AGU PUBLICATIONS

Geophysical Research Letters

RESEARCH LETTER
10.1002/2017GL073856

Key Points:
- The Bismarck and Solomon Seas are hot spots of dinitrogen fixation in the ocean
- Dinitrogen fixation is underestimated if the dissolved pool is not taken into account
- Regression analyses indicate that most of the dinitrogen fixation activity is attributed to *Trichodesmium*

Supporting Information:
- Supporting Information S1

Correspondence to:
H. Berthelot,
hugo.berthelot@gmail.com

Citation:
Berthelot, H., M. Benavides, P. H. Moisander, O. Grosso, and S. Bonnet (2017), High-nitrogen fixation rates in the particulate and dissolved pools in the Western Tropical Pacific (Solomon and Bismarck Seas), *Geophys. Res. Lett.*, *44*, 8414–8423, doi:10.1002/2017GL073856.

Received 18 APR 2017
Accepted 14 JUL 2017
Accepted article online 18 JUL 2017
Published online 17 AUG 2017

High-nitrogen fixation rates in the particulate and dissolved pools in the Western Tropical Pacific (Solomon and Bismarck Seas)

H. Berthelot[1,2], M. Benavides[3,4], P. H. Moisander[5], O. Grosso[1], and S. Bonnet[3]

[1]Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO) UM 110, Marseille, France, [2]Laboratoire des sciences de l'environnement marin, IUEM, Université de Brest-UMR 6539 CNRS/UBO/IRD/Ifremer, Plouzané, France, [3]Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO) UM 110, New Caledonia, France, [4]Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, Denmark, [5]Department of Biology, University of Massachusetts Dartmouth, North Dartmouth, Massachusetts, USA

Abstract Dinitrogen (N_2) fixation rates were investigated in the euphotic layer of the Bismarck and Solomon Seas using $^{15}N_2$ incubation assays taking into account both the particulate and the dissolved pools. Average depth-integrated particulate N_2 fixation rates were 203 (range 43–399) and 1396 (range 176–3132) μmol N m^{-2} d^{-1} in the Bismarck and Solomon Seas, respectively. In both seas, N_2 fixation measured in the dissolved pool was similar to particulate N_2 fixation, highlighting the potentially substantial underestimation of N_2 fixation in oceanic budgets when only particulate N_2 fixation is considered. Among the diazotroph phylotypes targeted using quantitative polymerase chain reaction amplification of *nifH* genes, *Trichodesmium* was the most abundant. Regression analyses suggest that it accounted for the major proportion of N_2 fixation. However, unicellular cyanobacterial and non-cyanobacterial diazotrophs were also occasionally abundant. This study reports high pelagic N_2 fixation rates and confirms that the Western Tropical South Pacific is a hot spot for marine N_2 fixation.

1. Introduction

Vast regions of the surface ocean, in particular subtropical gyres, are characterized by low nitrogen (N) availability that limits phytoplankton growth [*Moore et al*., 2013]. In these areas, biological dinitrogen (N_2) fixation (the reduction of atmospheric N_2 into bioavailable N) provides large amounts of "new" N fueling up to 50% of marine productivity and particle export [*Karl et al*., 2002, 2012; *Capone et al*., 2005]. Marine planktonic N_2 fixation is performed by diverse prokaryotes called diazotrophs, which include cyanobacteria such as the filamentous *Trichodesmium* that forms large blooms that accumulate at the surface [*LaRoche and Breitbarth*, 2005; *Bergman et al*., 2013], heterocystous cyanobacteria symbiotic to diatoms (diatom-diazotroph associations, DDAs), unicellular diazotrophs (UCYN) [*Zehr and Kudela*, 2011], and diverse non-cyanobacterial bacteria and archaea [*Riemann et al*., 2010].

The subtropical oligotrophic gyres of the North Atlantic and Pacific Oceans have long been studied and are now recognized to harbor high N_2 fixation rates [*Dore et al*., 2008; *Benavides and Voss*, 2015]. However, the southern counterparts of the Atlantic and the Pacific Oceans remain largely undersampled [*Luo et al*., 2012]. In the Western Tropical South Pacific (WTSP), *Bonnet et al*. [2015] and *Messer et al*. [2015] reported extremely high N_2 fixation rates (up to 90 nmol N L^{-1} d^{-1}) associated with diverse diazotrophs near Australia and in the Solomon Sea. A former study between the Solomon and the Bismarck Sea also reported extremely high N_2 fixation rates (up to 610 nmol N L^{-1} d^{-1}) [*Bonnet et al*., 2009]. These studies strongly suggest that the WTSP is a hot spot of diazotrophy that may provide a significant fraction of the overall Pacific Ocean N_2 fixation [*Bonnet et al*., 2015]. Yet the data available for this area are sparse [*Luo et al*., 2012] and have mainly been obtained during austral winter conditions. The geographical coverage and seasonality need to be extended to better understand the contribution of the WTSP to global fixed N inputs.

