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Trichodesmium 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 a major diazotroph in the Kuroshio. 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 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. 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 islands situated along 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.

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 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 modeling study indicated that dust deposition in the western North Pacific decreased exponentially from the continental shelf to the Philippine Sea (Jickells et al., 2005; Mahowald et al., 2009), and hence, deposition was not as high in the Kuroshio as in the adjacent waters. 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 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.

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). 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 $> 0.15\,\mathrm{mg\,m^{-3}}$ 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, 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, 09–17 September 2006; KT-07-22, 05–13 September 2007; KT-09-17, 08–13 September 2009; KT-10-19, 04–12 September 2010) and the T/V *Nagasaki-maru* (242, 19–28 July 2007) (Fig. 1a, Table S1). The stations from the KT-06-21, KT-07-22, and *Nagasaki-maru* 242 cruises were divided into three areas: 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 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, 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 at a detection limit of 3 nM (Hashihama et al., 2009) when the concentration was less than 0.1 µM. 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.

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). 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 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: $S1 = 0.097 \pm 0.043$ nM and $D2 = 0.91 \pm 0.17$ 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. 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 primary production using a dual isotopic ($^{15}N_2$ and ^{13}C) technique, the details of which are given in Shiozaki et al. (2009). 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). 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). 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).

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. In addition, phytoplankton other than *Trichodesmium* spp. was identified from the samples obtained during the KT-09-17 cruise.

2.3 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 release points of particles were selected at model grid points around the coastal waters of the Miyako Islands. The particle distribution at the surface was fixed throughout the experiment. To focus on transport during the summer season, particles were released on 01 June and were tracked until 30 September.

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 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. The algal bloom frequency was consistently $> 10\,\%$ in the west of the main stream of the Kuroshio because the average chl *a* was $> 0.15\,\mathrm{mg\,m^{-3}}$ (Fig. 1a and 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). 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 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. 4a and Kodama et al., 2011).

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). 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. ($> 20\,000$ 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 $\mu\mathrm{mol\,N\,m^{-2}\,d^{-1}}$ (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 $\mu\mathrm{mol\,N\,m^{-2}\,d^{-1}}$ 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)), 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 and 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). 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), indicating that St. T0904 was not located in the middle of the upwelling. The surface phosphate concentration was also highest at St. T0904 (23 nM). 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. 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. The count of *Thalassiosira* spp. was higher at St. T0907 than that at the other stations.

3.4 Numerical simulation

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 (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 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

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), 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, 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 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 physical conditions. Therefore, the environmental variables related to nitrogen fixation only slightly differed (see more detail in Supporting Methods and Supporting Results and Discussion).

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 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) (Fig. S3). The results demonstrated that the particles released from the other islands of the Miyako Islands were also delivered to the Kuroshio, with some exceptions.

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, we hypothesize that the high abundance of *Trichodesmium* spp. and active nitrogen fixation in the Kuroshio are 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 flourishes in the upstream Kuroshio near Luzon Island (Chen et al., 2008). Therefore, we suggest that *Trichodesmium* spp. abundances are generally increased around islands situated along the Kuroshio, and are 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.

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Author contributions. T. Shiozaki, S. Takeda, and K. Furuya designed the experiment and T. Shiozaki, S. Takeda, T. Kodama, X. Liu, F. Hashihama, and K. Furuya collected the samples at sea. T. Shiozaki 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. Liu did during the KT-10-19 cruise. T. Shiozaki analyzed datasets of satellite and climatological current field. S. Takeda analyzed concentration of dissolved iron. S. Itoh performed numerical experiments. T. Kodama and F. Hashihama determined nutrient concentration. T. Shiozaki prepared the manuscript with contributions from all co-authors.

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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*[a] [filaments L^{-1}]	N_2 fixation [μmol N L^{-1} d^{-1}]	Temperature[a] [°C]	MLD [m]	$NO_3^- + NO_2^-$[a,b] [nM]	PO_4^-[a,b] [nM]	DFe[a] [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

[a] Values in surface water.

