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Interactive comment on "*Trichodesmium* and nitrogen fixation in the Kuroshio" *by* T. Shiozaki et al.

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Anonymous Referee #1 General Comments: Shiozaki et al. look at the abundance of Trichodesmium and nitrogen fixation in and around the Kuroshio Current and attempt to determine the factors influencing the distribution pattern. The authors observed that abundances were lowest in the Philippine Sea and similar everywhere else, despite similar nutrient distributions at all sites. The manuscript is disorganized and lacks flow, particularly the introduction. Sentences contain fragments of several thoughts, complicating comprehension. No rationale, questions, or hypotheses are clearly presented in the manuscript. The manuscript lacks details about the methods used, particularly about how N2 fixation rates were measured. The authors conclude that there is a significant correlation between Trichodesmium abundance and N2 fixation, yet they do not

present the data.

We have revised the Introduction substantially in the revised manuscript. Marine nitrogen fixation is generally regulated by the supply of iron and phosphorus (Mahaffey et al., 2005), and Trichodesmium thrives in iron-rich oligotrophic regions (Moore et al., 2009; Shiozaki et al., 2010, 2014b). The abundance of Trichodesmium in the Kuroshio is much higher than that in neighboring seas (Marumo and Asaoka, 1974). Although the data of concentrations of dissolved iron and phosphate at nanomolar level are limited in this region, modeling studies and climatological phosphate data indicated that the limiting nutrients for nitrogen fixation did not increase in the Kuroshio compared with the adjacent waters (Jickells et al., 2005; Chen, 2008; Mahowald et al., 2009). Recent studies demonstrated that nitrogen fixation by Trichodesmium actively occurs around oceanic islands and that abundant Trichodesmium is delivered by the current to areas remote from the islands (Shiozaki et al., 2010, 2013, 2014c). Although this phenomenon was noted in the western Pacific warm pool and western South Pacific, it can also occur in and around the Kuroshio and may contribute to the distribution of Trichodesmium in this region. We have added these statements at L47-73.

Regarding the description of the Materials and Methods, because we used the same methods written before we omitted in the previous manuscript. We have written the detail in the revised manuscript. Please also see the following responses to each comment. We have added a new figure describing the relationship between Trichodesmium spp. abundance and bulk water nitrogen at the surface (Fig. 6)

Specific Comments: The title is very general and gives no information as to conclusions from the study. Also, the title implies that only the Kuroshio was studied, when in fact the whole area around the Kuroshio was studied.

We have changed the title to "Why does Trichodesmium become abundant in the Kuroshio?"

The introduction is disorganized and very short. It is not clear what hypothesis is being

tested by the study or how it is being tested. Some of the statements and generalizations made about nitrogen fixation are not entirely correct. For instance, it is true that phosphorus concentrations are thought to potentially the limit diazotrophs, but it is not because diazotrophs consume phosphate. They do consume phosphate, as does the rest of the microbial community. The conclusions of Moore et al. 2009 and Mather et al. 2008 are not completely integrated into the introduction.

We have revised the Introduction to be a more hypothesis-driven paper as written above. Regarding the description of phosphorus limitation for nitrogen fixation, we have revised as follows. (L59-61) “As for phosphorus limitation, iron-enhanced nitrogen fixation causes phosphorus depletion, and is consequently limited by phosphorus (Mather et al., 2008).”

The materials and methods section was lacking key information for interpreting results. In section 2.1 algal blooms were defined very well, but no details about how or what calculations were done are included. No definition of which months are considered summer is included. It is also unclear why summer chlorophyll is used when 4 of the 5 cruises were conducted in September. A description of how stations were categorized into areas (ECS, Kuroshio, etc.) should be included. The method used to determine in vivo chlorophyll fluorescence is not described. More details are needed for the nutrient methods and detection limits and microscopy counts. How are filaments defined? How were different colony morphologies addressed?