N_2 fixation is generally measured via the incorporation of ^{15}N into particulate organic N (PON) following incubation in the presence of $^{15}N_2$ (hereafter referred to as PN_2) [*Montoya et al*., 1996]. Nevertheless, the few studies that also measured the ^{15}N enrichment in the dissolved pool (hereafter referred to as DN_2) reported a variable but at times highly significant contribution of DN_2 (10–90% of total N_2 fixation, defined

as the sum of PN_2 and DN_2) [*Glibert and Bronk*., 1994; *Konno et al*., 2010; *Benavides et al*., 2013a]. The ^{15}N-enrichment in the dissolved pool has been attributed either to the release of recently fixed N_2 as dissolved organic N (DON) and ammonium (NH_4^+) by diazotrophs [*Glibert and Bronk*, 1994; *Mulholland et al*., 2006; *Benavides et al*., 2013a] or to the presence of ^{15}N-enriched cells small enough to pass GF/F filters (<0.7 μm pore size) in the filtrate [*Konno et al*., 2010]. The goals of this study were (1) to quantify the contribution of PN_2 and DN_2 fixation to total N_2 fixation in the euphotic layer of the Solomon and Bismarck Seas (WTSP), (2) to quantify the abundance of the major diazotrophic phylotypes, and (3) to assess the potential biogeochemical impact of N_2 fixation in this region during austral summer conditions.

2. Materials and Methods

2.1. Hydrography, Nutrients, and Chlorophyll *a*

The MoorSPICE cruise was carried out onboard the R/V *Thomas G. Thompson* from 1 to 30 March 2014, coinciding at the end of the austral summer. Samples were collected along three transects in the Bismarck (stations 1 to 6), North Solomon (stations 7 to 12), and South Solomon (stations 13 to 16) Seas. At each station four depths in the photic layer were sampled. The sampling depths were fixed (5, 15, 30, and 70 m) and corresponded to 55, 36, 10 and 1% of the surface irradiance. At each station, conductivity, temperature, and depth SBE 911 plus probe (Sea-Bird Electronics) equipped with a fluorometry (ECO-FL, WET labs) sensor was deployed on a rosette frame with 24 10 L Niskin bottles.

Samples for nitrate plus nitrite (NO_x) and phosphate (PO_4^{3-}) concentrations were collected and analyzed at stations 1 to 12 as described in *Benavides et al*. [2015]. Samples for NH_4^+ determination were collected in 40 mL borosilicate bottles and analyzed onboard by fluorometry according to *Holmes et al*. [1999] on a Trilogy fluorometer (Turner Designs, detection limit of 0.009 μmol L^{-1}). Samples for DON determination were collected in 40 mL borosilicate flasks after filtration through precombusted (450°C, 4 h) GF/F filters, stored at −20°C, and analyzed onshore by the wet oxidation procedure [*Pujo-Pay and Raimbault*, 1994]. For the measurement of Chlorophyll *a* (Chl *a*), 550 mL of seawater was filtered through GF/F filters at each station, immediately stored at −80°C, and analyzed by fluorometry (Trilogy fluorometer, Turner Designs) after extraction in methanol according to *Herbland et al*. [1985]. The Chl *a* concentrations were used to calibrate the fluorometry sensor.

2.2. N_2 Fixation and Primary Production Rates

Primary production (PP) and N_2 fixation rates were measured at stations 1 to 12 in triplicate 2.3 L transparent polycarbonate bottles at each of the four sampling depths using ^{13}C-labeled bicarbonate ($NaH^{13}CO_3$; ≥98 at. %, Sigma Aldrich, 10 at. % final enrichment) and $^{15}N_2$ ($^{15}N_2$ "dissolved" method, Cambridge Isotopes Laboratories, ≥98.9 at. %) as described in *Mohr et al*. [2010]. The level of contamination in $^{15}NO_3$ and $^{15}NH_4$ of the $^{15}N_2$ stock bottle was checked according to *Dabundo et al*. [2014] and was found to be lower than 2×10^{-8} mol per mol of $^{15}N_2$, leading to a potential overestimation of N_2 fixation rates <1%. The bottles were incubated for 24 h in on-deck incubators covered with blue light screening reproducing the light intensity at the corresponding sampling depths and cooled with continuously circulating surface seawater. Incubations were stopped by filtration through precombusted 25 mm GF/F filters to recover particulate organic carbon (POC) and PON. Filters were analyzed using an elemental analyzer coupled to a mass spectrometer (EA-IRMS, Integra CN, SerCon Ltd.) for the determination of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios and POC and PON concentrations. At each station, two 2.3 L bottles collected at the deepest and the shallowest depths were also filtered onto GF/F filters without any tracer amendment to measure the natural $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratio (time zero) in ambient photic waters. Discrete PP and N_2 fixation rate measurements were depth integrated over the photic layer using trapezoidal integration.

At each station, filtrate fractions from the shallowest ^{15}N incubations were recovered for the determination of DN_2 fixation. The samples were stored in 500 mL borosilicate bottles, poisoned with $HgCl_2$, and kept in the dark until analysis. The $^{15}N/^{14}N$ ratio analyses in the dissolved pool were performed following the diffusion method (*Slawyk and Raimbault* [1995], as modified by *Berthelot et al* [2015]) to recover NH_4^+ in a first step and DON in a second step. However, due to the low initial NH_4^+ concentrations, the measured ^{15}N enrichments were not significantly higher than those of the natural background. The contribution of the

NH_4^+ pool was thus not taken into account, and only the ^{15}N enrichment of the DON pool was considered. N_2 fixation rates were calculated as follows:

$$PN_2 \text{ or } DN_2 = \frac{R_{sample} - R_{t0}}{RN_2 - R_{t0}} \times \frac{[sample]}{t},$$

where R_{sample} represents the $^{15}N/^{14}N$ ratio of the measured PON or DON, R_{t0} the measured $^{15}N/^{14}N$ ratio of time zero samples, RN_2 the $^{15}N/^{14}N$ ratio of the N_2 source pool, [sample] the measured concentration of PON or DON, and t the incubation time. PP was calculated similarly from the measured $^{13}C/^{12}C$ ratios on POC.

