We have made modifications on the title of the manuscript and the new title is ‘Why does *Trichodesmium* become abundant in the Kuroshio?’ The manuscript has been revised substantially following the comments and suggestions from two reviewers. I believe we have covered all the points raised and incorporated the comments and annotations into the revised manuscript. For the revised manuscript, we have performed quantitative analyses of the numenrical simulations as reviewer #2 suggested. We have written details of the materials and methods as reviewer #1 suggest.

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 ^{13}C-labeled sodium bicarbonate (99 atom% ^{13}C; Cambridge Isotope Laboratories) was added to each bottle, 2 mL of $^{15}N_2$ gas (98+ atom% ^{15}N; 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 $^{15}N_2$ 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 $^{15}N_2$ 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 $^{15}N_2$ gas was undetectable (<nM level) in experiment 1. During the cruise experiments, we added 2 ml $^{15}N_2$ gas into 4.5 L seawater, and hence, the contamination level would be one order lower than that in experiment 1, indicating that ^{15}N-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 ^{15}N-labeled substrate would be <1/100 lower than that of seawater. In this case, the ^{15}N 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 ($r^2 = 0.80$; $p < 0.05$ [$r^2 = 0.55$; $p < 0.05$ 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)

Anonymous Referee #2

General Comments:

The main question posed in this manuscript seems to be whether observations support the hypothesis that nutrient supply through transport from the Ryukyu Islands fuels growth of Trichodesmium in the Kuroshio. The authors do not find evidence of a gradient in phosphorus and iron between the Islands and the Kuroshio. They do find elevated Trichodesmium abundances near the Ryukyu Islands and conclude that Trichodesmium grows in that region and is transported downstream to the Kuroshio. This is an interesting and significant result. However, the major evidence presented in support of this process is a series of numerical simulations demonstrating advection of particles from the Islands to the Kuroshio. Quantitative analysis of the simulations is not presented. Since this piece of evidence is so crucial to the paper, it should be evaluated and presented more thoroughly and quantitatively. The nutrient dynamics of the region and potential nutrient supply mechanisms could also be treated more thoroughly. Additionally, the manuscript would benefit from further revisions to improve organization, clarity, and flow. I have included some specific detailed comments below.

We have calculated the ratio of particles delivered to the Kuroshio to total released particles in the revised manuscript. In 2003–2009, 13–56% (30 ± 16%) of particles released from the Miyako Islands were delivered to the Kuroshio and passed the Tokara Strait. (L356-359) It should be noted that our numerical experiments contained the following two uncertainties. First, the distribution of *Trichodesmium* around the islands, which strongly influences the destinations of particles, was not able to be determined in advance. *Trichodesmium* is known to aggregate and not to occur uniformly in the ocean (Capone et al., 1997). Second, the model cannot reproduce the current very close to the islands. If a water mass very near the islands was delivered to the open ocean by tide and/or river plumes that were not considered in the model, seaward dispersions of particles was likely underestimated. We have added these statements and results in L235-243.

In addition, we have examined the particle tracks depending on the start time. When the start time was changed to June 1, 11, and 21, and July 1 in 2009, the particle tracks largely varied (Fig.S5). The ratio particles delivered to the Kuroshio varied from 6.2–38% (22 ± 13%) on day 120. (L361-364)

We have also mentioned about phosphorus limitation for nitrogen fixation and N/P ratio in each area in the revised manuscript. We have replied in detail below.

Specific Comments:

Abstract:

The major conclusion seems to be that Trichodesmium may be advected into the Kuroshio from the Ryukyu Islands, but this is not clear from the abstract.

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"

Introduction:

p.11063 lines 1-8: What is the relevance to the present study? This section could be shortened.

We have deleted these sentences from the revised manuscript.

p.11063 lines 28-29: "Phosphorus would ultimately limit diazotrophy because phosphorus in oligotrophic regions is consumed by diazotrophs, and is thus depleted." This sentence seems inaccurate, or at best, poorly worded, and could be removed. The discussion of previous findings related to the distribution of phosphorus in the region could be expanded.

We have revised the sentence as follows.

“As for phosphorus limitation, iron-enhanced nitrogen fixation causes phosphorus depletion, and is consequently limited by phosphorus (Mather et al., 2008).”

Materials and Methods:

p. 11067 lines 26-28: The distribution of Trichodesmium with depth can vary significantly. Perhaps the data from all depths could be presented where available.

We have added the figure of vertical distribution of *Trichodesmium* spp. abundance (Fig. S3).

p.11068 Section 2.3: Please provide more details on the methods used for the numerical experiments. What particle-tracking methods were used?

Numerical particle-tracking experiments were conducted to investigate the transport of water masses at the surface from areas around the Miyako Islands in the summer season from 2003 to 2009. Surface velocity data were derived from the FRA-JCOPE2 reanalysis product (Miyazawa et al., 2009), which is an eddy-resolving (1/12°) ocean model combined with three-dimensional variational data assimilation (satellites, ARGO floats, and shipboard observations), and is one of the most reliable models for the region around Japan for the above time period. The method of tracking particles was basically the same as in Itoh et al. (2009), but we did not include the random walk for simplicity. We have added these statements at L218-226.

p.11068 lines12-13: “The particle distribution at the surface was fixed throughout the experiment.” What does this mean?

This means the particle does not sink from the surface during the experiments. We have revised the sentences as follows. (L226-228)

“The release points of particles were selected at the surface of the model grid points around the coastal waters of the Miyako Islands. We assumed that the particles did not increase, die, or sink from the surface during the experiments.”

Results:

p.11068 line 24-p.11069 line 1: "The algal bloom frequency was consistently > 10% in the west of the main stream of the Kuroshio because the average chl a was > 0.15 mg/m-3." Please clarify.

We have revised the sentence as follows. (L253-255)

"In the west of the main stream of the Kuroshio, because the average chl *a* was over 0.15 mg m^{-3} (Fig. S2), the frequency of chl *a* values >0.15 mg m^{-3} was high (Fig. 1b)."

p. 11070 lines 3-8: Can the authors provide further justification for the basis of the analysis on surface Trichodesmium abundance? Perhaps a plot of depth-integrated vs. surface Trichodesmium abundance could be included.

The vertical distribution of *Trichodesmium* spp. abundance has been shown in Fig. S3. The abundance of *Trichodesmium* spp. was highest at the surface at almost all of the stations. Furthermore, the surface *Trichodesmium* spp. abundances were positively correlated with the depth-integrated abundances ($p < 0.05$, *t*-test) (Fig. 6a). (L291-293)

p. 11070 lines 25-27: Can the direction of the currents be verified with data?

The model data we assimilated satellite altimetry data, which means that satellite-based surface velocity field was reflected to the model outputs. Although shipboard ADCP recorded vertical velocity profiles along the ship track, it is difficult to eliminate tidal components that may change direction even within the same day.

p. 11071 lines 24-28: Quantitative analysis could strengthen the manuscript. For instance, what is the likelihood a trajectory starting in the island region will end up in the Kuroshio, based on the simulations? Also, to what extent does the start time influence the results?

We have calculated the ratio of particles delivered to the Kuroshio to total released particles in the revised manuscript. As the Kuroshio generally flows along the continental slope north of the Miyako Islands (Fig. 1b), particles around the Miyako Islands were not transported along the typical path of the Kuroshio to the northeast, especially at their initial stages (Fig. 7a). Some particles migrated around the Miyako Islands, or turned south after they passed the Tokara Strait. Nevertheless, the particles delivered to Area K east of the Tokara Strait increased as time elapsed, and the ratio of particles delivered to Area K to the total released particles ranged from 13–56% (30 ± 16%) by day 120 in 2003–2009 (Fig. 7b). The year-to-year variations in the ratio are mainly due to influences of mesoscale eddies as partly seen in the particle trajectories in Fig. 7a, and likely occurred over relatively short time scales (shorter than the seasonal time scale). This is supported by another series of experiments in which particles were released on June 1, 11, and 21, and July 1 in 2009, which yielded ratios of 6.2–38% (22 ± 13%) by day 120 (Fig. S5). (L352-364)

p.11073 line 17-p.11074 line 2: Please reword for clarity. Also, here iron is referred to as "the limiting nutrient", but phosphorus may also play a role.

We have added the sentences as follows. (L427-431)

Furthermore, the iron-enhanced active nitrogen fixation causes phosphorus depletion, and is consequently limited by phosphorus (Mather et al., 2008). No significant differences in surface iron and phosphate were observed among the study areas, which cannot explain the distribution of *Trichodesmium* spp. and nitrogen fixation in the study region.

p.11074 lines 13-15: This doesn't make sense. Do you mean "contribute to" rather than "be attributed to"?

Yes. We have changed to "contribute to". (L444)

p.11074 lines 15-22: The conclusion that "physical conditions" were similar in all regions based on consistent MLDs seems like a bit of a stretch. Perhaps the authors could reword this to make a more precise statement. Also, was there variation in N:P ratio among the regions analyzed?

We have changed "physical conditions" to "vertical mixing conditions". (L450)

The surface N/P (=N+N/phosphate) at each station has been shown in Table S1, respectively. The N/P (=N+N/phosphate) ratio at the surface varied from 0.28 to

6.40 except St. T0904 (N/P=16.3), and no significant difference was observed among the four areas ($p > 0.05$, Tukey’s HSD test). (L279-281)

p.11074 line 27 - p. 11075 line 1: What is the “inconsistency” between Trichodesmium abundance and iron and phosphate concentrations and how is it explained by the preceding part of the sentence?

This means why the *Trichodesmium* distribution in this region was not estimated from iron and phosphate concentrations. We have change the wording in L457-458. We described the inconsistency in L429-431.

p. 11075 lines 4-7: delete “This is because”

We have deleted as suggested.

Why does *Trichodesmium* become abundant in the Kuroshio? ~~and nitrogen fixation in the Kuroshio~~

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Abstract

~~Nitrogen fixation in the Kuroshio influences nitrogen balance in the North Pacific Ocean.~~ The genus *Trichodesmium* is recognized ~~as~~ as abundant and ~~a~~ major diazotroph in the Kuroshio, but ~~. Although its abundance is higher in the Kuroshio than in adjacent waters,~~ the reason for this ~~difference~~ remains unclear. The present study investigated the abundance of *Trichodesmium* spp. and nitrogen fixation together with concentrations of dissolved iron and phosphate~~, whose availabilities potentially control diazotrophy,~~ in the Kuroshio and its marginal seas. We performed the observations near the Miyako Islands, which form part of the Ryukyu Islands, situated along the Kuroshio, since our satellite analysis suggested that material transport could occur from the islands to the Kuroshio. *Trichodesmium* spp. bloomed (>20,000 filaments L^{-1}) near the Miyako Islands, ~~and the~~ abundance was high in the Kuroshio and the Kuroshio bifurcation region of the East China Sea, but was low in the Philippine Sea. The abundance of *Trichodesmium* spp. was significantly correlated with the total nitrogen fixation activity. The surface concentrations of dissolved iron (0.19–0.89 nM) and phosphate (<3–36 nM) were similar for all of the study areas, indicating that the nutrient distribution could not explain the spatial differences in *Trichodesmium* spp. abundance and nitrogen fixation. Numerical particle-tracking

experiments simulated ~~We used a numerical model to simulate~~ the transportation of water around the Ryukyu Islands to the Kuroshio. Our results indicate that *Trichodesmium* growing around the Ryukyu I~~i~~slands~~ situated along the Kuroshio~~ could be advected into the Kuroshio.

~~is potentially important for determining diazotrophy in this region.~~

1. Introduction

The Kuroshio is a western boundary current in the North Pacific Ocean that originates in the North Equatorial Current and bifurcates to the east of the Philippines. The main stream of the Kuroshio enters the East China Sea (ECS) northeast of Taiwan, flows out through the Tokara Strait, and runs along the Japanese islands of Shikoku and Honshu. While the Kuroshio and its adjacent waters are characterized by highly oligotrophic condition, ~~The Kuroshio transports large amounts of heat from south to north, thereby influencing the climatic variability of the surrounding countries via the ocean–atmosphere interaction (Nakamura et al., 2012; Sasaki et al., 2012; Hu et al., 2015). Furthermore, it plays an important role in delivering fish eggs and larvae from low to mid-latitudes. Pacific bluefin tuna (*Thunnus orientalis*), Japanese anchovy (*Engraulis japonicus*), and Japanese sardine (*Sardinops melanostictus*) spawn in the warm water region, and the fish eggs and larvae are transported by the Kuroshio to their nursery grounds in the Kuroshio-Oyashio transition region (Itoh et al., 2009; Kitagawa et al., 2010). In addition,~~ phytoplankton and zooplankton communities in the Kuroshio are distinct compared to those from adjacent waters (McGowan, 1971). McGowan (1971) suggested that some plankton species are delivered by the Kuroshio to the north from the equatorial region.