We defined as summer July through September. (L88-89) Thus, all cruises were conducted in summer. The bloom frequency was calculated from the ratio of counts of chl a over 0.15 mg $m-3$ to total counts which chl a detected at each pixel. The stations during the KT-06-21, KT-07-22, and Nagasaki-maru 242 cruises were divided into three areas based on the temperature-salinity (TS) diagram. (L102-104) Although the TS diagram was shown in Fig. 2 of Shiozaki et al. (2011), it was omitted in this manuscript due to the repetition. In vivo chlorophyll fluorescence was measured by a Minitracka fluorometer (Chelsea, UK). (L130-131) The N+N and phosphate concentrations were

determined at the nanomolar level using a supersensitive colorimetric system consisting of an AutoAnalyzer II (Technicon) and Liquid Waveguide Capillary Cells (World Precision Instruments, USA) (Hashihama et al., 2009). The detection limits of N+N and phosphate were both 3 nM. (L121-125) Trichodesmium greater than ca. 300 μm in length were counted as 1 filament and shorter lengths were counted as 0.5 filaments. (L202-203) Tuft-shaped colonies were found at St.T0706, T0723, CK-10, and T0906. (L302-303)

The way the authors approached interpreting their N2 fixation rates with consideration of the results of Mohr et al. 2010 and Dabundo et al. 2014 is inadequate. The authors need to add more information about how they measured N2 fixation, including how much N2 was added, the volume of the incubations, and the length of time for the incubations. The length of time and the time of day that the injections were made is critical for interpreting the results of Mohr et al. 2010. While the authors did look for potential contaminates in the 15N gas, they did not look at particulate isotopic species. Are the detection limits and associated errors of the nutrient measurement methods low enough to ensure that there was no significant contamination of the particulate 15N isotope signal? Perhaps the authors could include some calculations to address this.

All samples for nitrogen fixation activity were collected in duplicate in acid-cleaned 4.5-L polycarbonate bottles. After 13C-labeled sodium bicarbonate (99 atom% 13C; Cambridge Isotope Laboratories) was added to each bottle, 2 mL of 15N2 gas (98+ atom% 15N; SI Science Co. Japan) were injected directly into the incubation bottles through a septum using a gastight syringe. The bottles were covered with neutral-density screens to adjust the light level and incubated for 24 h in an on-deck incubator cooled by flowing surface seawater for 24 h. The start time of incubation in this study varied in each station (Table S1). Considering daily periodicity of nitrogen fixation in each diazotroph (Zehr, 2011) and the time to reach equilibration of the 15N2 gas bubble with seawater (>12 h, Mohr et al., 2010), the level of underestimation could vary at each

station. Meanwhile, the level of underestimation is known to be low in Trichodesmium dominant water because Trichodesmium can float to the top of the bottle and directly use the added 15N2 in the bubble method (Großkopf et al., 2012). Although the bias of underestimation could not be estimated from the results in this study, the actual nitrogen fixation rate would be higher than the obtained rate. We have added these statements in L163-187. As you mentioned, we did not determine the contaminates at the isotopic level. The contamination of nitrate, nitrite, and ammonium in the 250 ml of seawater with 2 ml 15N2 gas was undetectable (<nM level) in experiment 1. During the cruise experiments, we added 2 ml 15N2 gas into 4.5 L seawater, and hence, the contamination level would be one order lower than that in experiment 1, indicating that 15N-labeled substrates in the seawater were at most 10-2 nM. When the substrate concentration in the seawater was 3 nM (the detection limit of our analysis), the concentration of 15N-labeled substrate would be <1/100 lower than that of seawater. In this case, the 15N concentration would be too low to detect the uptake rate (Shiozaki et al., 2009). We have added these statements in L31-39 of Supporting Information.

In the supporting information the authors present an MDS and ANOSIM analysis. Did the authors look at co-variability between the parameters? This analysis was not mentioned in the results, but is important for interpreting the results.

