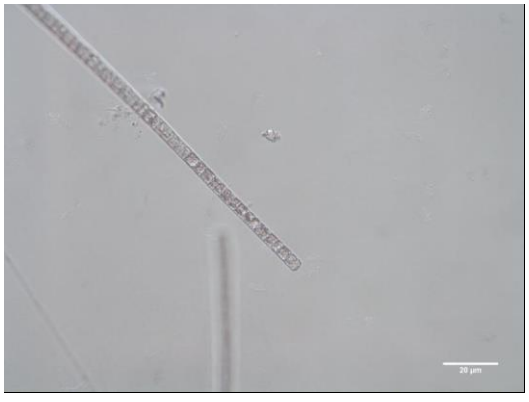
Author response:

We have confidence about the transfer of DDN to non-diazotrophs. First, the colony counting shows no heterogeneity among water samples (n=6) from the same depth. Secondly, the discrepancy of δ^{15}N value between two treatments ranged from 10 to as high as 770‰. This is much larger than the standard deviation of triplicates.

Page 8 Line 23 What confidence do you have that it is *T. thiebautii*.

Author response:

We used Nikon Eclipse 50i optical microscope to count the abundance and constrain the species of *Trichodesmium*. We have confidence about the *T. thiebautii*.



Page 9 Line 1-6 I suggest moving the water-column nitrogen fixation rates to the previous section on environmental conditions

Author response:

In this version, we adjust this section to 'Environmental conditions'

Page 9 Line 11-14 This should be in the same section as the NF-I

Author response:

In this version, we adjust this section to 'NF-I curves'

Page 9 How long were the incubations? The changes in POC are substantial and you should compare the increase in POC with the 13C-derived rate of productivity to make sure they agree.

Author response:

The incubations last for 24 hours. We added illustrations of comparison in table 2 in this version. The increases in POC were comparable with the ^{13}C-derived rate of productivity at light saturation conditions (larger than 400 μE m^{-2} s^{-1}). The two values were quite matched since the respiration rate was low. While the light intensity was under saturation value, the discrepancy increased due to the increasing of respiration rate.

Page 10 Section 3.4 This section cannot be included without statistical analysis

Author response:

In this version, we added statistical analyses at proper places to support our statements.

Page 10 I am not sure I follow your argument that the high light demand by *Trichodesmium* to fuel nitrogen fixation also help mitigate the problems caused by creating oxygen.

Author response:

Since the oxygen evolved by photosynthesis is toxic to nitrogenase, when *Trichodesmium* conduct NF processes, much energy was allocated to consume the oxygen to create the anaerobic microenvironment. Our statement is "The high energy requirement of *Trichodesmium* is not only for breaking the strong of 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

day time (Kana 1993)".

Page 11 Line 9 Did you ever consider conducting your incubations *in situ*? This would provide the light gradient you are after and as long as you are within the mixed layer then temperature would be constant (hopefully). I realize you lowest light levels might not attainable, but you should be able to cover 25-100% light levels.

Author response:

It's a good suggestion and actually many studies prefer *in situ* incubations now. But in this study, due to the cruise time schedule, we have no enough ship time to conduct *in situ* incubations. We hope to do it in future cruises.

Page 12 Line 14-24 I am not sure of the relevance of this paragraph to this study

Author response:

In this version, we added reasons to bridge the logic gap (Page 13, Line 11-15).

"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 dissolved pool and particulate pool (mainly *Trichodesmium*), the proportion of DDN released into the dissolved pool is a proper indicator. In this study, the increased proportion of DDN in the dissolved pool as the decrease of light intensity suggested that physiology status of diazotrophs modulated by light could take control on the DDN release process.".

Table 1 I increasingly see NOx being reported in the ocean literature and I dislike it application for describing nutrients due to the ambiguity. Report what was measured i.e. nitrate, nitrite. . .

Author response:

Changed as requested.

Figure 2 Given the presence of other diazotrophs, how do you attribute the measured rates to *Trichodesmium*

Author response:

We cannot exclude the NF by other diazotrophs, so we adapted the description of 'particulate NF' in proper places to be more precise

Figure 3. I suspect the x-axis shows PAR equivalent to 92, 54, 28, 14, 8, 1% of the daily averaged value, but this does not highlight the much higher intensities experienced. In Figure 1 you show PAR attaining values of 4000 uE and if this is true, it needs to be reflected.