The abundance of the cyanobacterial genus *Trichodesmium* in the Kuroshio is much higher than that in neighboring seas (Marumo and Asaoka, 1974). Because *Trichodesmium* is a major nitrogen fixer in the Kuroshio, ~~which is characterized by highly oligotrophic conditions,~~ it is believed to be the key genus for understanding the Kuroshio ecosystem (Chen et al., 2008, 2014; Shiozaki et al., 2014a). Nevertheless, the factors controlling the distribution of *Trichodesmium* in this region are poorly understood. 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~~ where the iron supply is high~~ (Moore et al., 2009; Shiozaki et al., 2010, 2014b). A major source of iron in the ocean is atmospheric dust deposition (Jickells et al., 2005; Mahowald et al., 2009). ~~A m~~Modeling studies~~y~~ indicate~~d~~ that dust deposition in the western North Pacific decreases~~d~~ exponentially from the continental shelf to the Philippine Sea (Jickells et al., 2005; Mahowald et al., 2009), and hence, deposition is~~was~~ not as high in the Kuroshio as in the adjacent waters. As for phosphorus limitation, iron-enhanced nitrogen fixation causes phosphorus depletion, and is consequently limited by phosphorus (Mather et al., 2008). The phosphate distribution has been examined in this study region using a conventional colorimetric method, and the surface phosphate concentration in the Kuroshio has been reported to be as low as that in the Philippine Sea (Chen, 2008). Therefore, the distinct high abundance of *Trichodesmium* in the Kuroshio is probably not explained by nutrient condition; however, distributions of dissolved iron and phosphate at nanomolar level have not been well studied in this region (Obata et al., 1997; Shiozaki et al., 2010; Kodama et al., 2011).

Nitrogen fixation by *Trichodesmium* has recently also been found to be active around oceanic islands (Shiozaki et al., 2010, 2013, 2014c). Furthermore, these studies demonstrated that abundant *Trichodesmium* is delivered by the current to areas that are remote from the islands. 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.

~~Although this suggests that the distribution of *Trichodesmium* in the Kuroshio is not due simply to the dust derived iron input, this theory has not been confirmed because the distribution of dissolved iron at sea has not been well studied in this region (Obata et al., 1997). Phosphorus would ultimately limit diazotrophy because phosphorus in oligotrophic regions is consumed by diazotrophs, and is thus depleted. Phosphorus limitation for diazotrophs can be indexed by the phosphate concentration at the nanomolar level (Mather et al., 2008; Hashihama et al., 2009). While the~~

~~phosphate concentration has been widely determined in the Kuroshio and its marginal seas using a conventional colorimetric method (Chen, 2008), studies on nanomolar phosphate dynamics are limited (Shiozaki et al., 2010, Kodama et al., 2011).~~

In the present study, we simultaneously determined *Trichodesmium* abundance and bulk water nitrogen fixation together with concentrations of dissolved iron and phosphate at the nanomolar level in the Kuroshio and its marginal seas. In addition, we conducted intensive observations around ~~examined whether islands affected the distribution of *Trichodesmium*. Marumo and Asaoka (1974) hypothesized that the Kuroshio entrains nutrients from coastal areas when it flows past islands, and that *Trichodesmium* grows using these entrained nutrients. Therefore, the study included~~ the Miyako Islands section of the Ryukyu Islands ~~because the Kuroshio flows close to these islands~~located close to the main stream of the Kuroshio.

2. Materials and Methods

2.1. Oceanographic database

Algal blooms in an oligotrophic region may indicate a nitrogen fixation hotspot (Wilson and Qiu, 2008; Shiozaki et al., ~~2014c~~2014c). To identify the locations of intensive algal blooms, we used a dataset of chlorophyll (chl) *a* observed by ~~a~~ satellite.

According to Wilson and Qiu (2008), an algal bloom in an oligotrophic region can be defined as a chl *a* value >0.15 mg m^{-3} in summer. In the present study, we used 8-day, moderate-resolution imaging spectroradiometer (MODIS) level 3 chl *a* with 9 km resolution during summer between July 2003 and September 2009. We defined as summer July through September. The bloom frequency for each pixel was calculated from the ratio of counts in which chl *a* was >0.15 mg m^{-3} to the total counts in which chl *a* was detected. ~~, and calculated the distribution of bloom frequency in the study areas.~~

To examine the current field, geoelectrokinetograph and ship-mounted acoustic Doppler current profiler (ADCP) data from the uppermost layer for the summers between 1953 and 2008 were obtained from the Japan Oceanographic Data Center (http://www.jodc.go.jp). Regridding, removal of anomalous values, and smoothing of the dataset were performed as described by Isobe (2008).

2.2. Cruise observations

Experiments were conducted during summer on-board the R/V *Tansei-maru* (KT-06-21, September 9–17, 2006; KT-07-22, September 5–13, 2007; KT-09-17, September 8–13, 2009; KT-10-19, September 4–12, 2010) and the T/V

Nagasaki-maru (242, July 19–28, 2007) (Fig. 1a, Table S1). The stations ~~from~~ during the KT-06-21, KT-07-22, and *Nagasaki-maru* 242 cruises were divided into three areas based on the temperature-salinity diagram (see Fig.2 of Shiozaki et al., 2011): the ECS, Kuroshio, and Philippine Sea ~~(Kodama et al., 2011; Shiozaki et al., 2011)~~. During the KT-09-17 cruise, we conducted experiments around the Miyako Islands which were distinguished ~~where algal blooms are frequent (see Results section), and distinguished the target area~~ from the other three areas. During the KT-10-19 cruise, we performed observations in the ECS, the Kuroshio, and around the Miyako Islands (Liu et al., 2013).

2.2.1. **Light intensity, hydrography, nutrients, and chl *a***

Water samples for all of the experiments, with the exception of determination of the dissolved iron concentration, were collected using an acid-cleaned bucket and Niskin-X bottles. The depth profile of light intensity was determined immediately before the water sampling using a light sensor (during the KT-06-22, KT-07-21, KT-09-17, and KT-10-19 cruises) or an empirical equation (during the *Nagasaki-maru* 242 cruise) (Shiozaki et al., 2011). Temperature and salinity profiles to a depth of 200 m were obtained using a conductivity, temperature, and depth (CTD) sensor. Mixed layer depth (MLD) was defined as the depth at which the sigma-t increased by 0.125 from its value at a depth of 10 m. Water samples for nitrate+nitrite (N+N) and phosphate were collected from 0, 10, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, and 200 m, and from depths at given light intensities. At all of the stations, 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. W~~at a detection limit of 3 nM (Hashihama et al., 2009) w~~hen the concentration was ~~less~~ greater than 0.1 µM, it was determined by conventional methods using a TRAACS 2000 autoanalyzer (Bran:Luebbe, UK). ~~.~~In addition to the observations at the stations, temperature, salinity, and the *in vivo* chl fluorescence of the surface water were monitored continuously during the cruises by a thermosalinograph (Ocean Seven, Idronaut, Italy) and a fluorometer (Minitracka, Chelsea, UK).

2.2.2. **Dissolved iron**

Water was sampled to estimate the dissolved iron concentration from 0.5-m depth during the KT-06-21 and KT-07-22 cruises and from 10-m depth during the KT-09-17 cruise using an acid-cleaned Teflon bellows pump (AstiPure PFD2; Saint-Gobain) with Teflon tubing (inner diameter = 12 mm). The water was filtered through an acid-cleaned 0.22 µm pore filter (Millipak100; Millipore) connected to the in-line of the Teflon tubing with a Teflon connector. Filtered seawater was collected in a 125 mL low-density polyethylene (LDPE) bottle (Nalgene, Nalge Nunc International), which~~. The sample bottles~~ had been washed using following technique: the sample bottles were sequentially cleaned by soaking in 5% alkali detergent for at least 2 days, in 4 N HCl for at least 1 day, in 0.3 N metal analysis-grade HNO_3 at 60°C overnight, and finally, in Milli-Q water at 60°C overnight. After rinsing with Milli-Q water, the bottles were dried in a laminar flow space and stored in double plastic bags. The filtrate samples were acidified to a pH <1.7 with trace-metal-grade HCl (Tamapure AA-100; Tama Chemicals) in a Class-100 clean-air bench, and stored at room temperature for more than 1 year.

The dissolved iron concentration was determined using an automatic Fe(III) flow injection analytical system (Kimoto Electric Co., Ltd.) using a chelating resin pre-concentration and chemiluminescence detection method (Obata et al., 1993). A buffer solution of 10 M formic acid and 2.4 M ammonium formate was added to the samples. The sample pH was adjusted to 3.0 with 20% ammonium hydroxide

(NH_4OH; Tamapure AA-10; Tama Chemicals) immediately prior to analysis. The detection limit of this method was 0.05 nM. The SAFe reference standards S1 and D2 were measured during the course of sample analysis, and the results were within the range of the published consensus values: S1 = 0.097 ± 0.043 nM and D2 = 0.91 ± 0.17 nM (Johnson et al., 2007).

2.2.3. **Nitrogen fixation and abundance of *Trichodesmium* spp.**

Samples for the incubation experiments were collected vertically at all of the stations, except at Sts. T0621, GN-3, and T0905, where samples were only collected from the surface. All samples were collected in duplicate in acid-cleaned 4.5-L polycarbonate bottles. During the *Nagasaki-maru* 242 cruise, water samples were collected from four different depths corresponding to 100%, 25%, 10%, and 1% of the surface light intensity. During the other cruises, samples were collected from a depth of 50% surface light intensity. Samples at 100% surface light intensity were collected from 0 m during all of the cruises, except during the KT-10-19 cruise in which the samples were collected from a depth of 5 m. The bulk water nitrogen fixation activity was determined ~~with~~ based on primary production using a dual isotopic ($^{15}N_2$ and ^{13}C) technique (~~, the details of which are given in~~ Shiozaki et al., ~~(~~2009). After ^{13}C-labeled sodium bicarbonate (99 atom% ^{13}C; Cambridge Isotope Laboratories) was added to each bottle, 2 mL of $^{15}N_2$ gas (98 + atom% ^{15}N; SI Science Co. Japan) was 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. We determined the nitrogen fixation activity using the $^{15}N_2$ gas bubble addition method (Montoya et al., 1996). This method is believed to underestimate the nitrogen fixation rate relative to the $^{15}N_2$ gas dissolution method (Mohr et al., 2010). The start time of incubation in this study varied at each station (Table S1). Considering daily periodicity of nitrogen fixation in each diazotroph (Zehr, 2011) and the time to reach equilibration of the $^{15}N_2$ 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 $^{15}N_2$ 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 ~~Thus,~~ the obtained ~~nitrogen fixation~~ rate. ~~was lower than the actual rate in the present study.~~

A recent study demonstrated that commercial $^{15}N_2$ gas could be contaminated by ^{15}N-labeled nitrate and ammonium (Dabundo et al., 2014). We tested the contamination in $^{15}N_2$ gas produced by SI Science Co., Ltd., which was used (from different batch numbers) in the present study (see Supporting ~~Methods~~Information). Briefly, the $^{15}N_2$ gas was dissolved in aged subtropical surface water, and concentrations of nitrate, nitrite, and ammonium at the nanomolar levels were determined using supersensitive colorimetric systems. The results showed that there were no significant differences between the control and samples to which $^{15}N_2$ had been added (Fig. S1), suggesting that the contamination of nitrate, nitrite, and ammonium in the $^{15}N_2$ gas was insignificant (~~see~~ Supporting ~~Results and Discussion~~Information).

Water samples were collected for microscopic analysis at all light depths during the *Nagasaki-maru* 242 and KT-07-21 cruises, and only from the surface during the KT-06-22, KT-09-17, and KT-10-19 cruises. The samples were fixed using acidified Lugol's solution. *Trichodesmium* spp. were counted using the Utermöhl method under inverted microscope observation. *Trichodesmium* greater than ca. 300 μm in length were counted as 1 filament and shorter lengths were counted as 0.5 filaments. In addition, phytoplankton other than *Trichodesmium* spp. ~~was~~ were identified from the samples obtained during the KT-09-17 cruise.

2.3. Statistical analysis of environmental variables

We used non-metric multi-dimensional scaling (nMDS) to investigate the spatial differences in the environmental variables that could influence *Trichodesmium* growth and bulk water nitrogen fixation; temperature, mixed layer depth, nitrate, dissolved iron, and phosphate. The environmental variables were transformed by $\log_{10}(x + 1)$ prior to analysis. A dissimilarity/similarity matrix between stations was constructed using the Bray-Curtis index. The nMDS was used to visualize similarities in the environmental variables among the stations. An Analysis of Similarity (ANOSIM) was used to test the differences in the environmental variables among the stations.