Yes. In the revised manuscript, we have moved the result of nMDS and ANOSIM analysis to the main text (L207-215, 286-289)

The authors state that there is a significant correlation between Trichodesmium abundances and N2 fixation, but do not show the data. This is a major conclusion of the study and the data should be shown. It is not clear if the authors are comparing surface Trichodesmium to depth integrated N2 fixation. The authors also state that Trichodesmium abundances decrease with depth. This data should be shown as well. It is unclear why the authors display only surface abundances of Trichodesmium and depth integrated fixation rates. The authors should display either surface data or depth integrated data for both parameters.

We have added a new figure describing the correlation between Trichodesmium spp. abundance and bulk water nitrogen fixation rate at the surface in the revised manuscript (Fig. 6c). Furthermore, the vertical distribution of Trichodesmium spp. abundance has been shown in Fig. S3. Although the vertical distribution was determined only during Nagasaki-maru 242 and KT-07-21 cruises, Trichodesmium spp. abundance was highest at the surface at almost all of the stations (Fig. S3), and the surface abundances were positively related with the depth-integrated ones (Fig. 6a). Thus, the surface abundance was used to discuss the geographical distribution of Trichodesmium spp. The surface abundance of Trichodesmium spp. in the entire study area was positively correlated with the nitrogen fixation rate at the surface ($r2 = 0.80$; $p < 0.05$ [$r2 = 0.55$; $p < 0.05$ if the datum taken at the Trichodesmium-bloom station T0906 is excluded]) (Fig. 6c), suggesting that they significantly contributed to nitrogen fixation in the study region. (L291-294, 311-315) As you mentioned, there could be a leap of logic in our way of data presentation. In the revised manuscript, we have shown the distribution of nitrogen fixation at the surface in Fig.2d instead of the depth-integrated ones to compare with the distribution of Trichodesmium spp. abundance at the surface (Fig. 2c). The surface nitrogen fixation rates were positively correlated with the depth-integrated rates ($p<0.05$, t-test) (Fig. 6b), suggesting that the distribution of nitrogen fixation was indexed by the surface activity. (L304-307)

It is not clear why diatom abundances are included in the manuscript and what impact they have on the conclusions. This should be removed.

In the northwest of the Miyako Islands, an upwelling occurred by the island wake effect. The microscopic analysis demonstrated that dominant phytoplankton in the upwelling was diatoms and was not Trichodesmium. This result indicated that high abundance of Trichodesmium near the islands was not directly caused by the upwelling, and would be useful information in future research. We have added these statements in L348-349.

The authors claim that Trichodesmium abundances are higher in the Kuroshio than in the surrounding areas, based on others' results, yet they do not use their own data to

test this. Looking at table 1, I do not think that there are any significant differences. The authors should test this and present the results.

In the present study, there was no statistically significant difference in Trichodesmium spp. abundance among the study areas ($p > 0.05$, Tukey's HSD test) probably because the data were limited and the variation was large. However, in the Kuroshio, Trichodesmium spp. were always observed and were abundant at almost stations. Furthermore, at St.CK-10 in the East China Sea, which is in the Kuroshio branch current, a high abundance of Trichodesmium spp. was observed. On the other hand, Trichodesmium spp. abundance in the Philippine Sea tended to be lower than that in the other areas. Such Trichodesmium distribution was also reported in the previous study (Marumo and Asaoka, 1974). The present study also showed lower surface nitrogen fixation in the Philippine Sea compared to that in the Kuroshio ($p < 0.05$, t-test). We have added these statements in L412-421.

Technical Corrections: Figure 1: It is very hard to see the station symbols. The print and symbols are very small. The color symbols overlaid on a color map also make it difficult.

We have moved the color map of average chlorophyll a to Fig. S2. The symbols have been enlarged (Fig.1a)

Figure 2: 'small box' – inset It is hard to read discrete values, compare the data points, or see any trends as the data is currently presented.

The areas of circles are proportional to the concentration, abundance, or activity. We have added the information in the figure legend (Fig. 2). The value is a subsidiary indicator. We have reduced the size. There was no significant relationship between abundance of Trichodesmium spp. and environmental variables around the Miyako Islands. (L404-405)

Figure 3: Each panel should have a number and the legend should identify which

panels correspond to the areas studied.