Author response:

Reviewer is right. In this version, we use the 2π PAR sensor we added the standard deviation of the average of light recorded on-deck for the day of incubation (Page 15, Line 17).

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Light effect on N_2 fixation and net nitrogen release of *Trichodesmium* in the field

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Abstract. Dinitrogen fixation (NF) by marine cyanobacteria is a crucial pathway to replenish the oceanic bioavailable nitrogen inventory. Light is the key to modulate NF, however, field studies regarding light response curve (NF-I curve) of NF rate and the effect of light on diazotroph derived nitrogen (DDN) net release are missing that may hamper an accurate nitrogen model prediction. Uncontaminated $^{15}N_2$ gas dissolution method was applied to examine how the light change 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 light effect on carbon fixation (CF) was measured in parallel using the ^{13}C labelling method specifically for a station characterized by *Trichodesmium* bloom. Both NF-I and CF-I curves showed I_k (light saturation coefficient) range of 193 to 315 μE m^{-2} s^{-1} with saturation light at around 400 μE m^{-2} s^{-1}. The proportion of DDN net release ranged from ~6% to ~50% revealing an increasing trend as the light intensity decreased. At the *Trichodesmium* bloom station, we found CF/NF ratio was light-dependent and the ratio started to increase as light was lower than the carbon compensation point of 200 μE m^{-2} s^{-1}. NF pathway was likely preferentially blocked under low light to conserve energy for photosynthesis, thus, there is a metabolism tradeoff between carbon and nitrogen fixation pathways under light stress. Results showed that short-term (<24h) light change modulates the physiological state, which subsequently determined the C/N metabolism and DDN net release of field *Trichodesmium*. Energy reallocation associated with the variations of field light intensity would be helpful for model prediction of global biogeochemical cycle involved with *Trichodesmium*.

Keywords: diazotroph derived nitrogen release, Nitrogen fixation irradiance curve, *Trichodesmium*

1. Introduction

The bioavailable nitrogen introduced via NF by cyanobacteria is crucial to fertilize the tropical and subtropical oligotrophic surface ocean (Karl et al., 1997). In such environments, nitrate supply from the subsurface is generally limited by thermostructure induced stratification and NF can directly input bioavailable nitrogen to euphotic zone (Capone et al., 2005). Among the variety of diazotrophs, the filamentous non-heterocystous cyanobacterium *Trichodesmium* is recognized as a major player, contributing to up to 80-110 Tg N annually, i.e. ~50% of global marine NF (Capone 1997). It often forms colonies or aggregates and under appropriate circumstances, 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 on CF and oxygen production in response to irradiance (P-I curve) and showed that photosynthetic rates of *Trichodesmium* were proportional to light intensities, and that *Trichodesmium* have a relatively high irradiance requirement and a high respiration rate to protect the nitrogenase enzyme from O_2 deactivation (Lewis et al., 1988; Carpenter 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 P-I curve. Similarly, by using $^{13}C/^{15}N$ isotope labelling 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 μE m^{-2} s^{-1}), and Bell and Fu (2005) observed increasing NF rates with the increase of light intensity (PAR 10–160 μE 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 the spatial variance of NF at 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 labelling 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, above mentioned 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 light effect on C and N fixation of bulk seawater, particularly during naturally-occurring *Trichodesmium* blooms. Moreover, to our knowledge, no study has been implemented yet by using the improved $^{15}N_2$ dissolution method (Mohr et al., 2010) to date.

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 DFAA) 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. In most NF rates measurements that 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 aforementioned potential underestimation of NF rates. On the other hand, diatom and dinoflagellate blooms have 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; Bonnet et al. 2016c; 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 study has reported a significant release of DDN and DOC in culture 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 Dongfanghong II during two cruises to the Western Equatorial Pacific Ocean (06 December 2015 to 12 January 2016) and the South China Sea (15 May to 07 June 2016). Experiments were conducted at three stations (Supplementary information Fig.1), among which one of them was characterized by the presence of a *Trichodesmium* bloom (Western Equatorial Pacific Ocean Sta. S0320), the other two were located at South China Sea (A3, D5).

2.1. Seawater sampling and experimental procedures

Water samples were collected from 3-5 m depth using 10 L Go-Flo bottles which were attached to a CTD rosette (Seabird 911 CTD). In our experiments, same 4.5L 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%, 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 a minute interval. We took the average light intensity of incubation light period (>1μE m^{-2} s^{-1}) as the surface irradiance to calculate light intensities of the six light gradients.