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

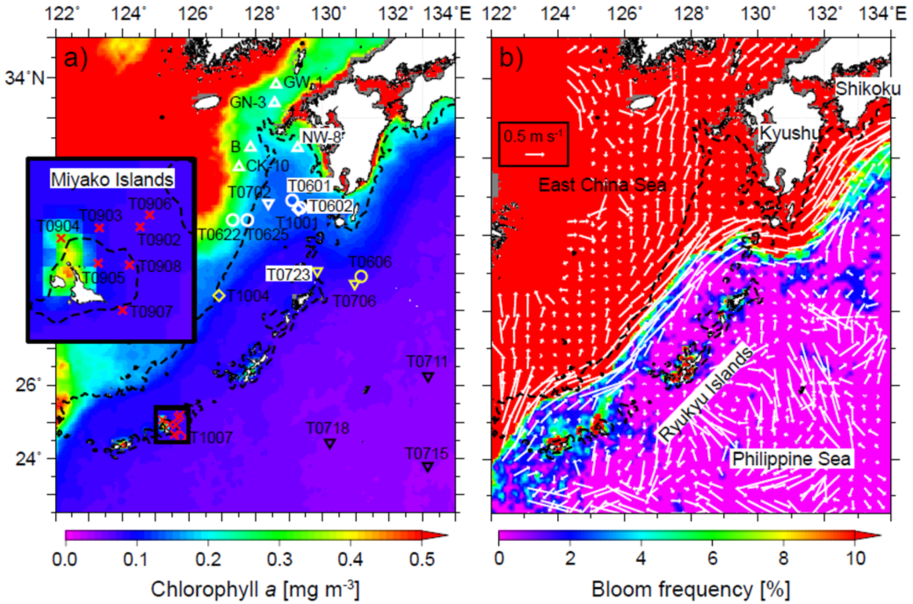

Figure 1. Sampling stations and distribution of chlorophyll *a* and bloom frequency. **(a)** Sampling stations during the KT-06-21 (circles), KT-07-22 (inverted triangles), KT-09-17 (crosses), KT-10-19 (diamonds), and 242 (triangles) cruises. Symbols of stations located in the East China Sea, the Kuroshio, the Philippine Sea, and near the Miyako Islands are indicated in white, yellow, black, and red, 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 $> 0.15\,\mathrm{mg\,m^{-3}}$ during summer between 2003 and 2009. Dashed lines indicate 200 m isobaths.

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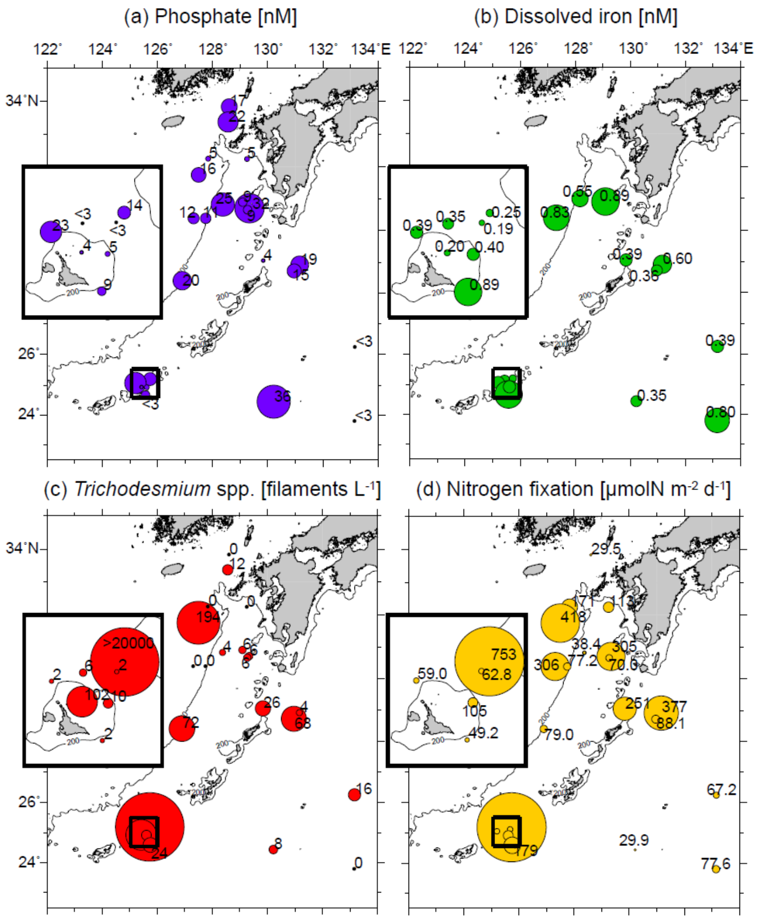

Figure 2. Distribution of **(a)** phosphate, **(b)** dissolved iron, and **(c)** *Trichodesmium* spp. at the surface and **(d)** depth-integrated nitrogen fixation. The parameters in the small boxes indicate results from the KT-09-17 cruise.