2.3. DNA Extraction and qPCR Assays

Seawater samples for *nifH* analyses were collected at the four sampling depths at all stations into 2.3 L polycarbonate bottles and filtered through 0.2 μm Supor filters using a peristaltic pump. Filters were then placed on sterile cryovials and stored at −80°C until analysis. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen), as modified by *Moisander et al*. [2008]. The abundance of diazotrophs was quantified by TaqMan quantitative polymerase chain reaction (qPCR) assays, using previously published primer-probe sets for UCYN-A1, UCYN-A2, UCYN-B, UCYN-C, *Trichodesmium*, the putative Gammaproteobacterium γ-24774A11, and Het1 (*Richelia-Rhizosolenia* or DDAs) [*Church et al*., 2005a, 2005b; *Foster et al*., 2007; *Moisander et al*., 2008, 2010; *Thompson et al*., 2014]. The γ-24774A11 is a *nifH* phylotype (clone name 24774A11) originally obtained from the South China Sea [*Moisander et al*., 2008] and representative of a cluster of phylotypes also termed Gamma A and UMB—uncultured marine bacteria—in other studies, where different qPCR assays have been used to target this group [*Bird et al*., 2005; *Church et al*., 2005b; *Langlois et al*., 2015]. The 20 μL reactions consisted of 10 μL ABI TaqMan Gene Expression Master Mix; 6.4 μL nuclease-free water; 0.4 μmol L^{-1} and 0.2 μmol L^{-1} final concentrations of primers and probe (FAM-TAMRA modified), respectively; and 1.6 μL DNA template. All samples were run in duplicate. Tenfold dilutions of linearized plasmids containing the relevant *nifH* targets were used as standards. The reactions were run on an ABI StepOnePlus Real-Time PCR system (Life Technologies). Amplification efficiencies were >90% for all reactions. Standard curves were calculated by linear regression of threshold cycle (C_t) and log gene copies per reaction using duplicate standards ranging from 10^7 or 10^8 to 10^1 gene copies. Duplicate no template control wells were included in all runs. Inhibition tests were carried out for all samples by adding 1.6 μL of the 10^5 standard to each sample well. The efficiencies of inhibition test runs ranged from 95.55 to 101.73%; thus, we consider that amplification in our samples was not inhibited. The limits of detection and quantification were considered as 1 and 8 gene copies per reaction, respectively.

3. Results and Discussion

3.1. N_2 Fixation Rates in Particulate and Dissolved Pools

PN_2 fixation was detected at all stations and all depths sampled at rates ranging from 0.1 ± 0.2 to 77.9 ± 5.9 nmol N L^{-1} d^{-1} (13.7 ± 20.5 nmol N L^{-1} d^{-1} on average; Figure 1). N_2 fixation rates were on average 2 orders of magnitude higher at the surface (5–30 m) as compared to deep samples (70 m) and 6 times higher in the North Solomon Sea (22.7 ± 25.5 nmol N L^{-1} d^{-1} on average) than in the Bismarck Sea (3.5 ± 3.9 nmol N L^{-1} d^{-1} on average). This difference may be strongly influenced by the hydrological context. The Bismarck Sea was characterized by higher NO_x availability, lower sea surface temperature (SST), and a shallower deep chlorophyll maximum as compared to the Solomon Sea (Figure 1 and Figures S1 and S2 in the supporting information), indicative of the upwelling of deep waters commonly occurring during this season due to the northwest monsoon winds [*Hasegawa et al*., 2010; *Delcroix et al*., 2014]. Diazotrophs are known to have lower growth rates compared to non-diazotrophs due to the high energetic cost of N_2 fixation [*Gallon*, 1992] and their high iron requirements [*Berman-Frank et al*., 2001]. The higher availability of NO_x in the Bismarck Sea may have favored non-diazotrophic phytoplankton [*Tyrrell*, 1999; *Dutkiewicz et al*., 2012; *Ward and Jensen*, 2014], likely explaining why N_2 fixation rates were higher in the NO_x-depleted Solomon Sea. This is further confirmed by the overall negative correlation of N_2 fixation rates with NO_x and PO_4 concentrations ($r = -0.52$, $p < 0.001$ and $r = -0.64$, $p < 0.001$, respectively, data not shown).

