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RESEARCH ARTICLE
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Physical Forcing Controls the Basin-Scale Occurrence of Nitrogen-Fixing Organisms in the North Pacific Ocean

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Key Points:
- Diazotrophs showed seasonal succession and different longitudinal patterns, which were explained with their different temperature preference
- Abundant diazotrophs were detected in the North Pacific transition zone in both western and eastern North Pacific
- Diazotrophs in western and eastern North Pacific were correlated with proxies of mesoscale eddies and coastal upwelling, respectively

Supporting Information:
- Supporting Information Data S1

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Abstract Biological nitrogen fixation is increasingly recognized as an important source of new nitrogen in a warming ocean. However, the basin-scale spatiotemporal distribution of nitrogen-fixing organisms (diazotrophs) in the ocean and its controlling environmental factors remain unclear. Here we examined the basin-scale seasonal distribution patterns of major diazotrophs (filamentous cyanobacterial *Trichodesmium*, unicellular cyanobacterial UCYN-A1, and proteobacterial Gamma-A) in surface waters of the North Pacific from 2014 to 2016 with unprecedented coverage and resolution. In general, UCYN-A1, *Trichodesmium*, and Gamma-A were abundant during spring-autumn, summer-autumn, and spring respectively. Regarding latitudinal patterns of abundance, UCYN-A1 showed dome shape; *Trichodesmium* was gradually decreasing from low- to high-latitude regions; and Gamma-A did not show a clear pattern, which were coincident with the distinct correlations between the diazotrophs and temperature. All three diazotrophs were abundant (reached 10^6–10^7 *nifH* gene copy number L^{-1}) in the North Pacific transition zone and subtropical gyre, where the cyanobacterial diazotrophs were more abundant in both the western and eastern North Pacific than in the central North Pacific. The diazotroph abundance in the western North Pacific was positively correlated with eddy kinetic energy and sea surface height anomaly, which implies an enhancement of diazotrophs in mesoscale eddies associated with the western boundary current Kuroshio and its extension. The cyanobacterial diazotrophs were positively correlated with wind stress curl, a measurable parameter of wind-driven upwelling, in the eastern North Pacific. Our study refines the biogeography of three major diazotrophs and highlights the importance of physical forcing in mediating their dynamics.

1. Introduction

Biological nitrogen fixation (BNF) reduces dinitrogen to bioavailable nitrogen, which fuels primary production and biological pump in the ocean (Capone et al., 2005; Capone & Carpenter, 1982; Karl et al., 1997) and also plays a major role in the global oceanic nitrogen budget (Galloway et al., 2004). A large portion of marine BNF occurs in the Pacific Ocean. A recent model-based study estimated that the Pacific Ocean contributed to more than half of biologically fixed nitrogen input in the global ocean (Wang et al., 2019), which is in agreement with the estimation that based on data synthesis of actual BNF rates (Luo et al., 2012). In the North Pacific subtropical gyre (NPSG), it has been suggested that the supply of bioavailable nitrogen from subsurface waters to the euphotic zone has been decreasing in recent decades, which could enhance BNF activities (Corno et al., 2007; Karl et al., 1997, 2001). Furthermore, nitrogen isotopic records in coral skeletons collected from Hawaiian archipelago indicated that BNF has increased in the NPSG since the end of Little Ice Age (Sherwood et al., 2014).

The BNF is carried out solely by prokaryotic organisms known as diazotrophs. Traditionally, marine nitrogen fixation was mainly attributed to a filamentous cyanobacterium, *Trichodesmium*, which could easily be observed under a light microscope (Capone et al., 1997; Dugdale et al., 1964). However, more recent studies have discovered that unicellular cyanobacterial diazotrophs contribute as much or even more than *Trichodesmium* to the BNF in some oceanographic regions (Langlois et al., 2005; Martinez-Perez et al., 2016; Moisander et al., 2010; Zehr et al., 1998, 2001). UCYN-A1 is the most abundant and widely distributed unicellular cyanobacterial diazotroph (Farnelid et al., 2016; Martinez-Perez et al., 2016), lacks the key enzymes that are involved in the oxygen-producing photosystem II complex of the photosynthetic apparatus (Tripp et al., 2010), and has a symbiotic relationship with eukaryotic haptophytes (Thompson et al., 2012). Besides, the significance of heterotrophic bacteria in BNF in the oceans has also received interest in recent years (Bombar et al., 2016; Moisander et al., 2017). However, to date, only the Gammaproteobacteria-affiliated phylotype Gamma-A has been accepted as a significant non-cyanobacteria diazotroph in the oceans (Langlois et al., 2015; Moisander et al., 2014; Shiozaki et al., 2014). Using quantitative polymerase chain reaction technique, the abundance of *Trichodesmium*, UCYN-A1, and Gamma-A can be estimated in DNA samples using specific TaqMan assays (Church et al., 2005; Moisander et al., 2008).

