

### **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 ( $<410 \mu\text{E m}^{-2} \text{s}^{-1}$ ) 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 ( $0^\circ \text{ N}$ ,  $142^\circ \text{ W}$ ), where the *Trichodesmium* abundance was up to 4227 trichomes/filaments.  $\text{L}^{-1}$ . The surface CF rate was up to  $3.6 \mu\text{M L}^{-1} \text{d}^{-1}$  and the surface NF rate was  $391 \text{ nM L}^{-1} \text{d}^{-1}$ , resulting in a turnover time (C, N based) of  $\sim 4\text{-}5$  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  $>1 \times 1$  degree ( $0^\circ \text{ N}$ ,  $141^\circ \text{ W}$ ). 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  $^{15}\text{N}$ -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  $>1^{\circ}\text{C}$  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 ( $\sim 60 \text{ L min}^{-1}$ ). The total volume of 10 incubator tanks was  $\sim 540 \text{ L}$ , so the water turnover time of every individual incubator was  $\sim 9 \text{ min}$ .

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**

It's 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 *nifH* gene of *Trichodesmium*  $>98.8\%$ . For A3 and D5, we assumed the pattern of light effect is mainly driven by *Trichodesmium* according to their *nifH* gene abundance of  $>89\%$  and  $>96\%$ .

**Referee #3, major comment #7:**

Why does Figure 1 show PAR of  $4000 \mu\text{E}$ ? I was under the impression that maximum sunlight was approx.  $2500 \mu\text{E}$ .

**Author response:**

We are really very grateful for review's this question. In this study, we have both spherical  $4\pi$  photosynthetically available radiation (PAR) sensor (QSL-2100; Biospherical instruments Inc.) and flat  $2\pi$  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 insufficient 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  $^{15}\text{N}$ -TDN.

**Author response:**

The detailed information about  $^{15}\text{N}$ -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

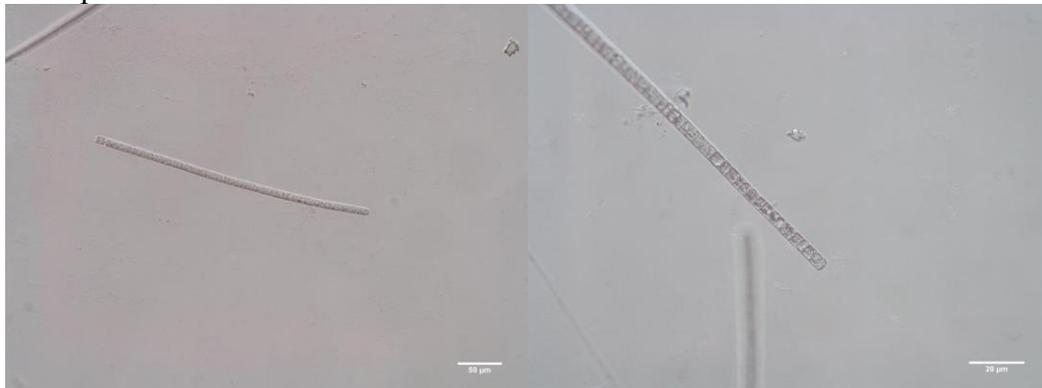
**Author response:**

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  $\delta^{15}\text{N}$  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  $^{13}\text{C}$ -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  $^{13}\text{C}$ -derived rate of productivity at light saturation conditions (larger than  $400 \mu\text{E m}^{-2} \text{s}^{-1}$ ). 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  $\text{N}_2$  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 NO<sub>x</sub> being reported in the ocean literature and I dislike its 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 2 $\pi$  PAR sensor we added the standard deviation of the average of light recorded on-deck for the day of incubation (Page 15, Line 17).

**Reference**

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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