We have added a number and changed the legend as suggested (Fig. 3).

Figure 5: The figures are small and it is difficult to identify islands.

We have put a mark on the islands (Fig. 7a).

Pg 11062: Lines 1-5: These sentences are more introductory. They should be shortened into one sentence.

We have shortened into one sentence as follows. (L19-20) "The genus Trichodesmium is recognized as abundant and major diazotroph in the Kuroshio, but the reason for this remains unclear."

Line 7: 'whose availabilities potentially control diazotrophy' This is introduction.

We have removed it as suggested.

Lines 9-10: 'since satellite. . .to the Kuroshio' More appropriate in the introduction.

This is our result. We have changed the sentence as follows. (L24-25) "since our satellite analysis suggested that material transport could occur from the islands to the Kuroshio."

Line 11: remove 'and the'

We have removed it as suggested.

Line 19-21: This sentence doesn't make sense. How can a diazotroph's presence be important for determining diazotrophy? Maybe something is missing?

We have changed the last sentence in the Abstract as follows. (L34-35) "Our results indicate that Trichodesmium growing around the Ryukyu Islands could be advected into the Kuroshio."

Pg 11063: Line 2: 'via the ocean-atm.' Remove 'the'.

We have removed it as suggested.

Line 3: Remove ‘furthermore’

We have removed it as suggested.

Line 8: Remove ‘in addition’

We have removed it as suggested.

Line 14: Remove ‘which is characterized by highly oligotrophic conditions’. This should be included in the general description of the Kuroshio.

We have moved it to L42-43.

Line 16: Remove ‘nevertheless’.

We have removed it as suggested.

Pg 11065: Lines 9-11:This sentence should be re-written.

We have rewritten as follows. (L105-107) “During the KT-09-17 cruise, we conducted experiments around the Miyako Islands which were distinguished from the other three areas.”

Pg 11066: Lines 11-16: Is this the protocol for preparing/cleaning the sampling bottles? If so, please state this, otherwise this method is unclear.

Yes, this is the protocol for cleaning the sampling bottles. We have added the information in L141.

Line 15: and ‘stored in’ double plastic bags?

Yes. We have changed as suggested. (L145)

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C6927/2015/bgd-12-C6927-2015-

supplement.pdf

Interactive comment on Biogeosciences Discuss., 12, 11061, 2015.

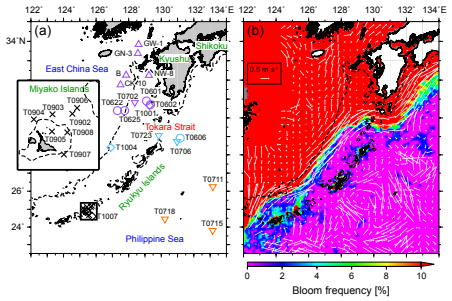

Fig 1 Shiozaki et al.

Fig. 1. (a) Sampling stations and (b) climatological surface current fields

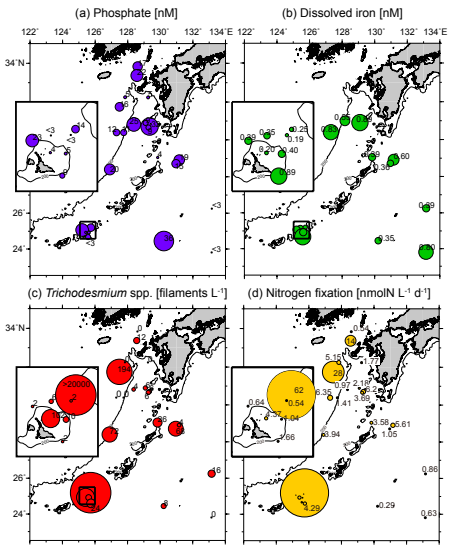

Fig 2 Shiozaki et al.

Fig. 2. Distribution of (a) phosphate, (b) dissolved iron, (c) Trichodesmium spp., and (d) nitrogen fixation at the surface.