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

Nutrient samples were collected in 100ml high density polyethylene (HDPE) bottles and kept frozen at -20 °C freezer until analysis. Nanomolar levels of 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 by chemiluminescent method (Garside, 1982) with a detection limit of 2 nM.

For Chl *a* concentrations 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 refreezing for 24 h and analysed fluorometrically according to 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 2ml sterile screw cap micro tube containing 0.2 g autoclaved glass beads and 0.8ml GTE buffer (100 mM EDTA, 50 mM Tris, 0.75M sucrose). The tubes were agitated three times for 40 s in a homogenizer (FastPrep-24, MP Bio, USA) at 4.5m/s, then froze-thaw 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., 2015a, 2015b) were used for qPCRanalysis. 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 20µL-QPCR mixtures were used for each sample and standard, reaction mixes contained 10ul 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 respectively. The Real-time Quantitative PCR was performed on an 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 1 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 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 4.5L PC incubation bottle (at bloom station S0320, 1.2L PC bottles were used) triplicated. The $^{15}N_2$ gas (98.9%) by Cambridge Isotope Laboratories was used. We conducted 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 to 20 mL headspace vials, sealed with septum stopper, and then shaken overnight. The $\delta^{15}N$ of TDN was measured and compared with the $\delta^{15}N$ of natural seawater samples. Values of $\delta^{15}N$ TDN of blank seawater and test seawater group were 4.7‰ and 5.0‰, respectively, revealing no contamination of the $^{15}N_2$ gas.

At Sta. 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 same bottle at a final tracer concentration of 70 μmol L^{-1} to simultaneously measure the CF and NF rates. At each irradiance level, triplicate water samples (4.5L /1.2L PC bottle) were incubated on-deck in incubators with surface seawater flow through.

After 24h incubation, water samples were gently filtered (<200mm Hg) onto pre-combusted (450℃, 4 h) 25 mm Whatman GF/F (0.7 μm) filters, preserved at -20℃and then dried at oven over night (50℃). The POC/PON concentrations and isotopic values were analysed on a Flash EA (Thermo Fisher Flash HT 2000)-IRMS (Thermo Fisher Delta V plus). International reference material (USGS40) with different amount of C/N and certified $\delta^{15}N$ and $\delta^{13}C$ value of -4.5‰ and -26.2‰, respectively, was inserted every 8 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

Follow the photosynthetic model by Webb et al., (1974):

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

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 , we constructed the irradiance curve 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

40 mL of the filtrate (passed through pre-combusted GF/F filters) of each NF incubation bottle was collected and preserved at -20℃ to determine the TDN concentration and δ ^{15}N-TDN according to Knapp et al., (2005). Briefly, TDN was oxidized to nitrate by persulphate oxidation reagent (purified by recrystallization 3-4 times) and the concentration was measured by the chemiluminescent method (Garside, 1982). The δ^{15}N-TDN-derived nitrate was analyzed by using the 'denitrifier method' (Sigman et al., 2001). The reproducibility for δ^{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 (24h) DDN transfer to non-diazotrophic plankton, we followed the method by Adam et al., (2016). Briefly, for the control group, 10μm sieve was used to remove most *Trichodesmium* colonies and the remaining community was incubated for 24h with $^{15}N_2$-enriched seawater. In another group, the whole community was incubated for 24 h and *Trichodesmium* colonies were removed after incubation terminated. Each experiment was performed in triplicates. The δ^{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 Figure 1. The sun rose at ~6 AM and set at ~6 PM. Value of PAR (sampling at ten second interval) varied rapidly in a wide range from 0 to 3000 μE m^{-2} s^{-1}, which are the typical 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 > 1μE m^{-2} s^{-1}) were applied for discussion. The average PAR values were1464 (± 888, 61%), 1293 (± 903, 70%) and 743 (± 619, 83%) μE 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 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℃). At the *Trichodesmium* bloom station (Sta. S0320), Chl *a* concentrations were 1.2 mg m^{-3}, much higher than those measured at the other stations (0.25, 0.39 mg m^{-3}, respectively). Result of the *nif*H phylotype abundances

showed that *Trichodesmium* accounted for >98.8%, 88.6% and 96.4% of the diazotrophic community in Sta. S0320, A3 and D5, respectively (Fig.2). The dominant *Trichodesmium* species were *Trichodesmium thiebautii* for Stas. S0320 and D5, with abundance of 4227 ± 679 (n=6), and 190 ± 50 (n=6) trichomes L^{-1}, respectively. POC /N concentration of the <10μm 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 at the surface light intensity were 390.6 ± 20.4, 12.2 ± 1.8, 9.9 ± 0.4 nM N d^{-1} at Sta. 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 were listed in supplementary information Table 1-3. However, trichomes-normalized rates were 92 and 52 pM N $trichomes^{-1}$ d^{-1}, respectively, for Stas. S0320 and D5 revealing a more consistent rate per *trichome*.