Environmental factors like temperature and the availability of iron and phosphate have been proposed to influence the distribution of diazotrophs in the oceans based on the published regional field observations, culture-based experiments, and model-based studies (Landolfi et al., 2015; Sohm et al., 2011; Ward et al., 2013; Weber & Deutsch, 2014). However, it should be noted that the dynamics of iron and phosphate in marine ecosystems could be strongly influenced by physical forcing, such as mesoscale eddies, upwelling, advection, and diffusion (Cullen et al., 2002). Although there is a knowledge gap regarding the effects and importance of physical forcing on the dynamics of diazotrophs, a number of regional studies have reported inconsistent responses of diazotrophs in eddy-influenced waters (Chen et al., 2007; Fong et al., 2008; Holl et al., 2007; Zhang et al., 2011). For example, both unicellular cyanobacterial diazotrophs and *Trichodesmium* were abundant in an anticyclonic eddy near the station ALOHA (Fong et al., 2008). Proteobacteria were suggested as the major diazotrophs in two cyclonic eddies in the South China Sea basin (Zhang et al., 2011). Less *Trichodesmium* were detected in a cyclonic eddy isolated from the Kuroshio (Chen et al., 2007). Unicellular diazotrophs were reported as the major contributors of BNF in the Leeuwin Current coastal eddies (Holl et al., 2007). Besides, active BNF was reported in the denitrified waters of upwelling systems (Fernandez et al., 2011).

A comprehensive understanding of the distribution and controlling factors of the various diazotrophs is essential, because the fixed nitrogen that arises from each group could have a different fate in marine food webs and a different influence on the biological pump (Sohm et al., 2011). However, such understanding is still lacking due to the limited sampling coverage (and resolution) and the ocean's vastness (Tang & Cassar, 2019). Indeed, even in the NPSG, where the importance of BNF has been known for decades (Karl et al., 1997), no trans-Pacific observation of the diazotroph groups has been conducted. Therefore, in the present study, we quantified three major diazotrophs, UCYN-A1, *Trichodesmium*, and Gamma-A in the surface waters of the North Pacific at 519 sampling stations along 37 trans-Pacific transects between Japan and North America during 2014–2016 to obtain a comprehensive picture of the dynamics of diazotrophs in this ocean. We also examined the key factors that are associated with the occurrence of these groups by examining not only basic hydrographic factors and nutrients but also the physical forcing (mesoscale eddies and wind-driven coastal upwelling).

2. Materials and Methods

2.1. Sample Collection and Environmental Analysis

In this study, observations were made across the entire North Pacific Ocean, from the tropics to the subarctic (Figure 1 and supporting information Table S1). Seawater samples were collected from a seawater intake installed at the bottom (approximately 5 m depth) of the M/V *New Century 2* (Fujitrans Corporation, Japan) around 14:00 (local time) from September 2014 to October 2016 (519 sampled stations along 37 trans-Pacific transects) as part of the ship-of-opportunity observation in the North Pacific between Japan and North America operated by the Japanese National Institute for Environmental Studies (NIES) (Figure 1 and Table S1). The detailed information on the observations is available at http://nc2.soop.jp/.

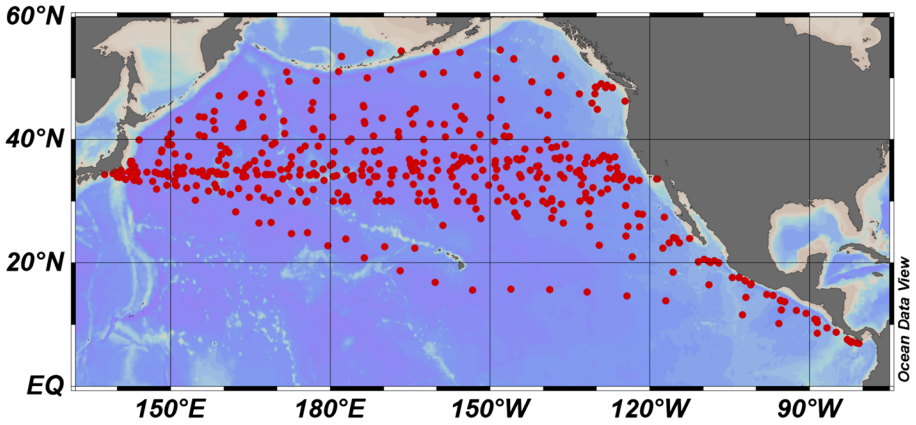


Figure 1. Locations of the samples in which UCYN-A1, *Trichodesmium*, and Gamma-A were quantified with TaqMan assays.

Each seawater sample (1.2 L) was filtered through a Millipore Express® PLUS polyethersulfone (PES) membrane filter (diameter, 25 mm; pore size 0.45 μm) with a gentle vacuum (0.013 MPa), following which the filtered samples were stored in freezer (<−70°C) or in liquid nitrogen until their analysis on land. During the cruises, the seawater temperature and salinity at the water intake were continuously monitored with an SBE 45 MicroTSG Thermosalinograph (Sea-Bird Scientific). In addition, the macronutrient contents of the seawater (nitrate + nitrite, ammonium, phosphate, and silicate) were measured by collecting additional samples into plastic tubes, stored frozen, and analyzed on land using an auto-analyzer (Yasunaka et al., 2018), while chlorophyll-*a* was measured by fluorometry (Welschmeyer, 1994). The detection limits of nitrate + nitrite, ammonium, phosphate, and silicate were 0.03, 0.04, 0.03, and 0.04 μM, respectively. The NP ratio was calculated by dividing the total concentration of dissolved inorganic nitrogen (DIN; nitrate + nitrite and ammonium) by the concentration of phosphate. The undetected data points of ammonium and nitrate + nitrite were treated as zero when calculating the concentration of DIN. The NP ratio was treated as not available (NA) when either the DIN concentration was zero or phosphate was not detected.