Depth-integrated PN_2 fixation rates were 203 ± 121 and 1396 ± 1181 μmol N m^{-2} d^{-1} in the Bismarck and North Solomon Seas, respectively. These rates are in the upper range of rates compiled in the global N_2 fixation database of *Luo et al*. [2012] (range 100–1000 μmol N L^{-1} d^{-1}). In the Solomon Sea, the N_2 fixation rates

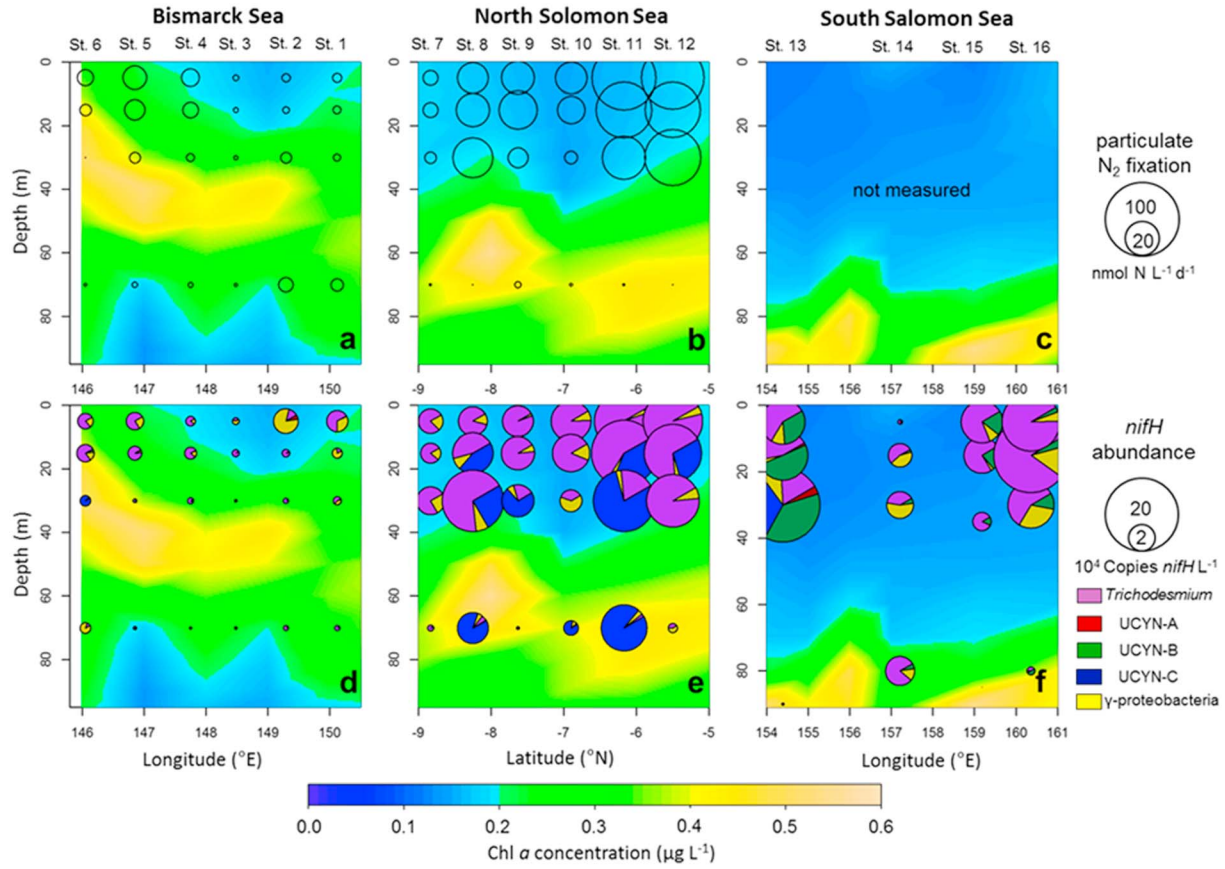

Figure 1. Vertical and horizontal distribution of the PN_2 fixation rates are shown in nmol N L^{-1} d^{-1} in the (a) Bismarck Sea, (b) North Salomon Sea, and (c) South Solomon Sea. Vertical and horizontal distribution of the *nifH* gene abundances in copies L^{-1} (sized dots) in the (d) Bismarck Sea, (e) North Salomon Sea, and (f) South Solomon Sea. Colors indicate the proportion of *Trichodesmium* (purple), UCYN-A (red), UCYN-B (green), UCYN-C (blue), and γ-24774A11 Gammaproteobacterium (yellow). DDAs abundances were several orders of magnitude lower than the other diazotrophs and hence are not shown. The color scale refers to Chl *a* concentrations in µg L^{-1}.

measured during austral summer conditions (this study) are significantly higher (Wilcoxon rank test, $p = 0.02$) than those measured during the austral winter (624 ± 1374 µmol N m^{-2} d^{-1}) [*Bonnet et al.*, 2015], suggesting a possible seasonal effect. These high rates might be induced by a strong stratification and warm waters (>25°C) in the austral summer providing an ideal environment for the bloom-forming diazotroph *Trichodesmium*, which was observed at high abundances during this study (see next section). Alternatively, the observed differences in integrated PN_2 fixation between these two studies may result from the different methodologies used to measure volumetric N_2 fixation rates in *Bonnet et al.*'s [2015] ("bubble method") and this study ("dissolved method"). In some studies, it has been reported that the bubble method [*Montoya et al.*, 1996] underestimates N_2 fixation rates by a factor of 2 to 6 [*Mohr et al.*, 2010; *Großkopf et al.*, 2012; *Wilson et al.*, 2012; *Böttjer et al.*, 2016], although other studies have not found any significant differences between both methods [*Benavides et al.*, 2013a; *Mulholland et al.*, 2014; *Shiozaki et al.*, 2015; *Bonnet et al.*, 2016b]. Nonetheless, N_2 fixation rates were generally high in both seasons, confirming that the region is a hot spot of diazotrophic activity and likely provides a significant amount of new fixed N year round, which is in accordance with recent high N_2 fixation rates reported further east in the WTSP [*Bonnet et al.*, 2017]. The reason for such high N_2 fixation rates measured all year long in the Solomon Sea need to be further investigated. However, the maintenance of warm temperatures (>25°C) [*Breitbarth et al.*, 2007], the low N/P ratio [*Sohm et al.*, 2011], and an important supply of iron through land runoff, hydrothermal, and volcanic activity [*Labatut et al.*, 2014] seem to provide optimal conditions for diazotrophs to bloom extensively in this region.