3.2. Light response of net (particulate) **N_2 fixation**

As shown in Figure 3, these NF-I curves showed a general pattern indicating that net NF rates increased significantly with light intensity from 10 to 400 μE m^{-2} s^{-1}, the R squares of fitted NF-I curves were 0.92, 0.71 and 0.95 at station S0320, A3 and D5, respectively and then saturated at around 400 μE m^{-2} s^{-1}. The simulated I_k values for NF were 271, 193 and 315 μE m^{-2} s^{-1}, respectively, for Stas. S0320, A3 and D5 with an average value 260 ± 51 μE m^{-2} s^{-1}.

Results of CF for Stas. S0320 showed a traditional P-I curve pattern without apparent light inhibition (solid curve in Fig. 4a). The fitted curve of CF showed consistent pattern with those of NF (dashed curve in Fig. 4a) giving an I_k value of 292 μE m^{-2} s^{-1} falling within the I_k range for the three NF-I curves and the R squares of fitted CF-I curve was 0.90.

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 μE m^{-2} s^{-1}.

The initial concentrations (n=3) of POC and PON were 13.4 ± 0.1 μM, 2.1 ± 0.0 μM, respectively, with a mean C:N molar ratio of 6.4 (horizontal lines in Fig. 4c an 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

μM to 10.9 ± 1.0 μM as the irradiance decreased. Below ~200 μE 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 μE m^{-2} s^{-1}. Similar light dependent pattern was found for PON, yet, final PON concentrations, varying from 2.1 ± 0.2 μM to 2.5 ± 0.2 μM, were always higher than the initial concentration (blue dashed line in Fig. 4c) without 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 observed also for the final C/N after incubation. The saturated irradiance of ~ 400 μE m^{-2} s^{-1} was likely a threshold, below the saturation light the final C/N tended to be lower than 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 ± 7.8 nM N d^{-1} for Sta. S0320, from 0.7 ± 0.2 to 1.0 ± 0.1 nM N d^{-1} for D5, and from 1.9 ± 1.5 to 5.0 ± 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% ± 5% for Stas. 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 value <0.05).

3.5. DDN transfer to non-diazotroph biomass

After 24 h incubation, the DDN transfer rates (transferred to the non-diazotrophic plankton) were 18.6± 3.6, 0.5 ± 0.3 and 0.7 ± 0.5 nM N d^{-1} corresponding to 5% ± 1%, 4% ± 3% and 5% ± 4% of total NF (net plus dissolved), respectively, for Stas. S0320, D5 and A3 (Fig. 6). Our fractions are consistent with previous reports by Bonnet et al., (2016a), in which 6% ± 1% of $DD^{15}N$ was transferred to non-diazotrophic plankton in naturally occurring *Trichodesmium* blooms and slightly lower than $DD^{15}N$ transfer (~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.

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 day time (Kana 1993). Thus, *Trichodesmium* is generally dwelled in the upper euphotic zone of 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 $\mu E\ m^{-2}\ s^{-1}$ in sunny days. By taking into account light extinction coefficient of seawater, the maximum depth for *Trichodesmium* to perform NF would be shallower than 15-40m. This result matches well with many field observations that most NF had occurred in the well-lit (0-45m) 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 appears at around 15 m depth and typically forms bloom in 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 (24h). Such result means 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 real ocean with greater applicability. The parameter consistency among our three stations in NF-I curves regardless the wide range of trichomes biomass and maximum NF rates, offers critical information for light-associated parameters in model predictions of global nitrogen fixation (Fennel et al., 2001; Hood et al., 2001).