2.2. DNA Extraction

DNA was extracted from the filtered seawater samples following Endo et al. (2013) and was then purified using a NucleoSpin® gDNA Clean-up kit (Macherey-Nagel) following the manufacturer's instructions. The concentrations of DNA in the purified extracts were quantified using a Thermo NanoDrop™ spectrophotometer (ND-1000).

2.3. Quantitative PCR of UCYN-A1, *Trichodesmium*, and Gamma-A

The *nifH* genes of UCYN-A1, *Trichodesmium*, and Gamma-A in the extracted DNA samples were quantified with the TaqMan qPCR assay using primers and probes that had been designed in previous studies (Church et al., 2005; Moisander et al., 2008). Standard curves were generated by 10-fold serial dilutions of the linearized plasmid pUC18, into which synthesized reference sequences of UCYN-A1 (accession number: AF059642), *Trichodesmium* (AY528677), and Gamma-A (EU052413) were inserted. These reference sequences were deposited in the GenBank of the National Center of Bioinformatic Information (NCBI). The qPCR reaction volume was 10 μl, which consisted of 1× Premix Ex Taq (Perfect Real Time; Takara, Shiga, Japan), 1.25 μM primers, 0.75 μM TaqMan probes, 0.5–1 μl of template DNA, and Ultrapure distilled water (Invitrogen, Thermo Fisher Scientific Corp.). The amplification reactions were performed in triplicate with the following conditions: 95°C for 2 min followed by 50 cycles at 95°C for 10 s and 60°C for 25 s. The qPCR efficiencies of the three targeted groups ranged from 80% to 118%. The detection limits of UCYN-A1 and Gamma-A were 1 *nifH* gene copy per reaction, while that of *Trichodesmium* was 10 *nifH* gene copy per reaction. The datasets for the abundance of the diazotrophs were divided into spring (April–June), summer (July–September), autumn (October–December), and winter (January–March), and the distributional

patterns of the diazotrophs were visualized with the Generic Mapping Tools version 4.5 (Wessel & Smith, 2016).

2.4. Remote Sensing Analysis

Mesoscale eddies (as inferred by the eddy kinetic energy [EKE] and sea surface height anomaly [SSHA]), wind-driven coastal upwelling (as inferred by wind stress curl), and sea surface temperature anomaly (SSTA) were estimated at the basin-scale using remote sensing analysis. The daily SSHA and the zonal (u) and meridional (v) components of the geostrophic currents were obtained from the Copernicus Marine Environment Monitoring Service (http://marine.copernicus.eu/) at a resolution of 0.25°, and the EKE was then calculated as $½ \times (u^2 + v^2)$. Daily SSTA data were obtained from the National Oceanic and Atmospheric Administration's Optimum Interpolation Sea Surface Temperature (https://www.ncdc.noaa.gov/oisst) at a resolution of 0.25°. The wind stress curl (τ, N m^{-3}) was estimated using the following equation:

$$\tau = \partial\tau_y/\partial x - \partial\tau_x/\partial y,$$

where τ_x and τ_y are the zonal and meridional wind stress values, respectively, which were calculated as $\tau_{(x,y)} = \rho C_D W^2(u, v)$, where ρ is the surface air density (1.22 kg m^{-3}), C_D is the drag coefficient, and W is the 10-m daily wind speed for the zonal and meridional components at 0.25° resolution obtained from the CCMP Wind Vector Analysis product (http://www.remss.com/measurements/ccmp). Satellite-derived variables were then matched with the ship's coordinates and the time of in situ sampling to extract the collocated data (Table S1).

Sea surface salinity (SSS) was used to estimate the seasonal boundaries of major biomes in the North Pacific Ocean during the sampling period (2014–2016). Seasonal average SSS was obtained from the Copernicus Marine Environment Monitoring Service (http://marine.copernicus.eu/) at a resolution of 0.25°. Shifts of SSS in 33.0–33.8 and 34.8–35.2 in the subtropical North Pacific Ocean (SNPO) have been defined as the subarctic and subtropical frontal zones, respectively (Roden, 1991). The salinity contour lines of 33.8 and 34.8 in the SNPO indicate the southern boundary of subarctic frontal zone and northern boundary of subtropical frontal zone (Roden, 1991), and they were used to delineate the subarctic gyre, North Pacific transition zone (NPTZ) and North Pacific subtropical gyre (NPSG).

2.5. Environmental Correlation Analysis

Correlations between the abundance of the major groups of diazotrophs and each of the environmental factors (temperature, salinity, concentration of macronutrients, NP ratio, SSTA, SSHA, EKE, and wind stress curl) were tested using the Spearman rank correlation analysis with the "corrplot" package in RStudio (Wei & Simko, 2013), and the results were then visualized with the "ggplot2" package in RStudio (Wickham, 2016). The correlation analysis was conducted with the entire basin-scale dataset of diazotroph abundance and environmental data, as well as with the partial datasets from the eastern subtropical North Pacific (ESNP; 23.5°–40°N, 80°–170°W) and the western subtropical North Pacific (WSNP; 23.5°–40°N, 130°E–170°W). For the correlation between diazotrophs, temperature, NP ratio, wind stress curl, and EKE, the trends in the relationships between the environmental factors and diazotroph abundance ($\log_{10}(X + 1)$ transformed) were examined using the generalized additive models (GAMs) "mgcv" package (Wood & Wood, 2015) and then visualized with the "ggplot2" package in RStudio (Wickham, 2016).