A parallel study conducted in the Solomon Sea indicates that this new N is transferred to non-diazotrophic plankton, primarily diatoms [*Bonnet et al.*, 2016a], potentially contributing to the export of organic matter

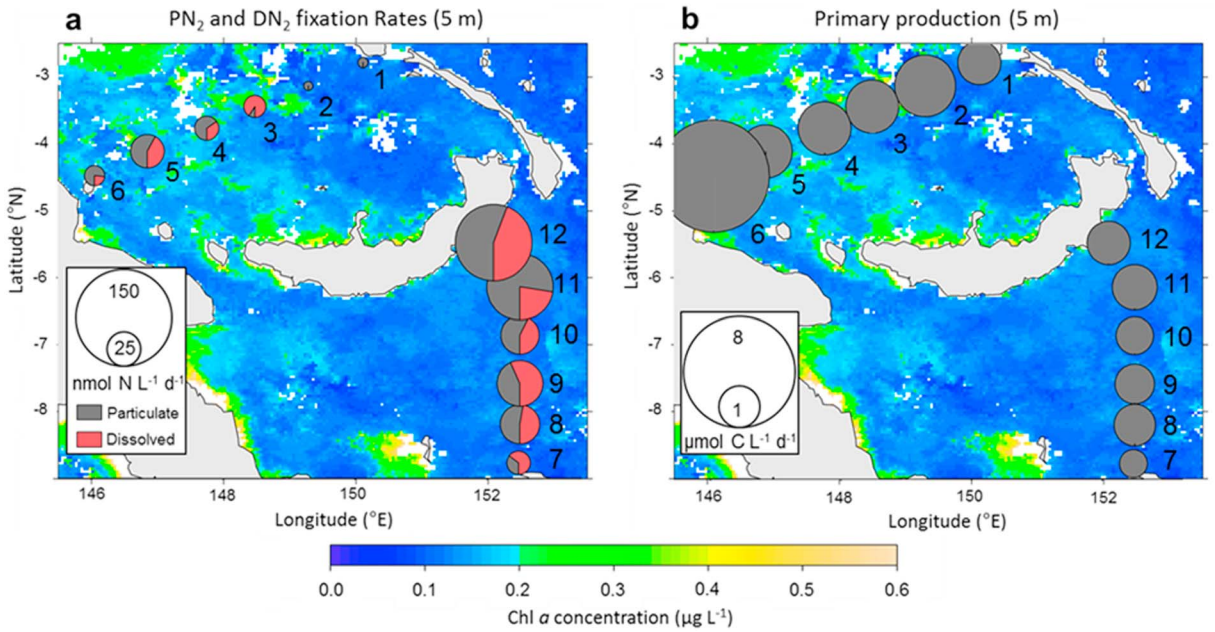

Figure 2. (a) PN_2 (grey) and DN_2 (red) fixation and (b) PP rates in surface waters (5 m) superimposed on a satellite image of the Chl *a* concentrations. Chl *a* data represent the average concentrations for March 2014 measured by AQUA/Moderate Resolution Imaging Spectroradiometer satellite and were obtained from the National Aeronautics and Space Administration (http://neo.sci.gsfc.nasa.gov/). The numbers (1 to 12) refer to the stations names.

out of the euphotic zone in this ecosystem [*Nelson et al.*, 1995]. The transfer of recently fixed N to surrounding planktonic communities takes place mainly through the dissolved pool [*Berthelot et al.*, 2016; *Bonnet et al.*, 2016a]. Significant ^{15}N-enrichment values were measured in the DON pool at all stations sampled (except at stations 1 and 2) and were used to compute the DN_2 fixation rates. DN_2 fixation rates ranged from 2.2 to 59.3 nmol N L^{-1} d^{-1} (17.6 nmol N L^{-1} d^{-1} on average) and accounted for 39.0 ± 25.8% of total N_2 fixation on average during the cruise (Figure 2 and Table 1). This proportion is twice that reported in a previous study in the subtropical Atlantic [*Benavides et al.*, 2013a] and is comparable to the results reported by *Konno et al.* [2010] and *Bonnet et al.* [2016a] in the Pacific Ocean, as well as those of *Mulholland et al.* [2006] in the Atlantic Ocean. These results highlight the need to take into account DN_2 fixation when routinely measuring N_2 fixation in order to avoid underestimating total N_2 fixation rates. DN_2 fixation measurements are largely disregarded in in situ N_2 fixation studies because they are considerably labor intensive. Since our understanding of new production in the surface ocean relies on accurate measurements of N_2 fixation, the determination of total N_2 fixation (including the contribution of the dissolved pool) becomes essential.