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, 4d). As reported in both culture and field studies, *Trichodesmium* usually exhibits a higher CF:NF ratio than 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 dissolved pool (Glibert and Bronk, 1994; Mulholland et al., 2004), 2) the

underestimation of N assimilation rate 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 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), 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 almost the same, so no apparent evidence support second hypotheses. Meanwhile, the third and fourth could not explain the increased CF:NF ratio trend with the decrease of light intensity over the low light condition (p value was 0.0005). In fact, the contribution from non-diazaotrophic phytoplankton to CF cannot be excluded during bulk water incubation; however, the contribution is limited even at low light after assessment (see Supplementary information). As aforementioned, *Trichodesmium* was the dominant phytoplankton species, thus, the variation pattern of CF rates and POC concentrations against different light intensity mainly reflects the carbon metabolism of *Trichodesmium*.

In fact, in uni-algal 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 prefer to allocate more energy for CF to alleviate the intensive carbon consumption by respiration. This is analogous to the *Trichodesmium* iron limitation metabolism, of which photosynthesis take the priority over NF to get iron (Shi et al., 2007). Since the short-term (<24h) light manipulation in our experiments resembles the natural variation of irradiance, such metabolism tradeoff between carbon and nitrogen fixation under low light for *Trichodesmium* may happen frequently and widespread in the field, such as

cloudy day and rainy day.

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 pathway 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 would decrease concurrently, resulting in reduced production of ATP and NADPH and increased activity of respiration. The negative feedback of POC consumption lead to more ATP and NADPH being reallocated to CF process, and in turn, the NF process would be down-regulated.

4.3. Light modulation of DDN net release fraction

In fact, previous study found that *Trichodesmium* trichomes contain only 15–20% of diazocytes cells capable of NF (Kranz et al., 2011 and reference therein). The remaining non-diazocytes 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 labelling and Nano-SIMS 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 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 dissolved pool and particulate pool (mainly *Trichodesmium*), the proportion of DDN released into the dissolved pool is a proper indicator. In this study, the increased proportion of DDN in the dissolved pool as the decrease of light intensity suggested that physiology status of diazotrophs modulated by light could take control on the DDN release process. At station A0320 high light intensities (>400μE 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% ± 6% to 23% ± 5%. Actually, several unialgal cultures 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 lack of exogenous factors 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., (2106).

Similar to their finding, we suggested the active cell status and exposure to the exogenous factor may only lead to slightly higher proportion DDN net release. Under the light limitation stress, the inactive physiology state condition of *Trichodesmium* was reflected by the decrease of 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 part of cells could breakdown and directly releases intracellular bioavailable nitrogen. The fraction of $DD^{15}N$ release in the dissolved pool ranged from 17% ± 4% to 50% ± 5% at low light conditions (400 μE 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 μmolL^{-1}) and high proportion of DDN release (20 ± 5 to 48 ± 5%) was observed, while in the exponentially growing bloom case, the proportion of DDN release only ranged from 13 ± 2 to 28 ± 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 by the difference between gross NF rates measured by acetylene reduction assays (ARA) and the net NF measured by the $^{15}N_2$ bubble labelling technique (Montoya et al., 1996). The known uncertainty of conversion factor for acetylene to N_2 for ARA method (Montoya et al., 1996; Shiozaki et al., 2010) may bias DDN release estimate, while potential underestimation of net NF by the $^{15}N_2$ bubble method may result in higher DDN net release. In this study the direct measurement of the $DD^{15}N$ in dissolved pool by the improved dissolution $^{15}N_2$ enriched seawater method (Mohr et al., 2010) was applied to assess the DDN net release, so our data were quite reliable.

5. Conclusions

Regarding the light response curves of *Trichodesmium*, most studies have concentrated on the photosynthesis behavior by using the oxygen evolution or $^{14}C/^{13}C$ assimilation measurement. In this study, we provide quantitative information on light effect on NF and DDN net release of field *Trichodesmium* and reveal that the NF was a function of light intensity and biomass. The light requirement of *Trichodesmium* NF was high relative to its photosynthesis light demand. The empirical I_k value suggests *Trichodesmium* population maxima should appear at <15 m depth to obtain sufficient light energy. Furthermore, diel light cycle is a crucial parameter to drive physiological state of *Trichodesmium*, which subsequently determined the C/N

metabolism and DDN net release. Accordingly, we suggest the necessity to provide field light data along with nitrogen fixation data obtained via on-deck incubation for the future studies.

Recent studies revealed that unicellular cyanobacteria diazotrophs, inhabit different niches, especially UCYN-A, distributed more widely in global ocean and may contribute equal 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 to better understand their light behavior and to complete the role of diazotroph in global NF models.