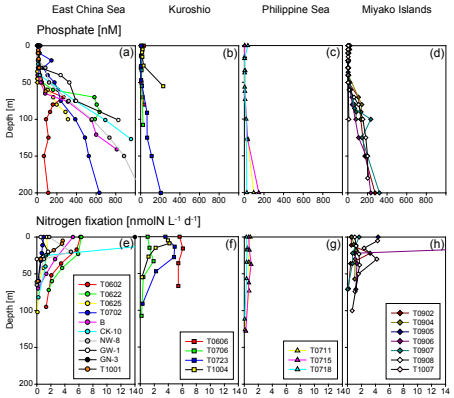

Fig 3 Shiozaki et al.

Fig. 3. Vertical profiles of phosphate and nitrogen fixation in the East China Sea (a and e), the Kuroshio (b and f), the Philippine Sea (c and g), and the Miyako Islands (d and h).

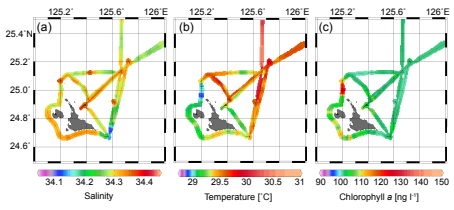

Fig 4 Shiozaki et al.

Fig. 4. Surface (a) salinity, (b) temperature, and (c) chlorophyll a during the KT-09-17 cruise.

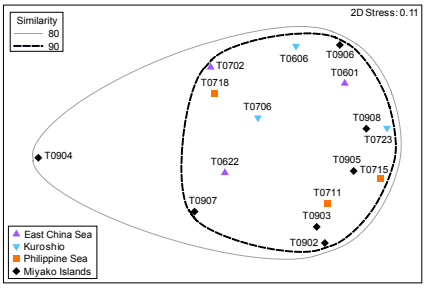

Fig 5 Shiozaki et al.

Fig. 5. nMDS ordination of sampling stations with environmental variables

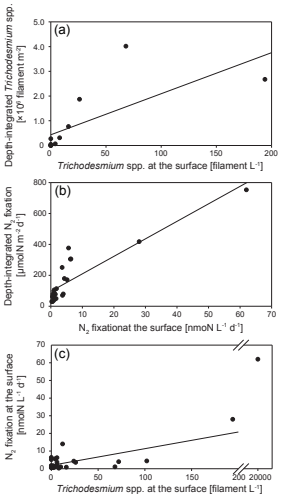

Fig 6 Shiozaki et al.

Fig. 6. Relationships (a) between surface and depth-integrated Trichodesmium spp. , (b) between surface and depth-integrated N2 fixation, and (c) between Trichodesmium spp. and N2 fixation at the surface.

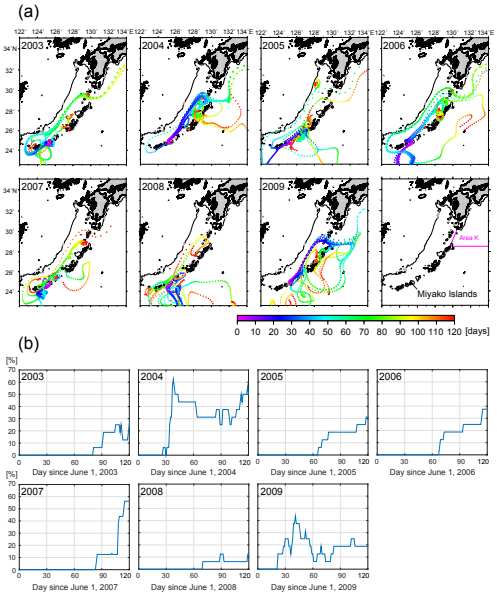

Fig 7 Shiozaki et al.

Fig. 7. (a) Trajectories of particles released from points around the Miyako Islands on June 1, 2003–2009. (b) The ratio of particles delivered to Area K to the total released particles.