3.2. Diazotroph Abundance and Distribution

Diazotrophs were present in both the Bismarck and Solomon Seas with abundances exceeding 1×10^4 *nifH* copies L^{-1} in half of the samples assayed and reaching up to 1.7×10^5 *nifH* copies L^{-1} (Figure 1 and Table S1). These abundances are within the highest decile of those reported in *Luo et al.* [2012], reinforcing the view that this region is a hot spot of diazotrophs [*Bonnet et al.*, 2009, 2015]. Coinciding with N_2 fixation rates, diazotroph abundances were higher in the Solomon Sea (6.5×10^4 *nifH* copies L^{-1} on average) as compared to the Bismarck Sea (5.0×10^3 *nifH* copies L^{-1} on average; Figure 1 and Table S1).

Trichodesmium was the most abundant diazotroph in the three transects (64% of the total; Figure 1 and Table S1), but abundances were 1 order of magnitude greater in the Solomon than in the Bismarck Sea (Figure 1). Consistent with previous studies performed in this area [*Bonnet et al.*, 2009, 2015], *Trichodesmium* reached abundances of 1×10^5 *nifH* copies L^{-1}. The highest abundances were measured at the surface (5–30 m), and numbers decreased with depth. Nevertheless, it is notable that in a parallel study performed in the mesopelagic layer of the same transects sampled here, sporadic *Trichodesmium nifH* genes were detected below the photic layer (between 200 and 1000 m), probably associated with sinking senescent cells

Table 1. Integrated (0–70 m) PP (mmol C m^{-2} d^{-1}), PN_2 and Total N_2 Fixation (μmol N m^{-2} d^{-1}), and PN_2 and total N_2 Fixation Contribution to PP (%) Assuming a C:N Ratio of 10.5 Calculated From Measurements Performed in the Bismarck and North Solomon Seas[a]

Station	Integrated PP (mmol C m^{-2} d^{-1})	Integrated PN_2 Fixation (μmol m^{-2} d^{-1})	Proportion of DN_2 (% of Total N_2 Fixation)[b]	Integrated Total N_2 Fixation (μmol m^{-2} d^{-1})[b]	PN_2 Fixation Contribution to PP (Particulate Only, %)	Total N_2 Fixation Contribution to PP (Total, %)[b]
			Bismarck Sea			
1	128	172	ND	172	0.6	0.6
2	182	242	ND	242	0.6	0.6
3	103	43	90.5	453	0.19	2.9
4	150	236	34.2	359	0.6	1.6
5	101	399	41.9	687	1.6	4.5
6	198	125	23.1	163	0.2	0.5
Average	144 ± 40	203 ± 121	31.6 ± 33.6[c]	415 ± 218	0.6 ± 0.5	2.4 ± 1.7
			North Solomon Sea			
7	62	176	64.7	499	1.2	5.3
8	124	1302	47.2	2466	4.3	13.1
9	89	788	56.7	1820	3.6	13.5
10	78	471	42.8	823	2.5	7.0
11	77	2508	22.6	3240	13.3	27.8
12	41	3132	44.2	5613	31.2	90.4
Average	79 ± 28	1396 ± 1181	46.4 ± 14.3	2410 ± 1869	9.3 ± 11.6	26.2 ± 32.4

[a]ND: Not detected.
[b]Assuming an equal proportion of DN_2 fixation to total N_2 fixation at all depths compared to those measured at the surface.
[c]Assuming that ND as 0%.

[*Benavides et al*., 2015]. Our results build upon previous evidence of the preferential growth of *Trichodesmium* at the surface and in warm waters (>25°C) [*Capone et al*., 1997]. *Trichodesmium* was more abundant close to the shore in the Solomon Sea (stations 11 and 12; Figure 1), likely due to the so-called "island mass effect," previously proposed by *Shiozaki et al*. [2014] for the WTSP, where the terrigenous supply of micronutrients and macronutrients near islands is suspected to enhance *Trichodesmium* development.

The UCYN groups assayed (UCYN-A1, UCYN-A2, UCYN-B, and UCYN-C) were much less abundant than *Trichodesmium*. UCYN-A1 was only detected in 12 out of 64 samples at abundances ranging between $<4 \times 10^1$ and 1.3×10^3 *nifH* copies L^{-1}. UCYN-A2 was detected only twice (Figure 1 and Table S1) at abundances of 1.3 and 7.0×10^3 *nifH* copies L^{-1}, in agreement with previous studies that have reported a generally more ubiquitous presence of the phylotype UCYN-A1 [*Thompson et al*., 2014; *Bonnet et al*., 2015]. UCYN-B was detected in 30 out of the 64 samples analyzed at abundances $<5 \times 10^2$ *nifH* copies L^{-1}, almost exclusively in the South Solomon Sea and to a lower extent in the Bismarck Sea (Figure 1 and Table S1). In contrast, UCYN-C was most abundant in the North Solomon Sea (4.4×10^4 *nifH* copies L^{-1} on average) and almost absent in the Bismarck and South Solomon Seas. The heterogeneous distribution of the different diazotrophs confirms that they occupy different ecological niches. The virtual absence of UCYN-A during our cruise, when seawater temperatures in the euphotic zone ranged from 27 to 31°C, confirms that these diazotrophs do not develop in waters >25°C, as previously observed in different oceanic areas [*Moisander et al*., 2010; *Agawin et al*., 2014; *Bonnet et al*., 2015]. Interestingly, UCYN-B and UCYN-C did not share the same spatial niche: UCYN-C thrived extensively and exclusively in the oligotrophic warm North Solomon Sea at SST of ~31°C, while UCYN-B was almost solely present in the Bismarck and South Solomon Seas at SST of ~29°C (Figure 1 and Table S1). This is in good agreement with results from *Bonnet et al*. [2015] where UCYN-B was found in similar abundances in the Solomon Sea during austral winter at SST ~28–29°C. Here again, temperature may have also played a major role in the distribution of photosynthetic UCYN phylotypes suggesting an adaptation of UCYN-C to relatively warmer waters compared to UCYN-B and UCYN-A. However, the covariation of temperature with other factors could also have influenced these patterns. Taken together, these results highlight the importance to consider seasonality in N_2 fixation studies, even in equatorial regions with narrow temperature ranges.