Table 1. Environment condition of three stations surface water. nd: not determined

Station	Salinity	Temperature (℃)	chl *a* (μg L^{-1})	SRP （nM）	Nitrate （nM）	DON (μM)	*Trichodesmium* Colonies (trichomesL^{-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 concentration, C/N, Carbon consumption and corresponding NF and CF rate, NF/CF in station S0320. Where the '<10μm-a' represent NF rate of <10μm community incubated with > 10μm *Trichodesmium* colonies, '<10μm-b' represent the background NF rate of <10 μm community, Carbon consumption were calculated by POC concentration variation from each irradiance point final concentration to initial POC concentration minus carbon fixation rate at 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 (<10μm-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 (<10μm-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)

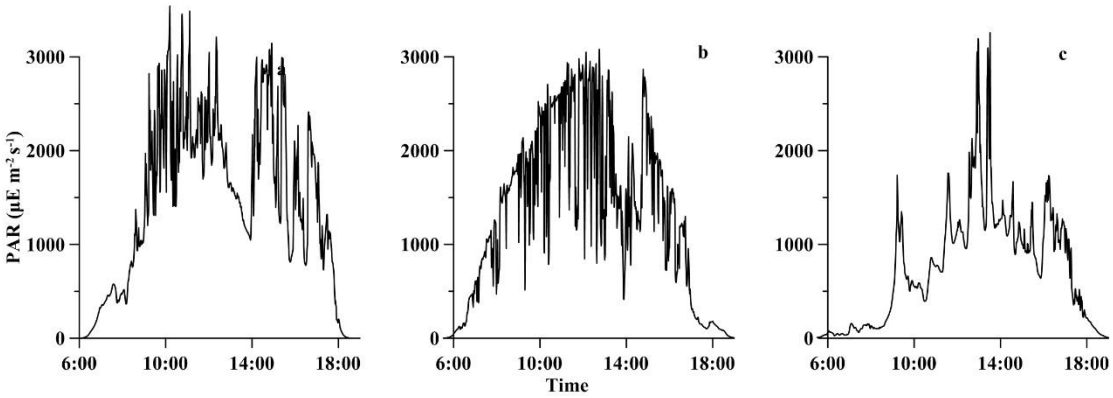

Figure 1. Temporal variations in photosynthetically active radiation (PAR μE m^{-2} s^{-1}) obtained on deck during the experiment periods, a) for station S0320; b) for station A3; c) for station D5.