3. Results

3.1. Spatial Variations in Environmental Conditions

Temperature increased, and the concentrations of macronutrients (dissolved inorganic nitrogen, phosphate and silicate) and chlorophyll-*a* decreased with decreasing latitude (southward) (Figures S1 and S2). Across the North Pacific, NP ratio was higher in the eastern North Pacific than in the western North Pacific (Figure S3b). SSTA was more positive in the eastern North Pacific than in the western North Pacific (Figure S3a). For the two proxies of mesoscale eddies, the western North Pacific had a highly variable SSHA and strong EKE, whereas the eastern North Pacific had less variation in SSHA and a lower magnitude of EKE (Figures S3c and S3d).

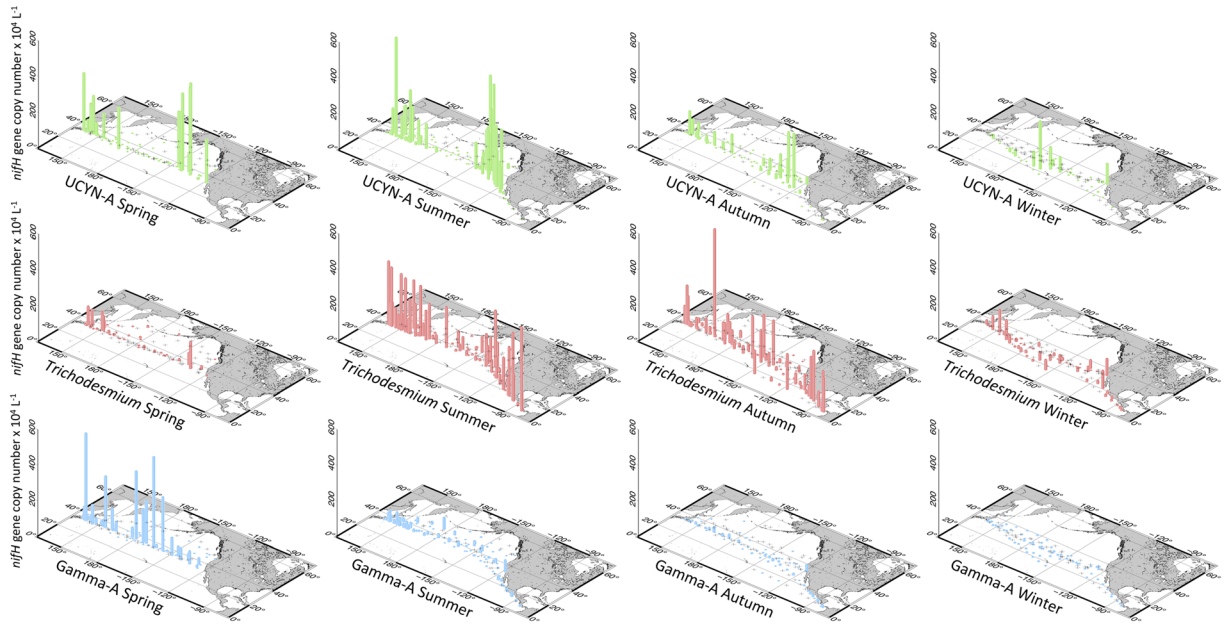


Figure 2. Basin-scale and seasonal dynamics of the distribution and abundance of UCYN-A1 (green), *Trichodesmium* (red), and Gamma-A (blue) in the North Pacific Ocean. The data points below detection limits were shown with black crosses.

Shifts of SSS at ~33.0–33.8 and 34.8–35.2 (Roden, 1991) were observed in the subtropical region (Figure S4). As inferred by the salinity contour lines of 33.8 and 34.8, the NPTZ ranged from ~30 to 40°N and separated the subarctic gyre from the NPSG (Figure S4).

3.2. Seasonal and Spatial Variations in UCYN-A1, *Trichodesmium*, and Gamma-A Abundance

The abundance of diazotrophs were binned by season and displayed in Figure 2. In spring and summer, observations were mainly made in the subarctic and subtropical regions, while in autumn and winter, they mainly covered the subtropical and tropical areas, resulting in the subtropical region being sampled in all four seasons (Figure 2). There were clear seasonal variations in the abundance of the diazotrophs, with UCYN-A1 being more abundant from spring to autumn (reached 10^7 *nifH* gene copies L^{-1}), *Trichodesmium* being more abundant in summer and autumn (reached 10^7 *nifH* gene copies L^{-1}), and Gamma-A being most abundant in spring (reached 10^6 *nifH* gene copies L^{-1}). The abundance of both UCYN-A1 and *Trichodesmium* peaked in summer (Figure 2). Thus, these diazotroph groups displayed seasonal succession in the North Pacific and were least abundant in winter.

Across the latitudes (6–55°N), UCYN-A1 was more abundant in the NPSG and NPTZ (21–40°N) than in both the tropical (6–20°N) and subarctic regions (41–55°N). The abundance of *Trichodesmium* decreased gradually from the tropical and subtropical regions to the subarctic regions, while Gamma-A did not show a clear latitudinal pattern compared to UCYN-A1 and *Trichodesmium*. Nevertheless, the abundance of all three diazotrophs were high (reached 10^6–10^7 *nifH* gene copy number L^{-1}) in the NPTZ and decreased from the NPTZ to the subarctic gyre (Figures 3 and S5). For the fraction of detectable data points, all three diazotrophs displayed latitudinal patterns similar to their abundance (Figure 3). Across the Pacific Ocean, UCYN-A1 and *Trichodesmium* were generally more abundant in the eastern and western North Pacific than in the central North Pacific, while the abundance of Gamma-A was relatively even throughout the studied region (Figure 2).