DDAs were detected in 28 out of the 48 samples at abundances generally $<5 \times 10^2$ *nifH* copies L^{-1} but at a maximal abundance of 5.1×10^3 *nifH* copies L^{-1} at 15 m depth of the station 16 (Table S1). These overall low abundances compared to *Trichodesmium* and UCYN-C are in agreement with previous findings in the

equatorial Atlantic Ocean [*Foster et al*., 2007, 2009a] and the Solomon Sea [*Bonnet et al*., 2015]. Nevertheless, their abundance is 1 or 2 orders of magnitude lower than those reported in the Amazon River plume [*Subramaniam et al*., 2008; *Yeung et al*., 2012] and in the North Pacific Ocean [*Church et al*., 2005a] where DDAs are thought to contribute significantly to N_2 fixation. Microscopic observations conducted on samples collected during the cruise in the Saint Georges Channel and in the Vitiaz Strait revealed a low abundance of *Rhizosolenia* (~400 cells L^{-1}) (V. Cornet, personal communication, 2016), but *Richelia* were not observed as either free-living cells or in association with diatoms. This indicates that the contribution of DDAs to the total diazotroph consortium in the Bismarck and Solomon Seas is probably very low during austral summer conditions.

The putative gammaproteobacterium γ-24774A11 was detected in 47 samples with an average abundance of 5.4×10^3 *nifH* copies L^{-1} ($>5 \times 10^3$ *nifH* copies L^{-1} in 17 of the 64 samples analyzed). Compared to *Trichodesmium* and UCYN, this diazotroph was more homogeneously distributed between the two seas and ranked as the third most abundant diazotroph considering all the phylotypes targeted (Figure 1 and Table S1). The ubiquitous presence of this non-cyanobacterial diazotroph at average abundances of thousands of *nifH* copies per liter compares well with previous reports from the WTSP Ocean [*Moisander et al*., 2014; *Bonnet et al*., 2015], the Tropical North Pacific [*Church et al*., 2005a] and Atlantic Oceans [*Langlois et al*., 2005], and the Red Sea [*Foster et al*., 2009b]. Their relatively homogeneous distribution when compared to cyanobacterial diazotrophs suggests that they are adapted to wider variations in environmental constraints (e.g., nutrients and temperature) and may have a largely cosmopolitan distribution in the tropical and subtropical oceans, as suggested by reports from wide latitudinal ranges and contrasting oceanic regions [*Luo et al*., 2012; *Moisander et al*., 2014; *Langlois et al*., 2015]. Nevertheless, their abundance was on average ~ 4.5 times higher in the Solomon as compared to Bismarck Sea suggesting that they also are more competitive under N scarcity. Given their relatively high abundance observed in this and other studies, the contribution of these non-cyanobacterial diazotrophs to global N_2 fixation may be considerable and deserves further study [*Bombar et al*., 2016].

The importance of N_2 fixation recovered in the dissolved pool has traditionally been attributed to the release of recently fixed N_2 to the dissolved pool as NH_4^+ or DON in the form of small organic compounds such as amino acids [*Capone et al*., 1994; *Glibert and Bronk*, 1994; *Mulholland et al*., 2004]. However, the presence of the non-cultivated, non-cyanobacterial diazotroph γ-24774A11 (whose size could be smaller than the GF/F cutoff; ~0.7 μm) at high abundances raises the question about the possibility of their recovery in the dissolved pool and their contribution to the high DN_2 fixation measured [*Konno et al*., 2010]. Abundances of *nifH* gene copies measured in this study do not necessarily translate into *nifH* expression and diazotroph activity. However, in an attempt to estimate the contribution of each diazotroph phylotypes to N_2 fixation, we assume a linear relationship between *nifH* abundances and diazotroph activity and correlate abundances to PN_2 and DN_2 fixation rates (Figure S3). Abundances of *Trichodesmium* were strongly positively correlated with both PN_2 and DN_2 fixation rates ($r = 0.974$, $p < 0.001$ and $r = 0.801$, $p = 0.002$, respectively). Conversely, despite their relatively high abundances at times, UCYN and γ-24774A11 did not correlate significantly neither with PN_2 nor DN_2 fixation rates, suggesting that their contribution to N_2 fixation was likely minor and that γ-24774A11 was not responsible for the high ^{15}N enrichment measured in the dissolved pool. It is possible that DN_2 fixation was primarily attributed to N release. Based on these considerations, we conclude that (1) nearly all the PN_2 fixation was attributed to *Trichodesmium*, (2) *Trichodesmium* was directly responsible for most of the DN_2 fixation through the release of DON compounds, and, when normalized by detected *nifH* copies, (3) *Trichodesmium* was much more efficient at fixing N_2 than UCYN and γ-24774A11.