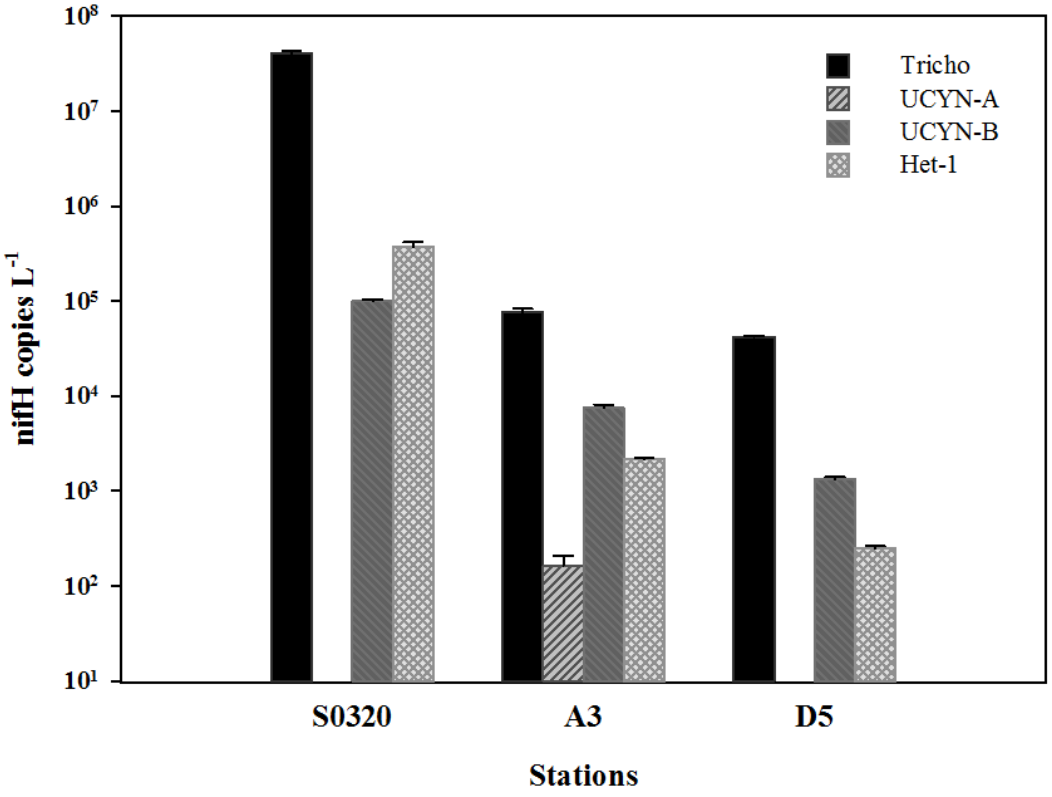

Figure 2. Cyanobacteria diazotrophs *nif*H phylotype abundances (*nif*H gene copies L^{-1}). 'Tricho' = *Trichodesmium* spp.; 'UCYN' = unicellular N_2-fixing cyanobacteria from Group A, B; 'Het-1'= heterocystous cyanobacteria from Group 1. Error bars represent the standard deviation for triplicate natural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

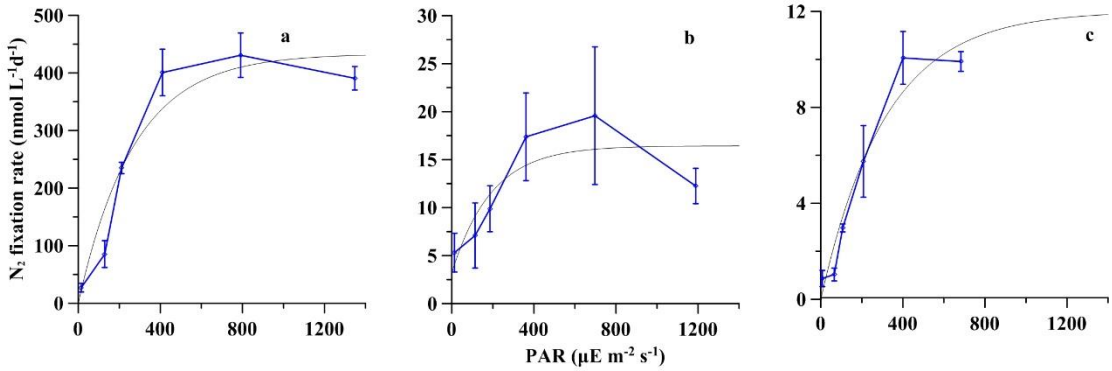

Figure 3. Net (particulate) NF versus irradiance. The gray curves represent the fitted NF-I curves. Error bar represents the standard deviation of triplicate incubations. a) for station S0320; b) for station A3; c) for station D5.