3.3. Relationship Between Diazotroph Abundance and Environmental Factors

Based on the Spearman Rho correlation coefficient, temperature and NP ratio were the major factors correlating with the general variances of diazotroph abundance (Figure 4a). Temperature was positively correlated with the abundance of diazotrophs, while macronutrients and NP ratio showed negative correlations with the abundance of diazotrophs. The abundance of Gamma-A was not significantly correlated with NP

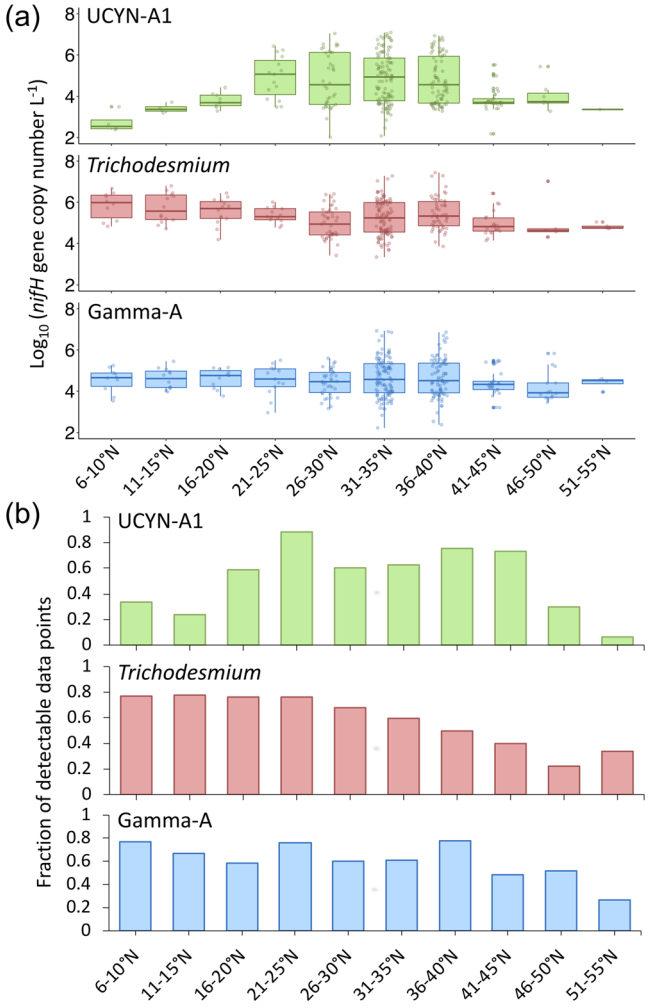


Figure 3. Boxplots showing latitudinal patterns of the abundance (a) and fraction of detectable data points (b) of UCYN-A1 (green), *Trichodesmium* (red) and Gamma-A (blue).

ratio, although it showed a similar relationship with NP ratio as the other two diazotrophs in the WSNP. As unraveled by GAMs, the abundance of UCYN-A1 peaked at 24°C, the abundance of *Trichodesmium* plateaued at 27–30°C, and the abundance of Gamma-A was linearly and weakly correlated with temperature compared to the other two diazotrophs (Figure 4b). Regarding the NP ratio, the abundance of UCYN-A1 clearly decreased with an increasing NP ratio when it was <15 and was highly variable at higher NP ratio, while the abundance of *Trichodesmium* showed a linear and negative correlation with the NP ratio (Figure 4b). In addition, the N:P ratio was weakly correlated with the abundance of Gamma-A compared to *Trichodesmium* and UCYN-A1, although it still showed a negative correlation.

Regarding the proxies of mesoscale eddies, all three diazotroph groups were positively correlated with EKE in the WSNP (Figure 4a), while both UCYN-A1 and *Trichodesmium* were positively correlated with SSHA. Within the ESNP, both *Trichodesmium* and UCYN-A1 showed significant and positive correlations with wind stress curl, with the former showing the strongest relationship (Figures 5a and 5b). However, Gamma-A did not correlate with the wind stress curl (Figure 5c). In addition, UCYN-A1 was positively correlated with SSTA throughout the North Pacific Ocean (Figure 4a).

4. Discussion

4.1. Seasonal Successions of the Diazotroph Groups at the Basin-Scale

The seasonal dynamics of diazotrophs and BNF rates was rarely explored at the basin-scale. Our results showed that all three diazotrophs were detectable during all four seasons in the SNPO (Figures 2 and S6), with the abundance of cyanobacterial diazotrophs peaking in summer and being lowest in winter. This basin-scale pattern is also supported by the previous regional observation at the station ALOHA that the BNF rate were generally higher in summer and lower in winter (Böttjer et al., 2017; Dore et al., 2002). More specifically, the *Trichodesmium* peaked at a higher temperature (27–30°C) than UCYN-A1 (23–24°C) (Figure 4b), matching the previous findings that cultured *Trichodesmium* strains showed high average optimal temperature at 26°C (Fu et al., 2014) and UCYN-A1 abundance was the highest at 24°C in the South Pacific Ocean (Moisander et al., 2010), which may explain why the high abundance of *Trichodesmium* was detected in summer and autumn while that of UCYN-A1 was detected from spring to autumn (Figure 2).