~~2.3.~~2.4. Numerical experiments

Numerical particle-tracking experiments were conducted to investigate the transport of water masses at the surface from areas around the Miyako Islands in the summer season from 2003 to 2009. Surface velocity data were derived from the FRA-JCOPE2 reanalysis product (Miyazawa et al., 2009), which is an eddy-resolving (1/12°) ocean model combined with three-dimensional variational data assimilation (satellites, ARGO floats, and shipboard observations), and is one of the most reliable models for the region around Japan for the above time period. The method of tracking particles was basically the same as in Itoh et al. (2009), but we did not include the random walk for simplicity. The release points of particles were selected at the surface ~~at~~ of the model grid points around the coastal waters of the Miyako Islands. We assumed that the particles did not increase, die, or sink from the surface during the experiments. ~~The particle distribution at the surface was fixed throughout the experiment.~~ To focus on transport during the summer season (July–September), particles were released one month before the summer (~~on~~ June 1) and were tracked until September 30.

To examine differences in the output depending on the start time within the same year, we also performed experiments starting on June 1, 11, and 21, and July 1 in 2009. The ratio of particles that reached areas downstream of the Tokara Strait (hereafter Area K) (Fig. 7), including the particles' entrainment to the Kuroshio, to total particles released from the Miyako Islands was computed in all experiments. It should be noted that these experiments contained the following two uncertainties. First, the distribution of *Trichodesmium* around the islands, which strongly influences the destinations of particles, was not able to be determined in advance.

Trichodesmium is known to aggregate and not to occur uniformly in the ocean (Capone et al., 1997). Second, the model cannot reproduce the current very close to the islands. If a water mass very near the islands was delivered to the open ocean by tide and/or river plumes that were not considered in the model, seaward dispersion of particles was likely underestimated.

3. RESULTS

3.1. The Kuroshio path and bloom frequency

The average surface current field indicated that the main stream of the Kuroshio flowed along the continental shelf in the ECS, and then passed to the south of the Kyushu and Shikoku Islands (Fig. 1b). In addition, the Kuroshio branch bifurcated northward at 25°N and 30°N at the continental shelf. Hence, all of the stations in the ECS were subject to the influence of the Kuroshio. While the northeastward stream of the Kuroshio was prominent in this region, smaller-scale flows and circulations were observed in the areas around and to the southeast of the Ryukyu Islands. In the west of the main stream of the Kuroshio, because the average chl *a* was over 0.15 mg m^{-3} (Fig. S2), the frequency of chl *a* values >0.15 mg m^{-3} was high ~~The algal bloom frequency was consistently >10% in the west of the main stream of the Kuroshio because the~~

~~average chl *a* was >0.15 mg m^{-3}~~ (Fig. 1~~a,~~ b). In contrast, the bloom frequency in the east of the main stream of the Kuroshio differed from the distribution of the average chl *a*; algal blooms occurred frequently in the Ryukyu Islands. Around the Miyako Islands, water of high bloom frequency was located to the west of the islands, extending to the north.

3.2. Region-wide environmental conditions, *Trichodesmium* spp., and nitrogen fixation

The sea surface temperature (SST) ranged from 25.1–30.5°C at all of the stations (Table S1), and there were no significant differences among the areas ($p>0.05$, Tukey's honestly significant difference [HSD] test). The MLD varied from 12–60 m at all of the stations, and was relatively deep around the Miyako Islands compared to the other areas (Table S1). The surface N+N concentration varied between <3 and 42 nM, except around the Miyako Islands (Shiozaki et al., 2010, 2011) (Table S1). The highest surface N+N concentration (374 nM) was observed at St. T0904 where upwelling occurred (see below). No significant difference in the surface N+N was observed among the four areas ($p>0.05$, Tukey's HSD test). The surface phosphate concentration varied between <3 and 36 nM at all of the stations (Fig. 2a). The phosphate concentration at the surface and within the MLD was not significantly different among the four areas (*p*>0.05, Tukey's HSD test). There was a greater increase in the phosphate concentrations below 40–50 m in the ECS compared to the other areas (Fig. 3a–d). Furthermore, the phosphate concentrations below 40–50 m near the Miyako Islands were higher than those in the Kuroshio and the Philippine Sea, which were depleted down to 100 m, except at St. T1004 located near the continental shelf. The N/P (= N+N/phosphate) ratio at the surface varied from 0.28 to 6.40 except at St. T0904 (N/P = 16.3) (Table S1), and no significant differences were observed among the four areas ($p > 0.05$, Tukey's HSD test). The surface dissolved iron concentration ranged from 0.19 to 0.89 nM at all of the stations (Fig 2b), with no significant spatial differences among the four areas (*p*>0.05, Tukey's HSD test). The surface dissolved iron concentration at Sts. T0622 and T0907 was elevated to 0.83 nM and 0.89 nM, respectively, with lower salinity water than in the adjacent waters (salinity data are shown in Fig. 4~~4~~a and Kodama et al., 2011). The nMDS showed that the environmental variables at all stations were the same at the >80% similarity level and were >90 % similar excepting station T0904 (Fig. 5). The ANOSIM indicated no significant differences among the stations ($p > 0.05$).

The abundance of *Trichodesmium* spp. was highest at the surface at almost all of the stations during the *Nagasaki-maru* 242 and KT-07-21 cruises (~~Shiozaki et al., 2010~~Fig. S3). The surface *Trichodesmium* spp. abundances were positively correlated with the depth-integrated abundances ($p < 0.05$, t-test) (Fig. 6a). Thus, the surface abundance was used to discuss the geographical distribution of *Trichodesmium* spp. The *Trichodesmium* spp. abundance at the surface varied widely, and there was no significant difference among the four areas ($p > 0.05$, Tukey's HSD test). *Trichodesmium* spp. were observed at all of the stations in the Kuroshio and around the Miyako Islands, whereas they were not always observed in the ECS and the Philippine Sea (Fig. 2c). The average surface abundance in the Philippine Sea was the lowest among all of the areas (Table 1). The highest abundance of *Trichodesmium* spp. (>20000 filaments L^{-1}) was observed near the Miyako Islands at St. T0906, where they bloomed (see below). Tuft-shaped colonies were found at Sts. T0706, T0723, CK-10, and T0906. The nitrogen fixation rate was highest in the upper 25% light depth, and decreased with increasing depth at all of the stations (Fig. ~~3b~~3e–h). The surface 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. Surface and d~~D~~epth-integrated nitrogen fixation ranged from 0.54 to 62 nmol N L^{-1} d^{-1} and from 29.5 to 753 μmol N m^{-2} d^{-1}~~-~~, respectively (Fig. 2d and

Table S1). Surface nitrogen fixation in the Philippine Sea was significantly lower than that in the Kuroshio ($p < 0.05$, *t*-test).~~Thus, the surface abundance was used to discuss the geographical distribution of *Trichodesmium* spp., which were observed at all of the stations in the Kuroshio and around the Miyako Islands, whereas they were not always observed in the ECS and the Philippine Sea (Fig. 2c). The highest abundance of *Trichodesmium* spp. (>20000 filaments L^{-1}) was observed near the Miyako Islands at St. T0906, where they bloomed (see below). Depth-integrated nitrogen fixation ranged from 29.5 to 753 μmol N m^{-2} d^{-1} (Fig. 2d). The nitrogen fixation rate was highest in the upper 25% light depth, and decreased with increasing depth at all of the stations (Fig. 3b). The average nitrogen fixation rate in the Philippine Sea of 58.3 ± 25.1 μmol N m^{-2} d^{-1} was the lowest among all of the areas (Table 1).~~

The surface abundance of *Trichodesmium* spp. in the entire study area was positively correlated with the nitrogen fixation rate at the surface ($r^2 = 0.80$; $p < 0.05$ [$r^2 = 0.55$; $p < 0.05$ 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. However, active nitrogen fixation occurred in the ECS where *Trichodesmium* abundance was low, and hence, the other diazotrophs could also be important for nitrogen fixation.

3.3. Observation around the Miyako Islands during the KT-09-17 cruise

The SST was lower to the northwest of the Miyako Islands than in adjacent waters, and chl *a* was enriched in the same location (Fig. ~~4b~~4b,~~–~~c). Therefore, the enhanced productivity was probably due to nutrient supply by upwelling. This upwelling generally occurs in the lee of islands (Hasegawa et al., 2009), suggesting that there was a northward current during the cruise. The surface salinity was lower east of the Miyako Islands than in the surrounding waters (Fig. ~~2a~~4a). The absence of any large river on the east side of Miyako-jima Island and the separation of low salinity water from the island suggest that the low salinity was caused by rainfall.

St. T0904 was located near the upwelling water; its SST of 29.0°C was lowest and its surface N+N concentration of 374 nM was highest among all of the stations. However, the N+N concentration at St. T0904 at the surface was higher than that at the subsurface (an approximate depth of 50 m; Fig. ~~S2~~S4), indicating that St. T0904 was not located in the middle of the upwelling. At St. T0904, t~~T~~he surface phosphate concentration was also highest ~~at St. T0904~~ (23 nM) and the N/P ratio (=16.3) was higher than the Redfield ratio.~~.~~ With the exception of the surface at St. T0904, the phosphate concentration was low (<3–9 nM) in the upper 50 m, with no noticeable variation among the stations (Fig. 2a). The dissolved iron concentration varied between 0.19 and 0.89 nM at the surface (Fig. 2b). The highest dissolved iron concentration was observed at St. T0907.

During the same cruise, we encountered a *Trichodesmium* spp.-bloom at St. T0906 (Fig. 2c), which had colored water at the surface. The abundance of *Trichodesmium* spp. at St. T0906 was >20,000 filaments L^{-1}, which was far higher than that at other stations (2–102 filament L^{-1}). The nitrogen fixation rate at the surface (61.9 nmol N L^{-1} d^{-1}) of this station was more than 30-fold that just below the surface, and was the highest among all of the stations (Fig. 3h~~4b~~). The diatom abundance was markedly higher at St. T0904 than that at the other stations. *Cylindrotheca closterium* was the most numerically dominant diatom (59%), followed by *Navicula* spp. (23%) and *Nitzschia* spp. (13%). *C. closterium* was not detected at the other stations, indicating that the high chl *a* induced by the island wake effect mainly consisted of diatoms.~~. The count of *Thalassiosira* spp. was higher at St. T0907 than that at the other stations.~~

3.4. Numerical simulation

As the Kuroshio generally flows along the continental slope north of the Miyako

Islands (Fig. 1b), particles around the Miyako Islands were not transported along the typical path of the Kuroshio to the northeast, especially at their initial stages (Fig. 7a). Some particles migrated around the Miyako Islands, or turned south after they passed the Tokara Strait. Nevertheless, the particles delivered to Area K east of the Tokara Strait increased as time elapsed, and the ratio of particles delivered to Area K to the total released particles ranged from 13–56% (30 ± 16%) by day 120 in 2003–2009 (Fig. 7b). The year-to-year variations in the ratio are mainly due to influences of mesoscale eddies as partly seen in the particle trajectories in Fig. 7a, and likely occurred over relatively short time scales (shorter than the seasonal time scale). This is supported by another series of experiments in which particles were released on June 1, 11, and 21, and July 1 in 2009, which yielded ratios of 6.2–38% (22 ± 13%) by day 120 (Fig. S5).

~~It should be noted that the model output could vary greatly depending on the start time because it assimilated observed datasets, and hence, quantitative assessments of the results are not straightforward. The model outputs demonstrated that, although there were some exceptions, the particles released from the islands were generally delivered to the Kuroshio in all years (Fig. 5).~~

4. DISCUSSION

4.1. Distribution of phosphate and dissolved iron concentrations

Phosphate concentrations were consistently low within the MLD in all of the studied areas, and the maximum abundance of *Trichodesmium* spp. and total nitrogen fixation activity generally occurred near the surface, suggesting that the phosphate conditions for surface *Trichodesmium* spp. and other diazotrophs were similar among all of the areas. Furthermore, with the exception of St. T1004 located near the continental shelf, the vertical distribution of phosphate in the Kuroshio was analogous to that in the Philippine Sea. Therefore, at least in the oceanic region of the two areas, phosphate availability for *Trichodesmium* spp. and the other diazotrophs was similar throughout the water column.

The surface distribution of the dissolved iron concentration demonstrated no significant variation among the areas. The dissolved iron concentration (0.19–0.89 nM) was higher than that ~~(0.15–0.4 nM)~~ in the western North Pacific subtropical region (0.15–0.4 nM) (Brown et al., 2005). Obata et al. (1997) demonstrated that the vertical distribution of the dissolved iron concentration in the ECS showed two peaks (at the surface and in the deep water), suggesting that aerial dust significantly contributes to the high dissolved iron concentration at the surface in all of our study areas. In accordance with our results, previous modeling studies estimated the amount of dust deposition to be similar in all four areas (Jickells et al., 2005; Mahowald et al., 2009). Therefore, iron availability for *Trichodesmium* spp. and the other diazotrophs was also likely similar across all of the study areas. Iron can be supplied from deep water to the surface by mixing processes (Johnson et al., 1999). However, if this were the case, the nitrate concentration would be expected to increase simultaneously at the surface (Johnson et al., 1999), and we observed no noticeable elevation in N+N in any of the areas, except at St. T0904. High concentrations of dissolved iron (>0.8 nM) corresponded with low salinity at Sts. T0622 and T0907, suggesting that wet deposition was an important process for iron supply. Dry deposition could also be important since the iron-enriched water at Sts. T0601 and T0715 did not correspond with low salinity.