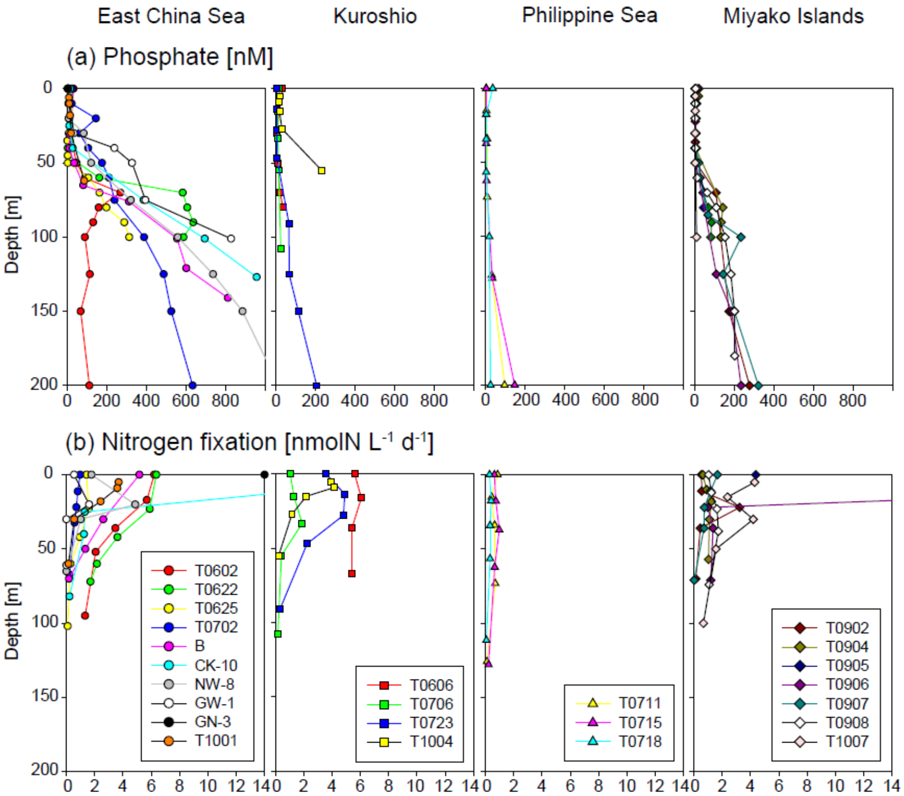

Figure 3. Vertical profiles of **(a)** phosphate and **(b)** nitrogen fixation in each area.

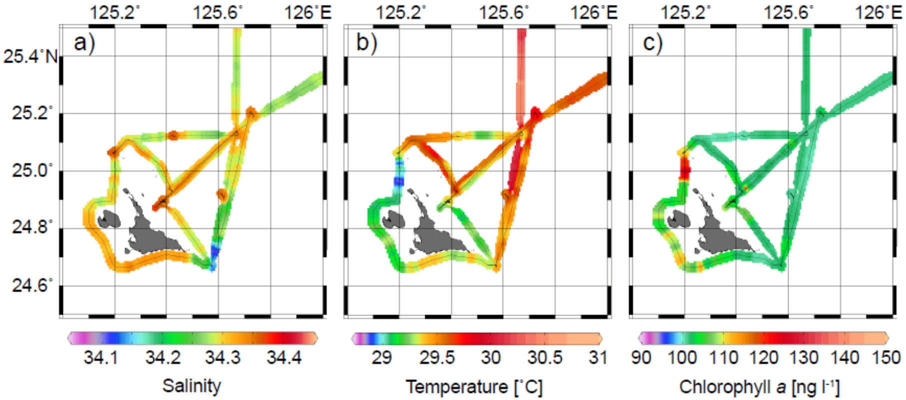

Figure 4. Surface **(a)** salinity, **(b)** temperature, and **(c)** chlorophyll *a* during the KT-09-17 cruise.

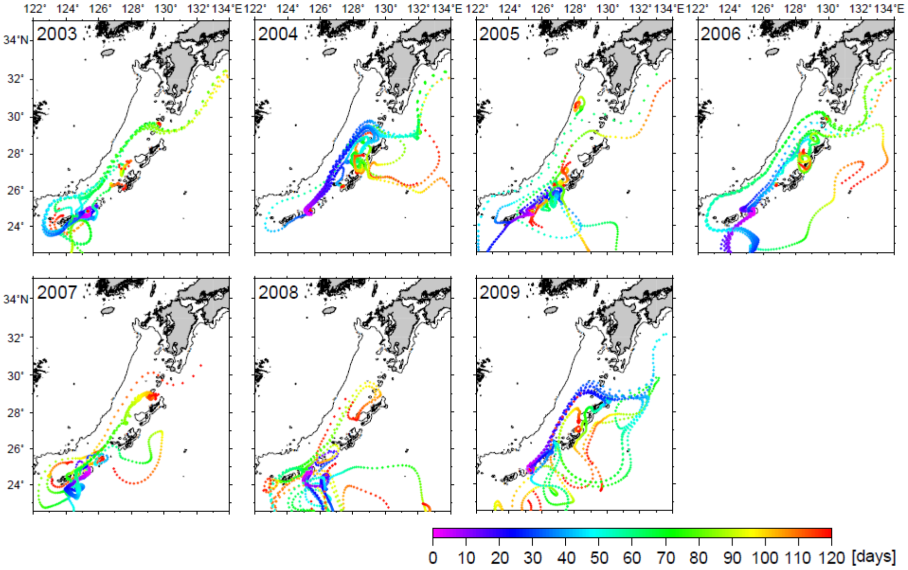

Figure 5. Particle trajectories from the release points around the Miyako Islands. The particles were released on 01 June 2009.