The seasonal pattern of Gamma-A could not be explained by temperature (Figures 2 and 4), while the high abundance of Gamma-A in spring maybe related to the spring blooms in the subtropical and subarctic North Pacific (particularly on the western side). The spring blooms may result in the depletion of nutrients (e.g., nitrate) (Saito et al., 2002), and the increased supply of labile dissolved organic carbon (DOC) (Kirchman et al., 1991). Gamma-A was speculated as a photoheterotrophic diazotroph based on its distributional pattern (Langlois et al., 2015; Moisander et al., 2014), although its genome is still not available. Our result suggests that Gamma-A could benefit from the low-nitrate and high-DOC conditions following the North Pacific spring bloom.

4.2. Latitudinal Patterns of the Diazotroph Groups at the Basin-Scale

The abundance of three diazotroph groups showed distinct latitudinal patterns (Figure 3), which were similar to the trends of their relationship with temperature (Figure 4b). *Trichodesmium* was more abundant and frequently detected in the tropical regions where SST ranged approximately 27–30°C; UCYN-A1 was most

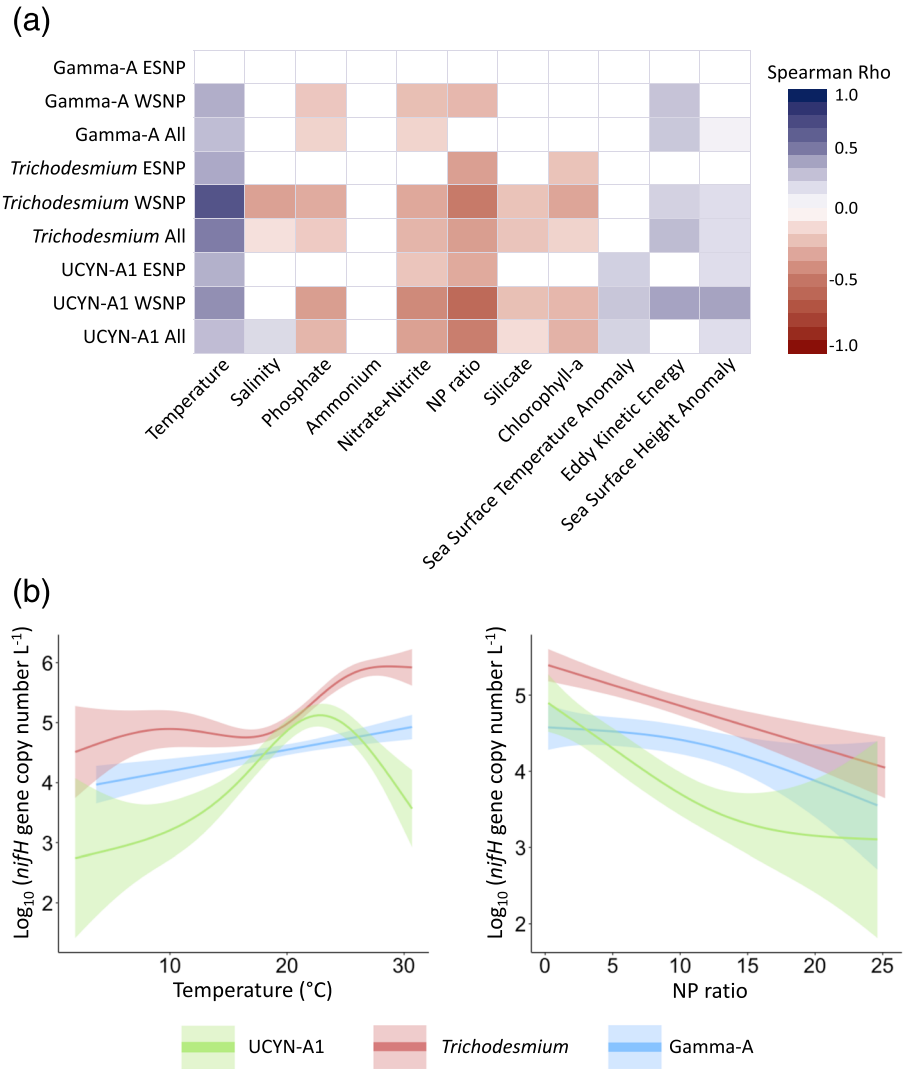


Figure 4. Spearman rank correlations between the diazotroph groups and environmental factors (a). Only the significant correlations ($p \leq 0.05$) are shown. Results of generalized additive models showing relationship between diazotroph abundance and temperature and the NP ratio (b).

abundant and frequently detected in the NPSG and NPTZ (20–40°N); and Gamma-A did not show a strong latitudinal pattern and was weakly correlated with temperature (Figures 3, 4b, and S1). Nevertheless, we found that *Trichodesmium* could reach 10^4–10^5 *nifH* gene copies L^{-1} in the subarctic region where temperature was below 10°C (Figures 3, 4b, and S1), which is considerably lower than the previously proposed temperatures that limit the growth of *Trichodesmium* (18–20°C) (Bergman et al., 2013; Fu et al., 2014). Coincidentally, *Trichodesmium* has also been detected in cold waters in the Arctic, North Atlantic, and Southern Oceans (Díez et al., 2012; Raes et al., 2020; Rees et al., 2016; Rivero-Calle et al., 2016), and it was suggested that *Trichodesmium* was transported to the cold waters by physical advection (Rees et al., 2016; Rivero-Calle et al., 2016). A case study in U.K. coastal waters reported that *Trichodesmium* was metabolically active but not fixing nitrogen at low temperature (Rees et al., 2016). Considering that *Trichodesmium* was increasingly reported in high latitude and cold waters, its actual contribution to BNF in these areas should be further verified.