Satellite data analysis indicated that there was a “pipeline” of material transport from the Miyako Islands to the Kuroshio, and this was supported by numerical simulations. According to the hypothesis of Marumo and Asaoka (1974), the growth of *Trichodesmium* in the Kuroshio could be maintained by the supply of iron and phosphorus from the islands situated along the Kuroshio, and the Miyako Islands were considered a possible nutrient source to the Kuroshio. Hence, assuming this hypothesis to be valid, the iron and phosphate concentrations near the Miyako Islands (especially in our observed area) would be expected to be higher than those in the other areas. However, we observed no significant difference in the iron and phosphate concentrations among the four areas. This suggested that there was no detectable washout of iron and phosphorus from the Miyako Islands during our observations, or that diazotrophs and other phytoplankton exhausted the nutrient supply close to the islands.

4.2. **Factors controlling the distributions of *Trichodesmium* spp. and nitrogen fixation**

Although there was no statistically significant difference in *Trichodesmium* spp. abundance among the study areas probably because the data were limited and the variation was large, *Trichodesmium* spp. were always observed in the Kuroshio and were abundant at most 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 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). ~~Trichodesmium spp. was abundant in the Kuroshio, as also reported by Marumo and Asaoka (1974).~~ Previous studies demonstrated that *Trichodesmium* spp. flourished in some regions of the subtropical ocean where the iron levels were high (Moore et al., 2009; Shiozaki et al., ~~2014b~~2014b), which can be attributed to the high iron requirement of *Trichodesmium* spp. for their growth compared to other diazotrophs and non-diazotrophs (Kustka et al., 2003; Saito et al., 2011). Therefore, the distribution of *Trichodesmium* spp. in the study area was expected to be associated with the dissolved iron concentration at the surface. Furthermore, the iron-enhanced active nitrogen fixation causes phosphorus depletion, and is consequently limited by phosphorus (Mather et al., 2008). No significant differences in surface iron and phosphate were observed among the study areas, which~~Furthermore, iron is the limiting nutrient not only for *Trichodesmium*, but also for other diazotrophs. Accordingly, bulk water nitrogen fixation appeared to also be related to the dissolved iron concentration (Moore et al., 2009; Shiozaki et al., 2014b), although not significantly, which indicates that the dissolved iron concentration~~ cannot explain the distribution of *Trichodesmium* spp. and nitrogen fixation in the study region.

Johnson et al. (1999) reported that the iron supply increased around the continental shelf because re-suspension from the bottom to the euphotic zone becomes significant. However, in the continental shelf of the ECS, the abundance of *Trichodesmium* spp. and nitrogen fixation were low (Marumo and Asaoka, 1974; Zhang et al., 2012). Zhang et al. (2012) suggested that the low nitrogen fixation in the continental shelf was attributable to mixing processes and the influence of the Changjiang River. Turbulence near the sea floor influences the surface water in the shallower bottom region (Matsuno et al., 2006), and Zhang et al. (2012) suggested that the physical disturbance reduces diazotrophy since diazotrophs including *Trichodesmium* favor calm seas. Furthermore, the water in the continental shelf of the ECS is strongly influenced by the Changjiang River. The N/P ratio of the Changjiang River plume is significantly higher than the Redfield ratio, which results in phosphorus limitation, and can ~~be attributed~~contribute to the low nitrogen fixation (Zhang et al., 2012). In the present study, despite the fact that the surface phosphate concentration was low throughout the study areas, the N/P ratio was generally lower than the Redfield ratio, suggesting that biological production was limited by the availability of nitrogen compared to phosphate (Moore et al., 2008, 2013). Furthermore, the insignificant difference in MLD among the ECS, the Kuroshio, and the Philippine Sea ($p>0.05$; Tukey HSD test) indicated similar vertical mixing~~physical~~ conditions. Therefore, the environmental variables related to nitrogen fixation only slightly differed as demonstrated by the nMDS plot.~~ (see more detail in Supporting Methods and Supporting Results and Discussion)~~.

In our study, we found a *Trichodesmium* spp. bloom near the Miyako Islands.~~Why did *Trichodesmium* spp. become abundant in the Kuroshio?~~ Recent studies demonstrated that *Trichodesmium* spp. thrived near oceanic islands (Shiozaki et al., 2010, 2014c; Dupouy et al., 2011)~~, which was attributable to the terrigenous nutrient supply (Shiozaki et al., 2014c)~~.~~ In fact, we observed a *Trichodesmium* spp. bloom near the Miyako Islands.~~ Given that some aspect of the environment around the islands increases *Trichodesmium* spp. abundance and that they are transported from the islands to the Kuroshio, this can explain why the *Trichodesmium* distribution was not estimated from environmental variables. ~~the inconsistency between *Trichodesmium* spp. abundance and concentrations of iron and phosphate.~~ Accordingly, the low abundance of *Trichodesmium* spp. in the Philippine Sea was likely due to the low density of islands. Furthermore, higher nitrogen fixation in the Kuroshio than in the Philippine Sea might be explained in the same manner. ~~This is because~~ *Trichodesmium* is ~~considered~~ a major nitrogen fixer in the Kuroshio (Chen et al., 2008, 2014; Shiozaki et al., 2014a), and our results showed that the bulk water nitrogen fixation was positively correlated with *Trichodesmium* abundance.

The numerical simulation demonstrated that released particles from the Miyako Islands were generally transported to the northeast and flowed along the Kuroshio during summer between 2003 and 2009. Thus, if *Trichodesmium* increases and active nitrogen fixation usually occurs around the Miyako Islands, the water would be delivered to the Kuroshio. Furthermore, we performed additional particle tracking experiments whose particle release points were set at major islands in the Ryukyu Islands (Amami Islands, Okinawa Main Island, and the Ishigaki Islands) (Figs. ~~S3~~S6 and S7). The results demonstrated that the particles released from the other islands of the Miyako Islands were also delivered to the Kuroshio, with some exceptions. Based on the calculations for 2003–2009, 13–56% (30 ± 16%) of particles released from the islands reached Area K by day 120 (Fig. S7).

Studies on nitrogen fixation around islands in the study region are fairly limited (Liu et al., 2013), and the present study is the first report of a *Trichodesmium* bloom around islands in the area. The Miyako Islands are surrounded by reefs, and studies have shown that *Trichodesmium* blooms can be associated with reef environments

(Bell et al., 1999; McKinna et al., 2011). However, the factors causing the *Trichodesmium* blooms around islands are not well understood (Shiozaki et al., 2014c). Further studies are required to identify which characteristics of the near island environment are important for the growth and/or accumulation of *Trichodesmium* and other diazotrophs.

5. CONCLUSIONS

~~Based on our results, w~~We hypothesize that the high abundance of *Trichodesmium* spp. and active nitrogen fixation in the Kuroshio ~~are~~ were ascribable not to the unique nutrient environment, but rather to the supply of *Trichodesmium* spp. and other diazotrophs from the surrounding islands. The Ryukyu Islands would not be the only islands with abundant *Trichodesmium* spp., as *Trichodesmium* spp. also flourish~~es~~ in the upstream Kuroshio near Luzon Island (Chen et al., 2008). Therefore, ~~we suggest that~~ the abundance of *Trichodesmium* spp. ~~abundances are~~would be generally increased around islands situated along the Kuroshio, and the abundant *Trichodesmium* spp. ~~are~~ would likely be transported to the mainstream of the Kuroshio. *Trichodesmium* is a major diazotroph in the Kuroshio (Chen et al., 2008, 2014; Shiozaki et al., 2014a), and diazotrophy in the Kuroshio is considered to influence the nutrient stoichiometry in the North Pacific (Shiozaki et al., 2010). Thus, our results indicate that phenomena around the islands located along the Kuroshio are important for determining the partial nitrogen inventory in the North Pacific.

Author Contributions

T.S., S.T., S.I., and K.F. designed the experiment and T.S., S.T., T.K., X.L., F.H., and K.F. collected the samples at sea. T.S. determined nitrogen fixation and abundance of *Trichodesmium* spp. during the KT-06-21, KT-07-21, KT-09-17, and *Nagasaki-maru* 242 cruises, and X.L. did during the KT-10-19 cruise. T.S. analyzed datasets of satellite and climatological current field. S.T. analyzed concentration of dissolved iron. S.I. performed numerical experiments. T.K. and F.H. determined nutrient concentration. T.S. prepared the manuscript with contributions from all co-authors.

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References

Bell, P.R.F., Elmetri, I., Uwins, P.: Nitrogen fixation by *Trichodesmium* spp. in the central and northern Great Barrier Reef lagoon: relative importance of the fixed-nitrogen load, Mar. Ecol. Progr. Ser., 186, 119-126, 1999.

Brown, M.T., Landing, W.M., Measures, C.I.: Dissolved and particulate Fe in the western and central North Pacific: Results from the 2002 IOC cruise, Geochem. Geophys. Geosyst., 6(10), Q10001, 2005.

Capone, D.G., Zehr, P.J., Paerl, H.W., Bergman, B., Carpenter, E.J.: *Trichodesmium,* a globally significant marine cyanobacterium, Science, 276, 1221-1229, 1997.

Chen, C.T.A.: Distributions of nutrients in the East China Sea and the South China Sea connection, J. Oceanogr., 64, 737-751, 2008.

Chen, Y.L.L., Chen, H.Y., Tuo, S.H., Ohki, K.: Seasonal dynamics of new production from *Trichodesmium* N_2 fixation and nitrate uptake in the upstream Kuroshio and South China Sea basin, Limnol. Oceanogr., 53(5), 1705-1721, 2008.

Chen, Y.L.L., Chen, H.Y., Lin, Y.H., Yong, T.C., Taniuchi, Y., Tuo, S.H.: The relative contributions of unicellular and filamentous diazotrophs to N_2 fixation in the South China Sea and the upstream Kuroshio, Deep-Sea Res. I, 85, 56-71, 2014.

Dabundo, R., Lehmann, M.F., Treibergs, L., Tobias, C.R., Altabet, M.A.: The contamination of commercial $^{15}N_2$ gas stocks with ^{15}N-labeled nitrate and ammonium and consequences for nitrogen fixation measurements, PLoS one, 9(10), e110335, 2014.

Dupouy, C., Benielli-Gary, D., Neveux, J., Dandonneau, Y., Westberry, T.K.: An algorithm for detecting *Trichodesmium* surface blooms in the South Western Tropical Pacific, Biogeosciences, 8, 3631-3647, 2011.

Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M.M.M., Lavik, G., Schmitz, R.A., Wallace, D.W.R., LaRoche, J.: Doubling of marine dinitrogen-fixation rate based on direct measurements, Nature, 488, 361-364, 2012.

Hasegawa, D., Lewis, M.R., Gangopadhyay, A.: How islands cause phytoplankton to bloom in their wake, Geophys. Res. Lett., 36, L20605, 2009.

Hashihama, F., Furuya, K., Kitajima, S., Takeda, S., Takemura, T., Kanda, J.: Macro-scale exhaustion of surface phosphate by dinitrogen fixation in the western North Pacific, Geophys. Res. Lett., 36, L03610, 2009.

~~Hu, D., Wu, L., Cai, W., Gupta A.S., Ganachaud, A., Qiu, B., Gordon, A.L., Lin, X., Chen, Z., Hu, S., Wang, G., Wang, Q., Sprintall, J., Qu, T., Kashino, Y., Wang, F., Kessler, W.S.: Pacific western boundary currents and their roles in climate, Nature 522, 299-308, 2015.~~

Isobe, A.: Recent advances in ocean-circulation research on the Yellow Sea and East China Sea shelves, J. Oceanogr., 64, 569-584, 2008.

Itoh, S., Yasuda, I., Nishikawa, H., Sasaki, H., Sasai, Y.: Transport and environmental temperature variability of eggs and larvae of the Japanese anchovy (*Engraulis japonicus*) and Japanese sardine (*Sardinops melanostictus*) in the western North Pacific estimated via numerical particle-tracking experiments, Fish. Oceanogr., 18(2), 118-133, 2009.

Jickells, T.D., An, Z.S., Andersen, K.K., Baker, A.R., Bergametti, G., Brooks, N., Cao, J.J., Boyd, P.W., Duce, R.A., Hunter, K.A., Kawahata, H., Kubilay, N., LaRoche, J., Liss, P.S., Mahowald, N., Prospero, J.M., Ridgwell, A.J., Tegen, I., Torres, R.: Global iron connections between desert dust, ocean biogeochemistry, and climate, Science, 308, 67-71, 2005.

Johnson, K.S., Chavez, F.P., Friederich, G.E.: Continental-shelf sediment as a primary source of iron for coastal phytoplankton, Nature, 398, 697-700, 1999.