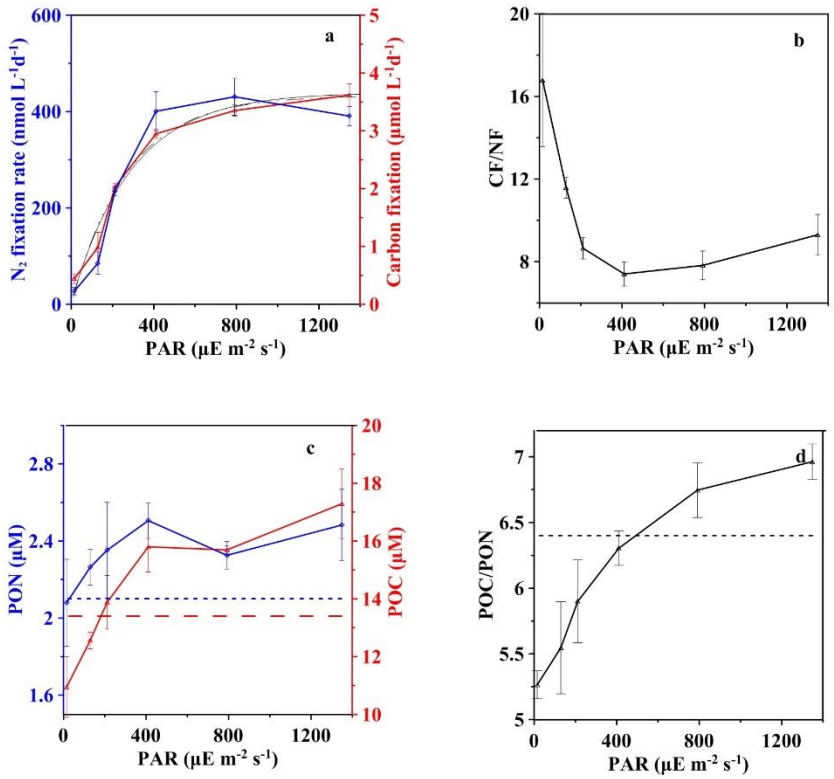

Figure 4. Light effect on carbon and nitrogen budget at Station S0320 with *Trichodesmium thiebautii* bloom. (a) Carbon (red solid line), nitrogen (blue solid line) fixation at different light intensity with fitted light response curves (black solid line for CF and black dotted line for NF); (b) The CF/NF ratios at different light intensity; (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.

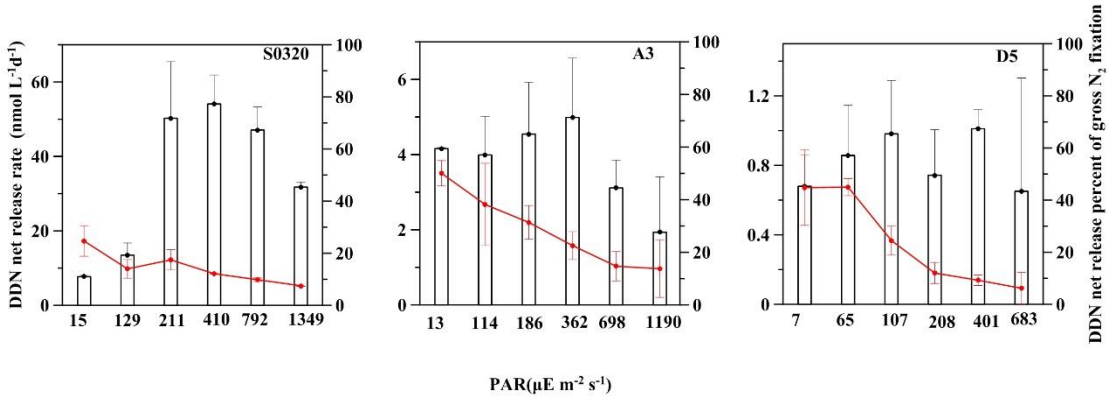

Figure 5. DDN net release rate (bar charters) and percentage of total NF (red lines) under different light intensities for stations S0320, A3 and D5. Error bars represent the standard deviation of triplicates.

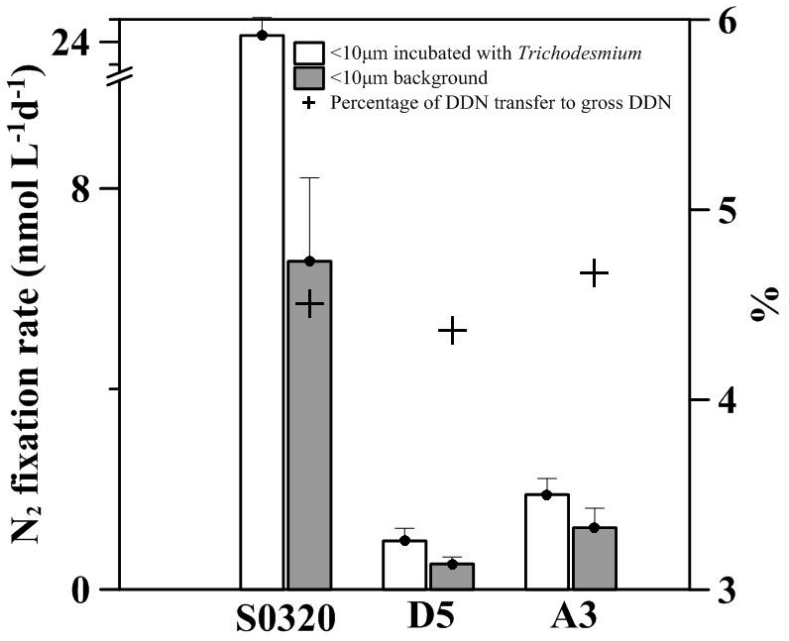

Figure 6. The NF and DDN transfer measured in two treatment groups for Stations S0320, D5 and A3. The black bars represent background NF rate of <10μm community. White bars represent NF rate of <10μm community incubated with > 10μm *Trichodesmium* colonies. Error bar represent the standard deviation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

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