While the domain of marine BNF has been broadened with ongoing studies and active BNF in the Arctic region (Harding et al., 2018; Shiozaki et al., 2018; Sipler et al., 2017), diazotrophs and BNF were found to be rare or absent in the NPTZ where constitutes approximately 12% of the surface area of the North

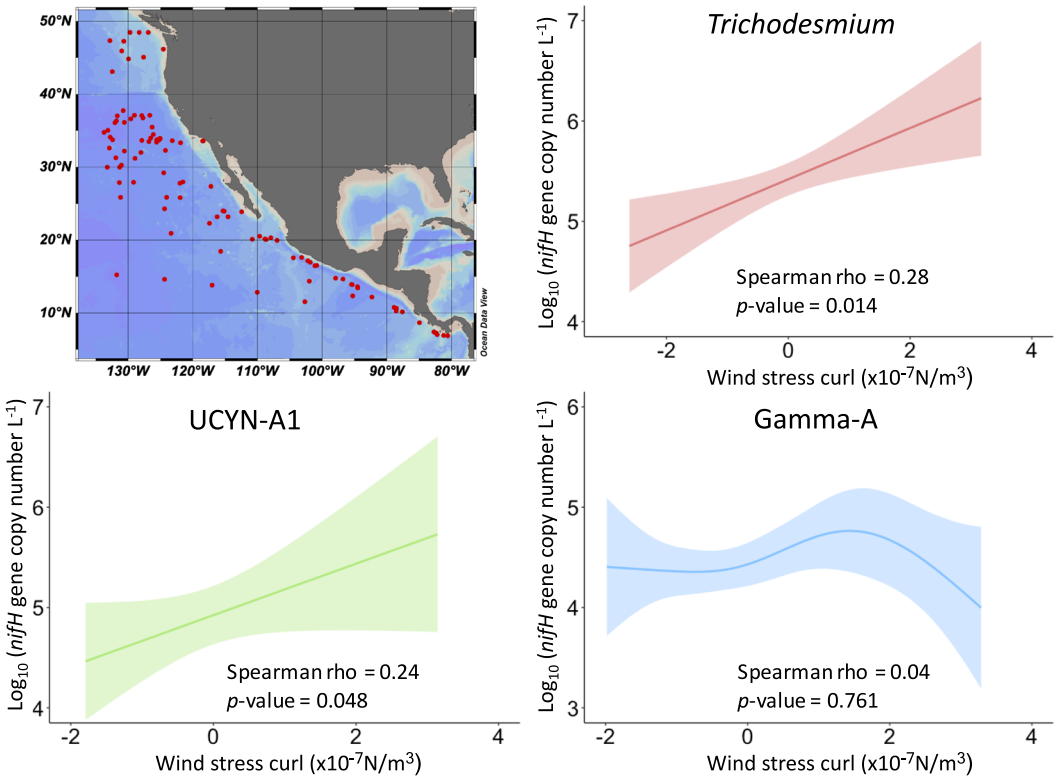


Figure 5. Results of Spearman rank correlations and generalized additive models showing the relationship between the diazotrophs (*Trichodesmium* (a), UCYN-A1 (b), and Gamma-A (c)) and wind stress curl (strength of upwelling-favorable wind) in the eastern North Pacific Ocean (5–50°N, 80–135°W).

Pacific Ocean (Church et al., 2008; Gradoville et al., 2020; Shiozaki et al., 2017). The most recent survey of diazotrophs in the NPTZ was conducted along latitudinal transects in the central Pacific along 160°W during spring (Gradoville et al., 2020), in which the diazotroph abundance decreased suddenly at the southern edge of the NPTZ (~32°N) and became rare or undetected in the NPTZ (~32–40°N). A similar study conducted along 170°W also found undetectable diazotroph abundance with qPCR and relatively low BNF in the NPTZ between 35 and 40°N (Shiozaki et al., 2017). In our study, the latitudinal range of the NPTZ was approximately 30–40°N, in which UCYN-A1 and *Trichodesmium* were highly abundant (reached 10^7 *nifH* gene copy number L^{-1}) (Figures 2, S4, and S5). Indeed, *Trichodesmium* and UCYN-A1 were more abundant at both eastern and western regions than in the central region of the NPTZ (Figure 2). All these results indicate that diazotrophs were indeed thriving with distinct longitudinal patterns in the NPTZ, which led to the undetectable (or rare) BNF and diazotrophs in the NPTZ in previous studies that were conducted in the Central North Pacific (Gradoville et al., 2020; Shiozaki et al., 2017). The BNF activity of the abundant diazotrophs in both the eastern and western regions of the NPTZ should be further verified.