Johnson, K.S., Boyle, E., Bruland, K., Coale, K., Measures, C., Moffett, J., Aguilar-Islas, A., Barbeau, K., Bergquist, B., Bowie, A., Buck, K., Cai, Y., Chase, Z., Cullen, J., Doi, T., Elrod, V., Fitzwater, S., Gordon, M., King, A., Laan, P., Laglera-Baquer, L., Landing, W., Lohan, M., Mendez, J., Milne, A., Obata, H., Ossiander, L., Plant, J., Sarthou, G., Sedwick, P., Smith, G.J., Sohst, B., Tanner, S., Van den Berg, S., Wu, J.: The SAFe iron intercomparison cruise: an international collaboration to develop dissolved iron in seawater standards, Eos, 88, 131-132, 2007.

~~Kitagawa, T., Kato, Y., Miller, M.J., Sasai, Y., Sasaki, H., Kimura, S.: The restricted spawning area and season of Pacific Bluefin tuna facilitate use of nursery areas: A modeling approach to larval and juvenile dispersal processes, J. Exp. Mar. Biol. Ecol., 393, 23-31, 2010.~~

Kodama, T., Furuya, K., Hashihama, F., Takeda, S., Kanda, J.: Occurrence of rain-origin nitrate patches at the nutrient-depleted surface in the East China Sea and the Philippine Sea during summer, J. Geophys. Res., 116, C08003, 2011.

Kustka, A., Sañudo-Wilhelmy, S., Carpenter, E.J., Capone, D.G., Raven, J.A.: A revised estimate of the iron use efficiency of nitrogen fixation, with special reference to the marine cyanobacterium *Trichodesmium* spp. (Cyanophyta), J. Phycol., 39, 12-25, 2003.

Liu, X., Furuya, K., Shiozaki, T., Masuda, T., Kodama, T., Sato, M., Kaneko, H., Nagasawa, M., Yasuda, I.: Variability in nitrogen sources for new production in the vicinity of the shelf edge of the East China Sea in summer, Cont. Shelf Res., 61-62, 23-30, 2013.

McKinna, L.I.W., Furnas, M.J., Ridd, P.V.: A simple, binary classification algorithm for detection of *Trichodesmium* spp. within the Great Barrier Reef using MODIS imagery, Limnol. Oceanogr.; Methods, 9, 50-66, 2011.

Mahaffey, C., Michaels, A.F., Capone, D.G.: The conundrum of marine N_2 fixation, Am. J. Sci., 305, 546-595, 2005.

Mahowald, N.M., Engelstaedter, S., Luo, C., Sealy, A., Artaxo, P., Benitez-Nelson, C., Bonnet, S., Chen, Y., Chuang, P.Y., Cohen, D.D., Dulac, F., Herut, B., Johansen, A.M., Kubilay, N., Losno, R., Maenhaut, W., Paytan, A., Prospero, J.M., Shank, L.M., Siefert, R.L.: Atmospheric iron deposition: Global distribution, variability, and human perturbations, Annu. Rev. Mar. Sci., 1, 245-278, 2009.

Marumo, R., Asaoka, O.: *Trichodesmium* in the East China Sea 1. Distribution of *Trichodesmium thiebautii* GOMONT during 1961-1967, J. Oceanogr. Soc. Japan, 30, 298-303, 1974.

Mather, R.L., Reynolds, S.E., Wolff, G.A., Williams, R.G., Torres-Valdes, S., Woodward, E.M.S., Landolfi, A., Pan, X., Sanders, R., Achterberg, E.P.: Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres, Nat. Geosci., 1, 439-443, 2008.

Matsuno, T., Lee, J.S., Shimizu, M., Kim, S.H., Pang, I.C.: Measurements of the turbulent energy dissipation rate ε and an evaluation of the dispersion process of the Changjiang Diluted Water in the East China Sea, J. Geophys. Res., 111, C11S09, 2006.

McGowan, J.A.: Oceanic biogeography of the Pacific, in: The microplaleontology of the oceans, Cambridge University Press, Cambridge, 3-74, 1971.

Miyazawa, Y., Zhang, R., Guo, X., Tamura, H., Ambe, D., Lee, J.S., Okuno, A., Yoshinari, H., Setou, T., Komatsu, K.: Water mass variability in the western North Pacific detected in a 15-year eddy resolving ocean reanalysis, J. Oceanogr., 65, 737-756, 2009.

Mohr, W., Großkopf, T., Wallace, D.W.R., LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rate, PLoS one, 5(9), e12583, 2010.

Montoya, J.P., Voss, M., Kähler, P., Capone, D.G.: A simple, high-precision, high-sensitivity tracer assay for N_2 fixation, Appl. Environ. Microbiol., 62(3), 986-993, 1996.

Moore, C.M., Mills, M.M., Langlois, R., Milne, A., Achterberg, E.P., LaRoche, J., Geider, R.J.: Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean, Limnol. Oceanogr., 53(1), 291-305, 2008.

Moore, C.M., Mills, M.M., Achterberg, E.P., Geider, R.J., LaRoche, J., Lucas, M.I., McDonagh, E.L., Pan, X., Poulton, A.J., Rijkenberg, M.J.A., Suggett, D.J., Ussher, S.J., Woodward, E.M.S.: Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability, Nat. Geosci., 2, 867-871, 2009.

Moore, C.M., Mills, M.M., Arrigo, K.R., Berman-Frank, I., Bopp, L., Boyd, P.W., Galbraith, E.D., Geider, R.J., Guieu, C., Jaccard, S.L., Jickells, T.D., LaRoche, J., Lenton, T.M., Mahowald, N.M., Marañon, E., Marinov, I., Moore, J.K., Nakatsuka, T., Oschlies, A., Saito, M.A., Thingstad, T.F., Tsuda, A., Ulloa, O.: Processes and patterns of oceanic nutrient limitation, Nat. Geosci., 6, 701-710, 2013.

~~Nakamura, H., Nishina, A., Minobe, S.: Response of storm tracks to bimodal Kuroshio path states south of Japan, J. Clim., 25, 7772-7779, 2012.~~

Obata, H., Karatani, H., Nakayama, E.: Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection, Anal. Chem., 65, 1524-1528, 1993.

Obata, H., Karatani, H., Matsui, M., Nakayama, E.: Fundamental studies for chemical speciation of iron in seawater with an improved analytical method, Mar. Chem., 56, 97-106, 1997.

Saito, M.A., Bertrand, E.M., Dutkiewicz, S., Bulygin, V.V., Moran, D.M., Monteiro, F.M., Follows, M.J., Valois, F.W., Waterbury, J.B.: Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocosphaera watsonii*, Proc. Natl. Acad. Sci. USA, 108, 2184-2189, 2011.

~~Sasaki, Y.N., Minobe, S., Asai, T., Inatsu, M.: Influence of the Kuroshio in the East China Sea on the early summer (Baiu) rain, J. Clim., 25, 6627-6645, 2012.~~

Shiozaki, T., Furuya, K., Kodama, T., Takeda, S.: Contribution of N_2 fixation to new production in the western North Pacific Ocean along 155ºE, Mar. Ecol. Progr. Ser., 377, 19-32, 2009.

Shiozaki, T., Furuya, K., Kodama, T., Kitajima, S., Takeda, S., Takemura, T., Kanda, J.: New estimation of N_2 fixation in the western and central Pacific Ocean and its marginal seas,. Glob. Biogeochem. Cycles, 24, GB1015, 2010.

Shiozaki, T., Furuya, K., Kurotori, H., Kodama, T., Takeda, S., Endoh, T., Yoshikawa, Y., Ishizaka, J., Matsuno, T.: Imbalance between vertical nitrate flux and nitrate assimilation on a continental shelf: Implications of nitrification, J. Geophys. Res., 116, C10031, 2011.

Shiozaki, T., Chen, Y.L.L., Lin, Y.H., Taniuchi, Y., Sheu, D.S., Furuya, K., Chen, H.Y.: Seasonal variations of unicellular diazotroph groups A and B, and *Trichodesmium* in the northern South China Sea and neighboring upstream Kuroshio

Current, Cont. Shelf Res., 80, 20-31, 2014a.

Shiozaki, T., Ijichi, M., Kodama, T., Takeda, S., Furuya, K.: Heterotrophic bacteria as major nitrogen fixers in the euphotic zone of the Indian Ocean, Glob. Biogeochem. Cycles, 28, 1096-1110, 2014b.

Shiozaki, T., Kodama, T., Furuya, K.: Large-scale impact of the island mass effect through nitrogen fixation in the western South Pacific Ocean, Geophys. Res. Lett., 41, 2907-2913, 2014c.

Wilson, C., Qiu, X.: Global distribution of summer chlorophyll blooms in the oligotrophic gyres, Progr. Oceanogr., 78, 107-134, 2008.

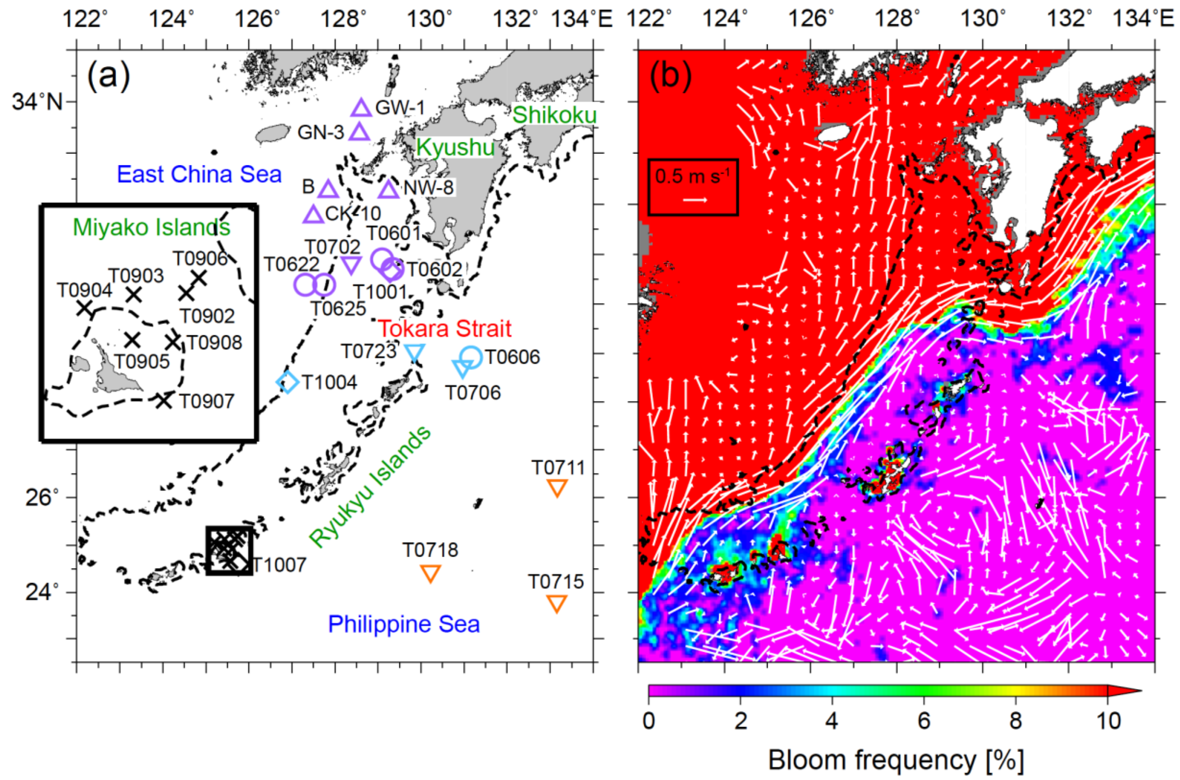
Zhang, R., Chen, M., Cao, J., Ma, Q., Yang, J., Qiu, Y.: Nitrogen fixation in the East China Sea and southern Yellow Sea during summer 2006, Mar. Ecol. Progr. Ser., 447, 77-86, 2012.

Table 1 Summary of *Trichodesmium* at the surface, and depth-integrated nitrogen fixation and its related parameters in the four representative study areas.

Area	*Trichodesmium*[*] [filaments l^{-1}]	N_2 fixation [µmolN L^{-1} d^{-1}]	Temperature[*] [°C]	MLD [m]	NO_3^-+$NO_2^{-*,†}$ [nM]	$PO_4^{3-*,†}$ [nM]	DFe[*] [nM]
East China Sea	21±58	170±140	28.5±1.2	24±12	19±11	15±9	0.76±0.18
Kuroshio	43±33	199±142	29.4±0.81	27±8	9±8	15±7	0.45±0.13
Philippine Sea	8±8	58.3±25.1	29.4±0.1	23±3	8±3	14±19	0.51±0.25
Miyako Islands	3019±8478	201±274	29.3±0.3	40±12	61±128	8±7	0.38±0.24

[*] values in surface water

[†] When the concentration was below the detection limit (3 nM), we assumed a concentration of 3 nM to calculate the mean.



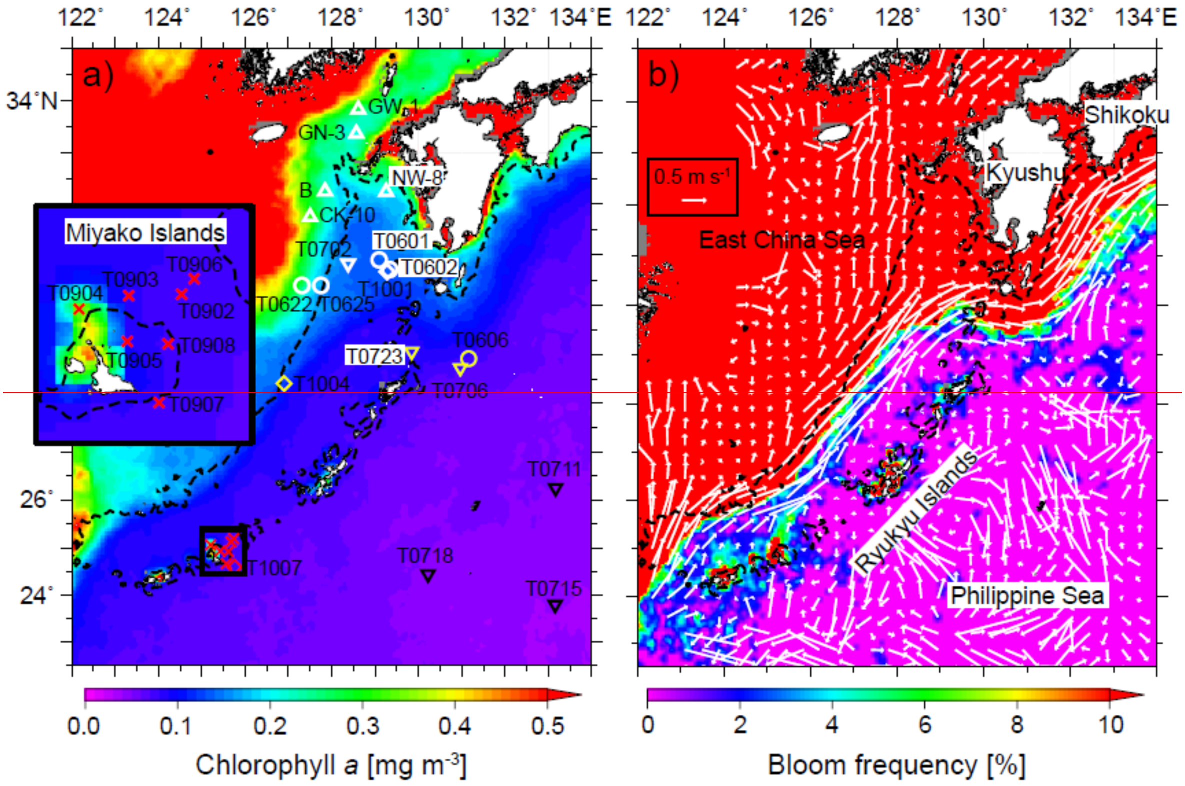
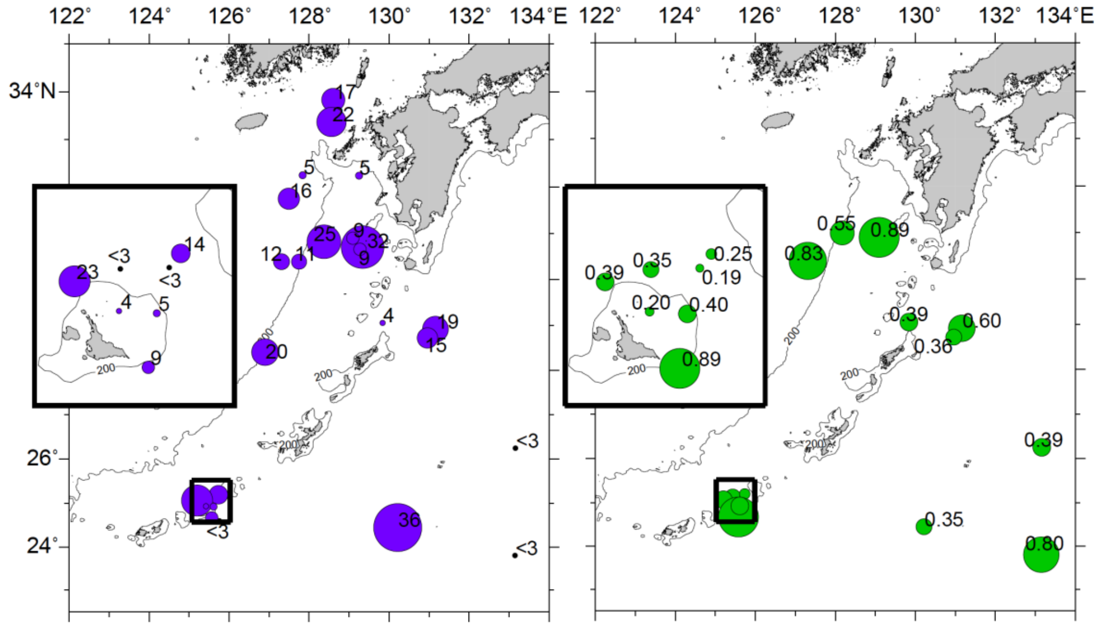


Figure 1. ~~Sampling stations and distribution of chlorophyll *a* and bloom frequency.~~

(a) Sampling stations during the KT-06-21 (circles), KT-07-22 (inverted triangles), KT-09-17 (crosses), KT-10-19 (diamonds), and 242 (triangles) cruises. Symbols of stations located in the East China Sea, the Kuroshio, the Philippine Sea, and near the Miyako Islands are indicated in ~~white~~purple, ~~yellow~~light blue, ~~black~~orange, and ~~red~~black, respectively. ~~The background contour denotes satellite-derived average chlorophyll *a* during the summer from July 2003 to September 2009.~~ (b) Climatological surface current fields during summer (1953–2008) from geoelectrokinetograph measurements and ship-mounted ADCP data. The background contour represents the percentage of chlorophyll *a* of >0.15 mg m^{-3} during summer between 2003 and 2009. Dashed lines indicate 200 m isobaths.

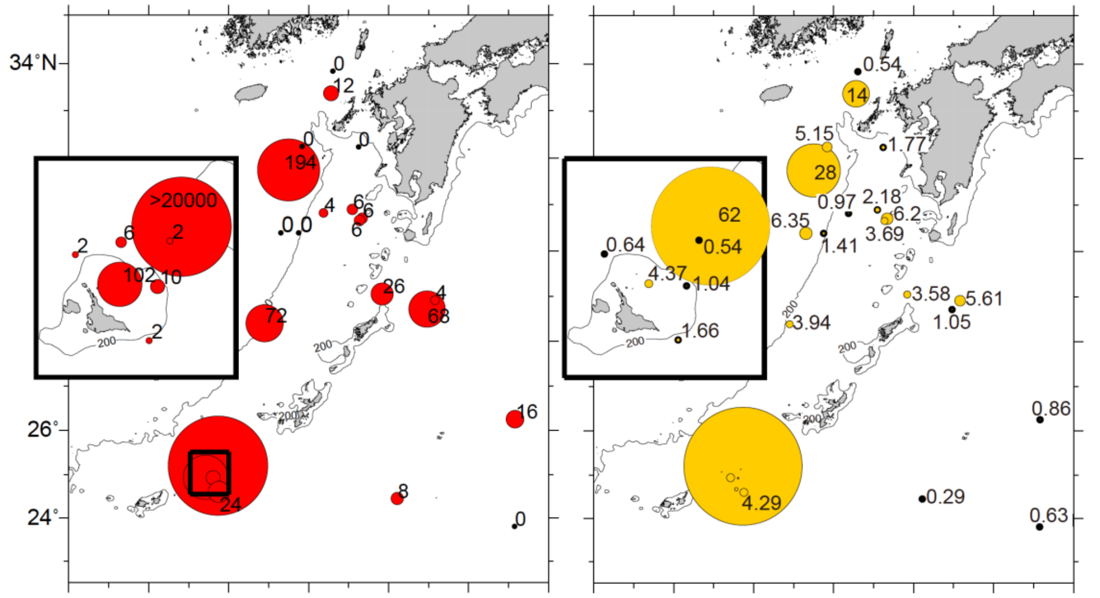
(a) Phosphate [nM]

(b) Dissolved iron [nM]



(c) *Trichodesmium* spp. [filaments L^{-1}]

(d) Nitrogen fixation [nmolN L^{-1} d^{-1}]



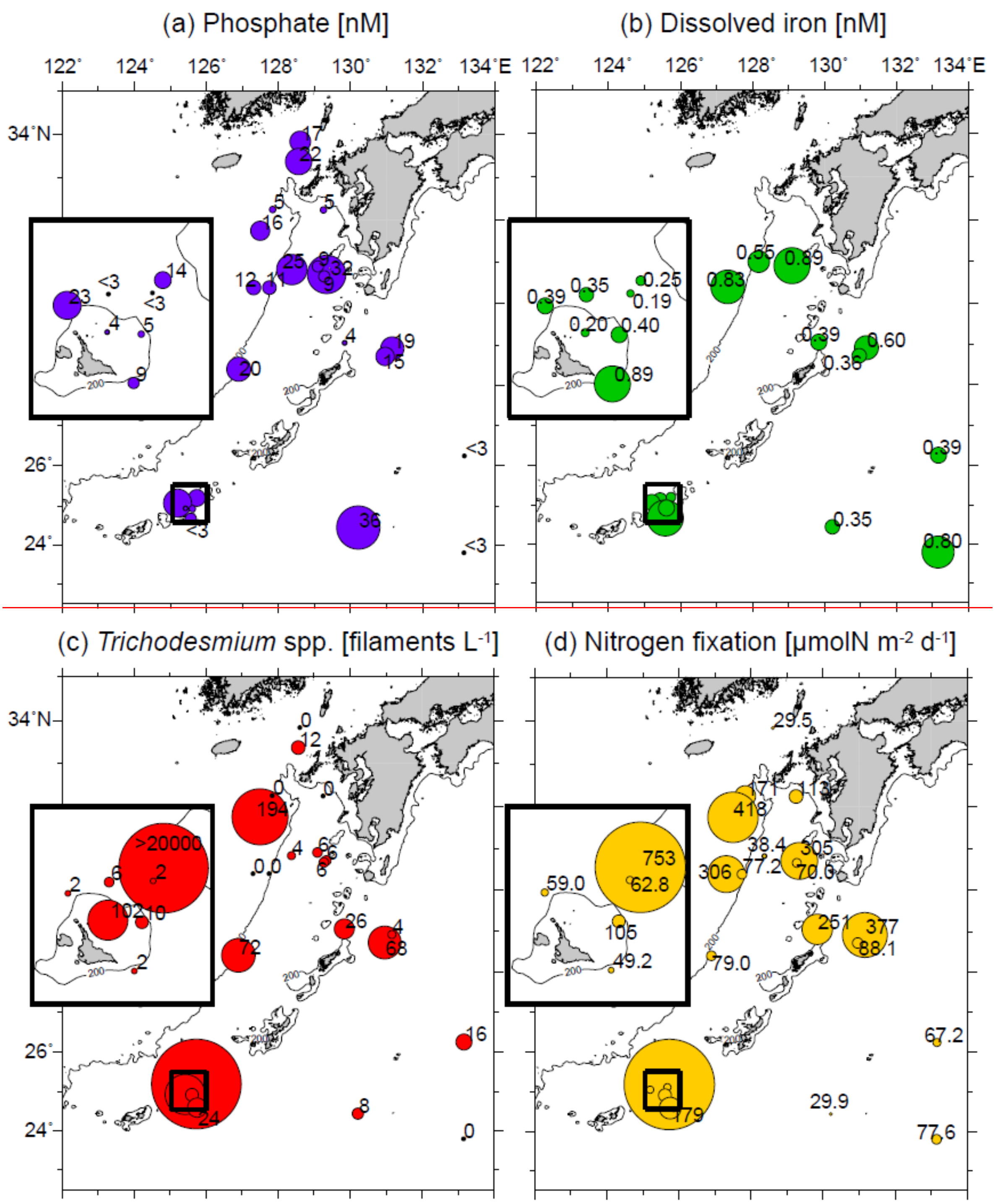
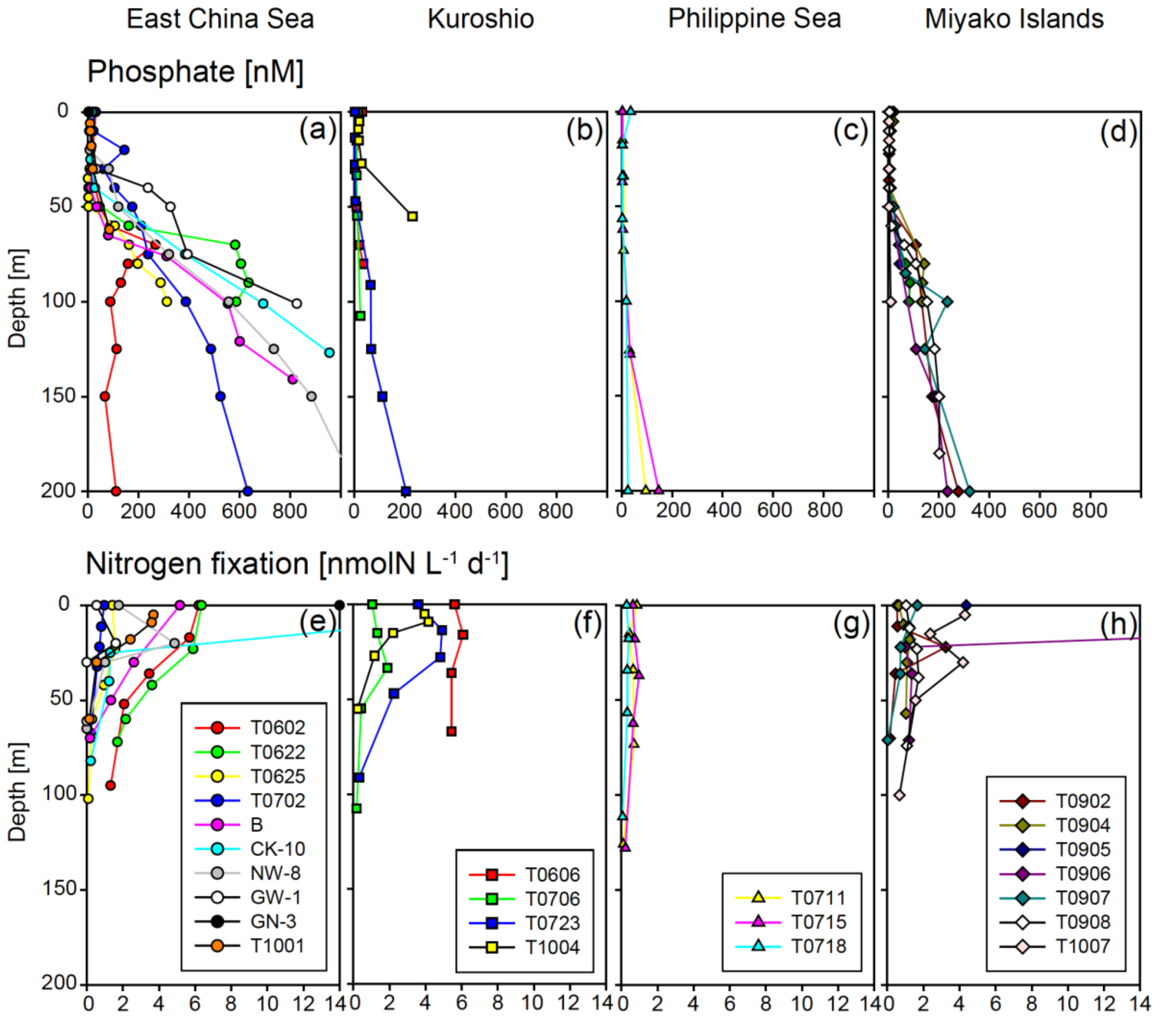