4.3. Crucial Role of Physical Forcing in Shaping the Abundance of Diazotrophs

In the WSNP, our results highlight the positive correlation between mesoscale eddies and the abundance of diazotrophs. The WSNP was characterized by water masses with strong EKE and highly variable SSHA (Figures S3c and S3d), reflecting the strong mesoscale eddy activities associated with Kuroshio and its Extension (KE) (Cheng et al., 2014; Qiu et al., 1991). The KE is formed when the western boundary current, the Kuroshio, turns eastward off the east coast of Japan at a latitude of ~35°N (Qiu, 2001) and is accompanied by strong, pinched-off mesoscale eddies (Qiu et al., 1991), making the WSNP the most active region for eddies in the North Pacific (Cheng et al., 2014). The positive correlation between the three diazotrophs and EKE suggests that the hydrographic condition of the mesoscale eddies in the WSNP is favorable for diazotrophs (Figures 4a and S7). Besides, the positive correlation between the cyanobacterial diazotrophs and

SSHA was consistent with previous finding at station ALOHA, in which high BNF rates were correlated with high SSHA that inferred to anticyclonic eddies (Church et al., 2009). In particular, UCYN-A1 showed a stronger correlation with SSHA compared to *Trichodesmium*. Coincidentally, the positive correlation between UCYN-A1 and SSTA in the WSNP further indicates that UCYN-A1 may benefit more from the anticyclonic eddies than *Trichodesmium*. In the WSNP, values of positive SSHA were more than the negative SSHA (Figure S3d) and in combination with positive SSTA and strong EKE values suggest that there were more intensive anticyclonic (warm) eddies than cyclonic (cold) eddies, which are in agreement with previous studies (Aoki & Imawaki, 1996; Ebuchi & Hanawa, 2000). The enhancement of diazotrophs and BNF in anticyclonic eddies has been observed in a number of studies (Church et al., 2009; Davis & McGillicuddy, 2006; Fong et al., 2008; Robidart et al., 2014; Wilson et al., 2017), and it has been suggested that diazotrophs may take advantage over other organisms in the nitrite depleted conditions resulted from the isopycnic downwelling in anticyclonic eddies (Church et al., 2009; Wilson et al., 2017). Nevertheless, the variations of sea surface height might also be associated with other physical phenomena including Rossby waves and ocean fronts (Barone et al., 2019). Therefore, the underlying mechanisms of the positive correlation between SSHA and diazotroph abundance should be further explored. In the WSNP (the most active region for eddies in the North Pacific), the positive correlation between diazotrophs and the proxies of mesoscale eddy suggests that mesoscale eddies could play a crucial role in mediating the distribution of diazotrophs in the region. On the other hand, diverse diazotrophs were found to be enriched in the sinking particles collected from 150 m depth in an anticyclonic eddy (Farnelid et al., 2019), which implies direct contribution of BNF to nitrogen export. The dynamics of different diazotrophs in mesoscale eddies and their contribution to nitrogen export should be further explored with high-resolution time-series sampling in the WSNP.

In the eastern North Pacific, our results highlight the important role that wind-driven coastal upwelling may play in shaping the diazotroph community in this region. The eastern North Pacific had water masses with low NP ratio compared to western North Pacific (Figure S3b). Low NP ratio in the eastern North Pacific has previously been observed in the surface waters of the wind-driven coastal upwelling regions of eastern tropical North Pacific (ETNP) and ESNP, which is considered to be a result of the upwelling of denitrified water from the subsurface oxygen minimum zone (Paulmier & Ruiz-Pino, 2009; Puigcorbé et al., 2015). The abundance of UCYN-A1 and *Trichodesmium* was higher in coastal upwelling regions of the ETNP and ESNP than in the central Pacific (Figure 2) and was positively correlated with wind stress curl (the strength of the upwelling favorable wind), suggesting that the conditions in the coastal upwelling regions could benefit these diazotrophs (Figures 5a and 5b). Moreover, the negative correlation between both of these diazotrophs and the NP ratio suggests that they might be benefited from the excess phosphate in these regions (Figures 4a and S3b). The traditional concept considered that most of the marine BNF occurred in the central gyre, while a recent study highlighted intense BNF in coastal waters (Tang et al., 2019). In the eastern North Pacific, diazotrophs and significant BNF activity were detected in the surface waters of coastal upwelling regions in both ESNP and ETNP (Hamersley et al., 2011; Selden et al., 2019; White et al., 2013), although the BNF was low or highly variable during some observations (Gradoville et al., 2017; Selden et al., 2019). Our results further show that the cyanobacterial diazotrophs were more abundant in the surface waters of coastal upwelling regions than in the central Pacific, which can be explained by the upwelling of subsurface water with a low N:P ratio and remineralized iron (Klar et al., 2018; White et al., 2013). Besides, the strong 2015/2016 El Niño occurred during the sampling period, which caused a temporal elevation of SSTA in the eastern North Pacific (Jacox et al., 2016; Peterson et al., 2017), that was also observed in our study (Figure S3a). The positive correlation between UCYN-A1 and SSTA in the ESNP implies a positive relationship between UCYN-A1 and El Niño (Figure 4a), which is worth further verification through comparative studies in both El Niño and non-El Niño periods.

5. Conclusions

Our findings refine the biogeography of three major diazotrophs and highlight the crucial role that physical forcing may play in shaping the basin-scale dynamics of diazotrophs in the North Pacific Ocean. The high abundance of diazotrophs in the NPTZ and the distinct longitudinal patterns of cyanobacterial diazotrophs were previously unknown due to limited coverage of sampling (Luo et al., 2012; Tang & Cassar, 2019), which highlights the importance of conducting high-resolution basin-scale observations to achieve a better understanding of the dynamics and determinants of important functional groups in the ocean. It would be worth

applying a similar approach to other oceans in the future to gain a more comprehensive understanding of the fate of diazotrophs and nitrogen fixation in the current and future oceans.

Data Availability Statement

The dataset of the diazotroph abundances and the environmental parameters are available in SEANOE (10.17882/75415).

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