Figure 2. Distribution of (a) phosphate, (b) dissolved iron,~~ and~~ (c) *Trichodesmium* spp.~~,~~ ~~at the surface~~ and (d) ~~depth-integrated~~ nitrogen fixation at the surface. The parameters in the small boxes indicate results from the KT-09-17 cruise. The areas of the circles

are proportional to the concentration, abundance, or activity.



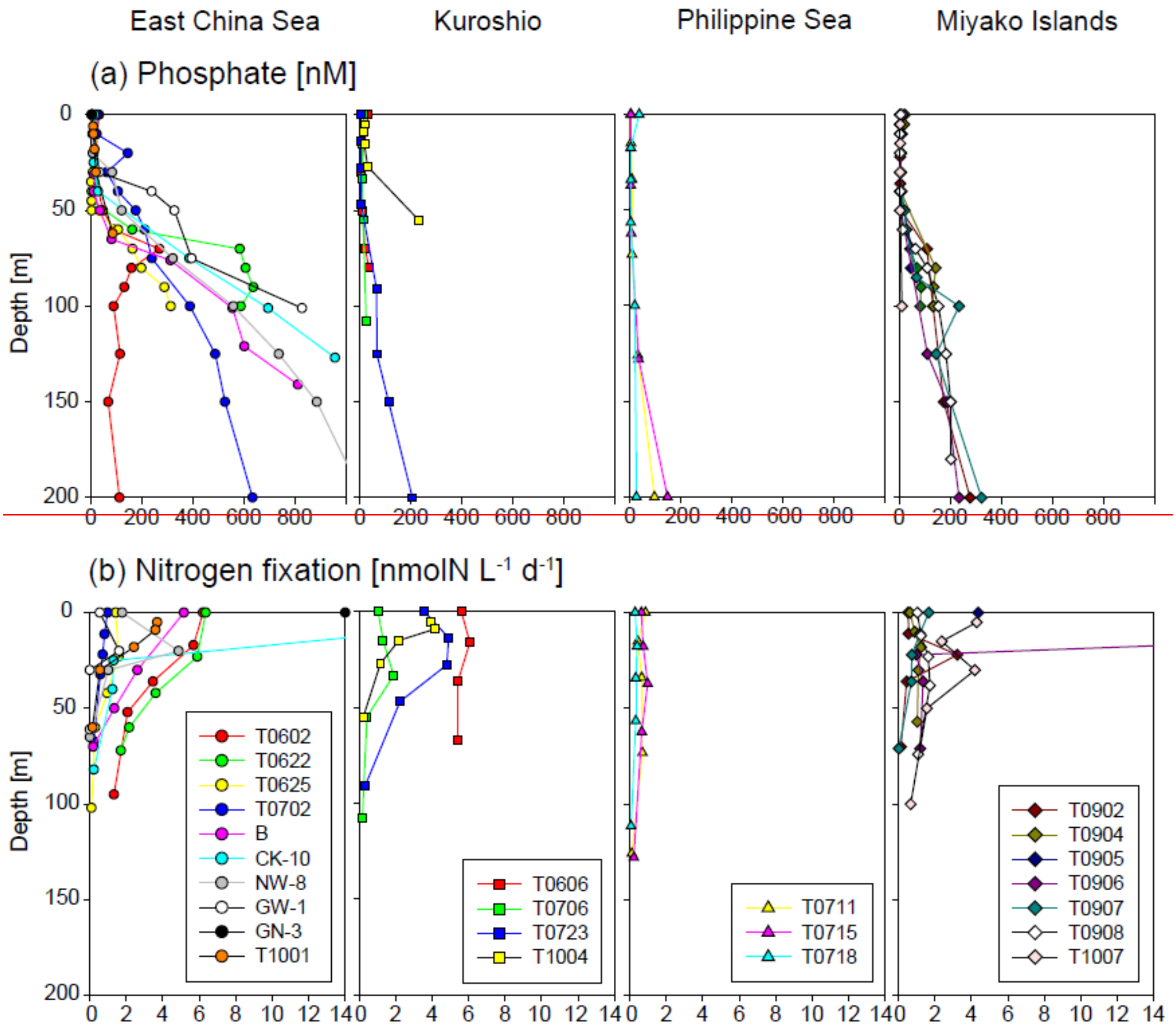
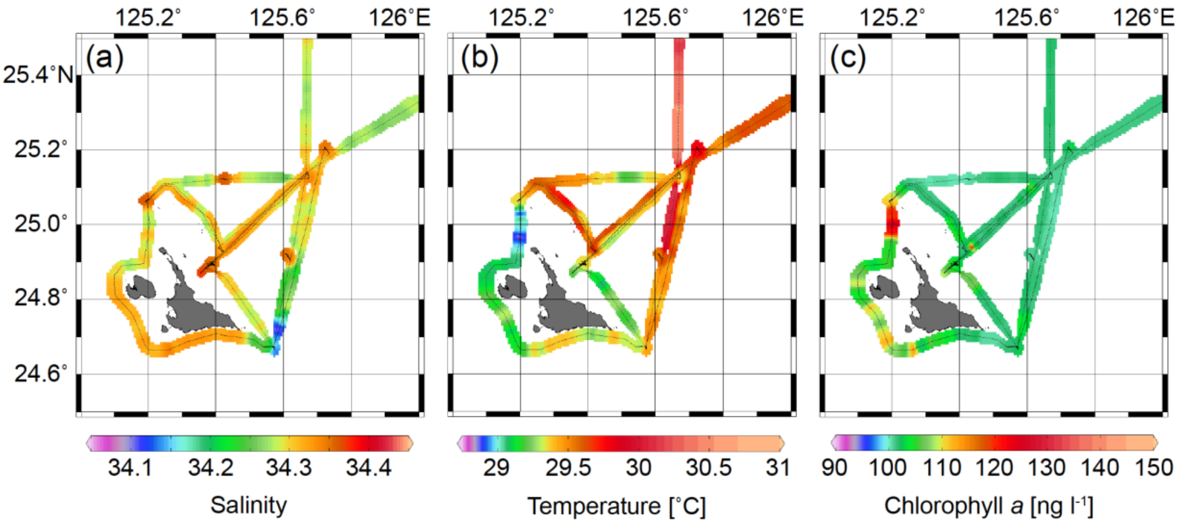


Figure 3. Vertical profiles of ~~(a)~~ phosphate and ~~(b)~~ nitrogen fixation in the East China Sea (a and e), the Kuroshio (b and f), the Philippine Sea (c and g), and the Miyako Islands (d and h)~~each area~~.



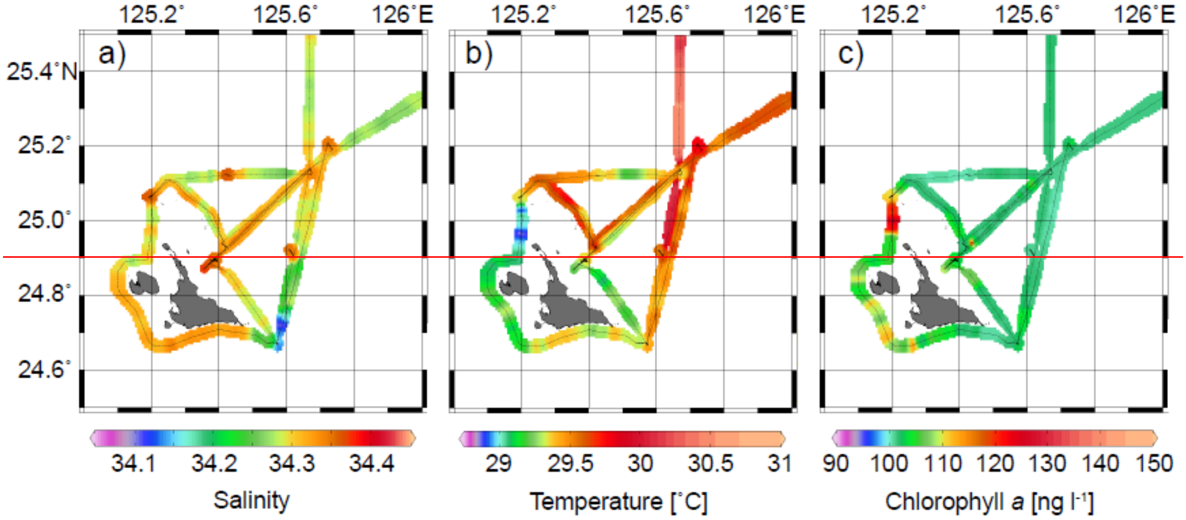


Figure 4. Surface (a) salinity, (b) temperature, and (c) chlorophyll *a* during the KT-09-17 cruise.

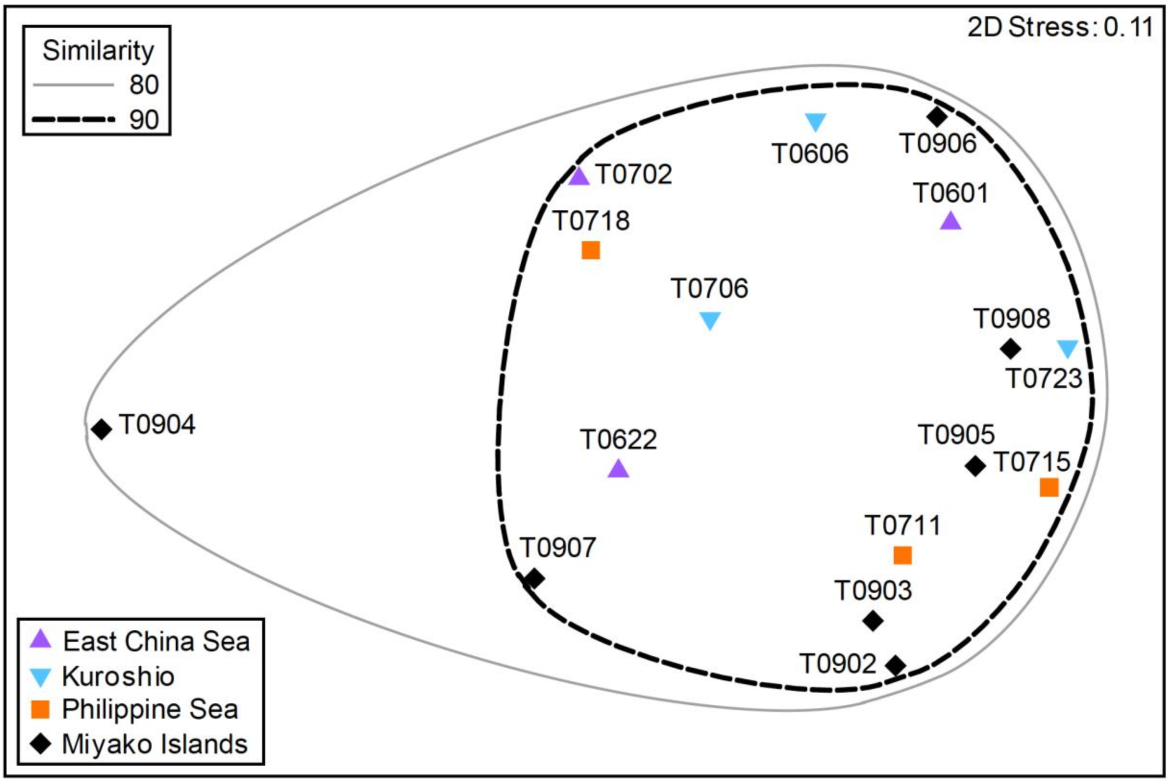


Figure 5. nMDS ordination of sampling stations with environmental variables

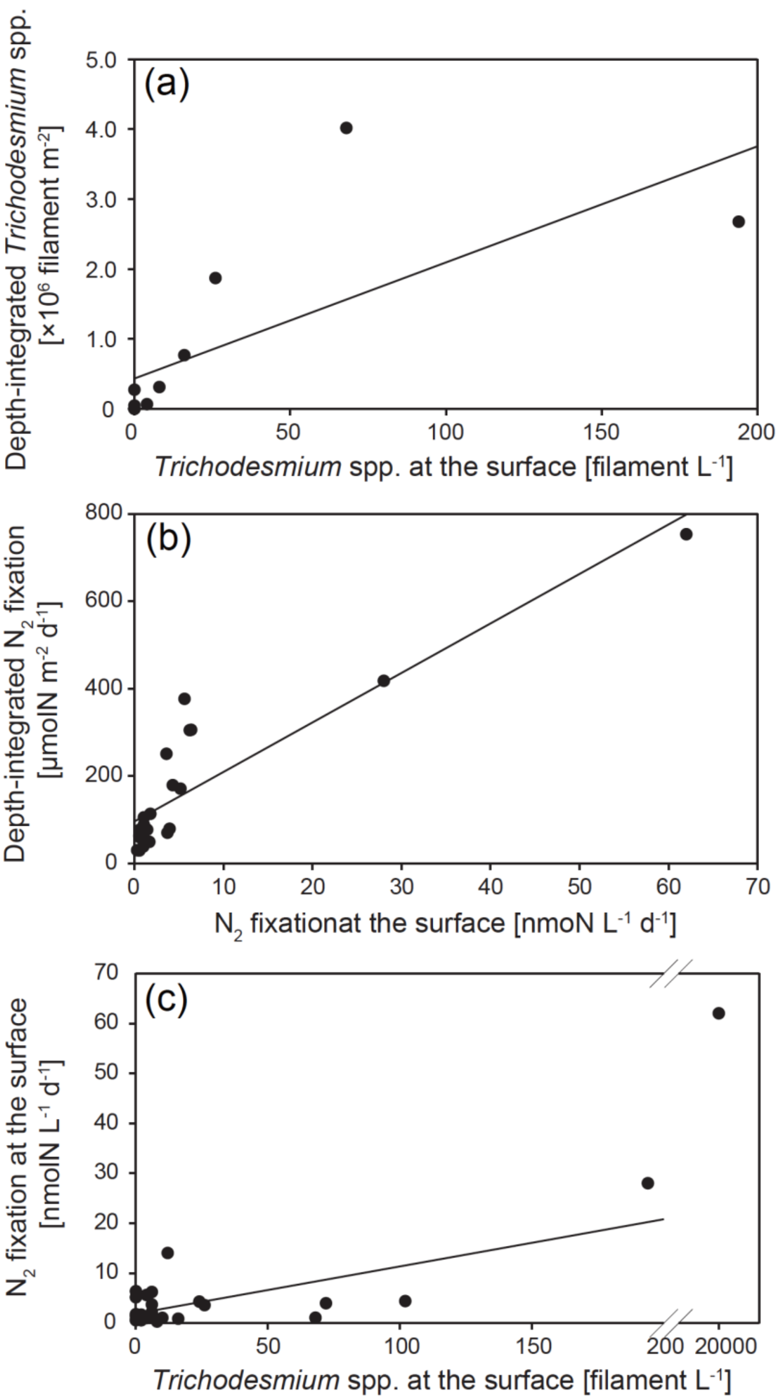
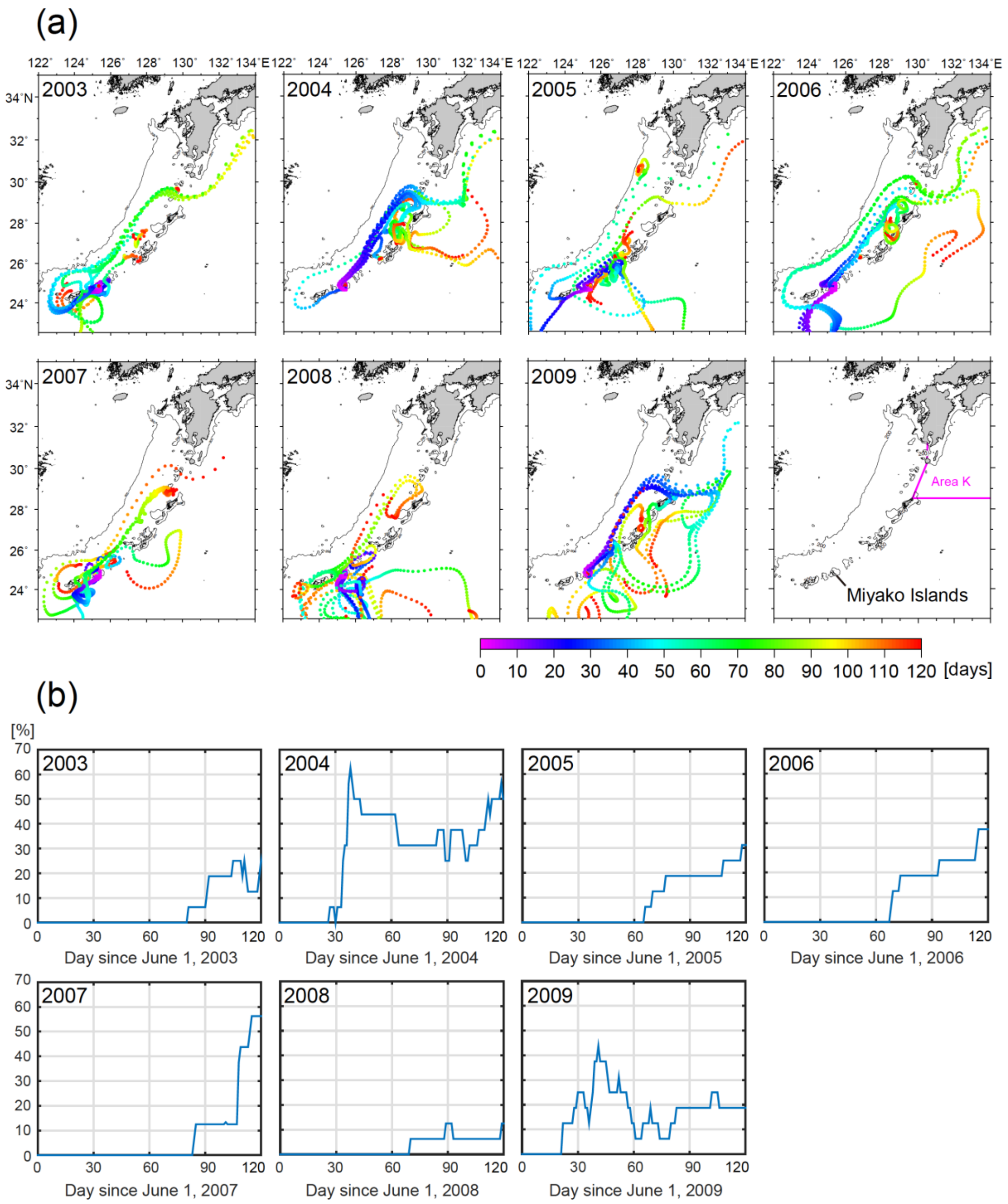


Figure 6. Relationships (a) between surface and depth-integrated *Trichodesmium* spp. abundance, (b) between surface and depth-integrated nitrogen fixation rates, and (c) between *Trichodesmium* spp. abundance and nitrogen fixation rate at the surface.


(a)
122° 124° 126° 128° 130° 132° 134°E
34°N
32°
30°
28°
26°
24°
2003
2004
2005
2006
2007
2008
2009
Area K
Miyako Islands
0 10 20 30 40 50 60 70 80 90 100 110 120 [days]
(b)
[%]
0 10 20 30 40 50 60 70
0 30 60 90 120
Day since June 1, 2003
Day since June 1, 2004
Day since June 1, 2005
Day since June 1, 2006
Day since June 1, 2007
Day since June 1, 2008
Day since June 1, 2009

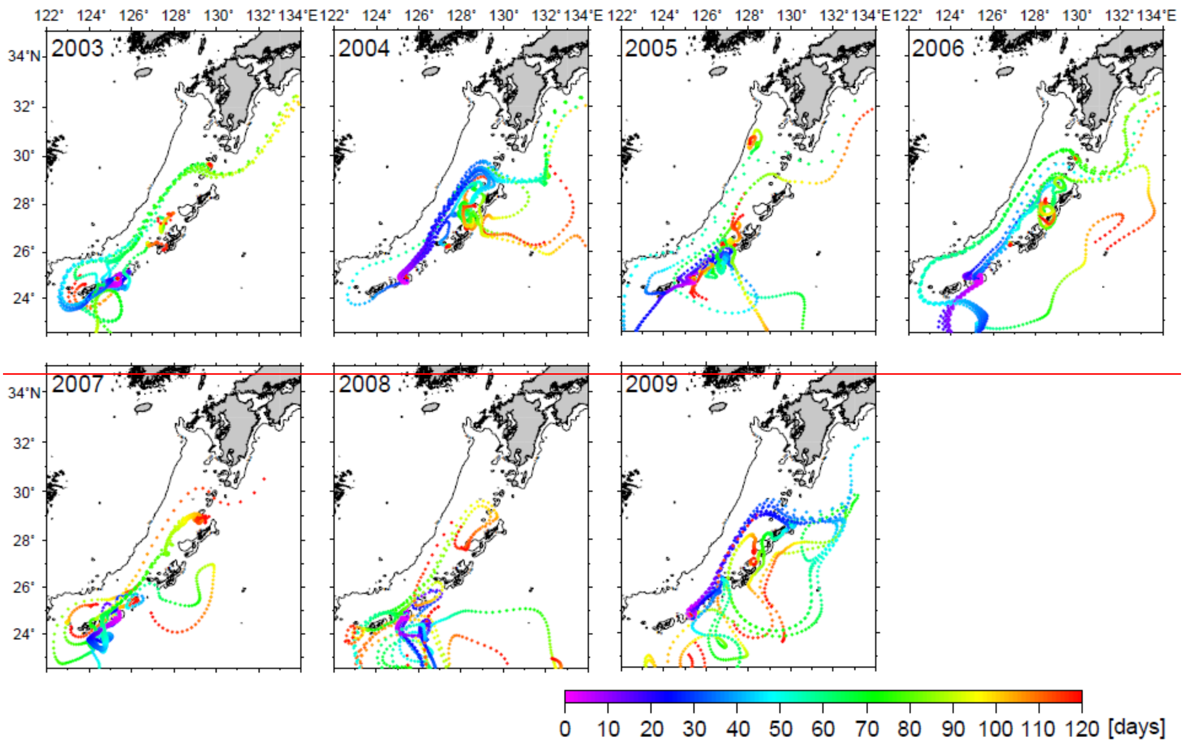


Figure 7~~5~~. (a) ~~Particle t~~Trajectories of particles released from ~~the release~~ points around the Miyako Islands~~. The particles were released~~ on June 1, ~~2009~~2003–2009.

(b) The ratio of particles delivered to Area K to the total released particles.