3.3. Biogeochemical Implications and Conclusions

Depth-integrated PP rates were higher in the Bismarck Sea (125 ± 67 μmol C m^{-2} d^{-1}) than in the Solomon Sea (78 ± 28 μmol C m^{-2} d^{-1}) (Figure 2 and Table 1). This is consistent with the higher-surface Chl *a* concentrations and nutrient availability in the Bismarck Sea (Figure S3). The computation of the N demand derived from this PP (assuming a C:N ratio of 10.5 ± 3.2 as measured in the POC and PON samples) indicates that PN_2 fixation fueled 0.6% (range 0.2 to 1.6%) and 9.4% (range 1.2 to 31.2%) of PP in Bismarck and Solomon Seas, respectively (Table 1). The contribution of PN_2 fixation to PP in the Solomon Sea is remarkably high compared to other oceanic regions such as the Northwestern Pacific [*Shiozaki et al*., 2013], the Eastern

tropical South Pacific [*Raimbault and Garcia*, 2008], subtropical Northeast Atlantic [*Benavides et al*., 2013b], or the Mediterranean Sea [*Bonnet et al*., 2011; *Ridame et al*., 2013], where the contribution is generally <5%. It is, however, in the range of previous studies performed in the Solomon Sea during the austral winter [5.5%; *Bonnet et al*., 2015]. If we consider total N_2 fixation (DN_2 + PN_2 fixation) and assume a similar proportion of DN_2 fixation to total N_2 fixation at depth compared to the surface, the contribution of N_2 fixation to PP reaches 1.5% (ranging from 0.3 to 2.8%) and 16.2% (ranging from 3.3 to 56.0%) in the Bismarck and the Solomon Seas, respectively (Table 1). To the best of our knowledge, these are among the highest contributions reported, which further confirms the importance of N_2 fixation in supplying new N to the surface waters of the Solomon Sea. Nitrate uptake was not measured in this study, and it is thus difficult to assess the relative importance of N_2 fixation in sustaining new production (in the sense of *Dugdale and Goering* [1967]). However, new PP rarely exceeds 30% of the total PP in N-depleted waters [*Yool et al*., 2007; *Raimbault and Garcia*, 2008; *Bonnet et al*., 2015], suggesting that new production in the Solomon Sea was probably importantly sustained by N_2 fixation.

Accurate estimates of N_2 fixation are of outmost importance to constrain the global N cycle. The results from this study highlight the importance of considering both PN_2 and DN_2 fixation when quantifying rates and report a hot spot of N_2 fixation in the equatorial and WTSP that needs to be taken into account in future global budgets.

Acknowledgments
The MoorSPICE cruise is a contribution to the CLIVAR/SPICE International program. It has been cofounded by NSF grant OCE1029487, ANR- 09-BLAN-0233-01, INSU/LEFE projects Solwara and SPICEMoor and Institut de Recherche pour le Développement (IRD). MoorSPICE data can be found through cruise DOI: 10.7284/903044. We are grateful to the crews of the R/V *Thomas G. Thompson*. We are also grateful to the engineers, especially from the IRD US-IMAGO team and DT-INSU, and scientists who carefully sampled, recorded, and treated the data. We thank particularly V. Cornet for the help at sea. M.B. was funded by the Marie Sklodowska-Curie Actions of the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement 625185. S.B. was funded by IRD. We thank M. Daley (UMass) for assistance with sample analyses and NSF OCE 1130495 to P.M. for additional funding.

References

Agawin, N. S. R., M. Benavides, A. Busquets, P. Ferriol, L. J. Stal, and J. Arístegui (2014), Dominance of unicellular cyanobacteria in the diazotrophic community in the Atlantic Ocean, *Limnol. Oceanogr.*, *59*(2), 623–637, doi:10.4319/lo.2014.59.2.0623.

Benavides, M., and M. Voss (2015), Five decades of N_2 fixation research in the North Atlantic Ocean, *Front. Mar. Sci.*, *2*(June), 1–20, doi:10.3389/fmars.2015.00040.

Benavides, M., D. A. Bronk, N. S. R. Agawin, M. D. Pérez-Hernández, A. Hernández-Guerra, and J. Arístegui (2013a), Longitudinal variability of size-fractionated N_2 fixation and DON release rates along 24.5°N in the subtropical North Atlantic, *J. Geophys. Res. Oceans*, *118*, 3406–3415, doi:10.1002/jgrc.20253.

Benavides, M., J. Arístegui, N. S. R. Agawin, X. A. Álvarez-Salgado, M. Álvarez, and C. Troupin (2013b), Low contribution of N_2 fixation to new production and excess nitrogen in the subtropical northeast Atlantic margin, *Deep Sea Res., Part I*, *81*(3), 36–48, doi:10.1016/j.dsr.2013.07.004.

Benavides, M., P. H. Moisander, H. Berthelot, T. Dittmar, O. Grosso, and S. Bonnet (2015), Mesopelagic N_2 fixation related to organic matter composition in the Solomon and Bismarck seas (Southwest Pacific), edited by D. A. Campbell, *PLoS One*, *10*(12), e0143775, doi:10.1371/journal.pone.0143775.

Bergman, B., G. Sandh, S. Lin, J. Larsson, and E. J. Carpenter (2013), *Trichodesmium* - a widespread marine cyanobacterium with unusual nitrogen fixation properties, *FEMS Microbiol. Rev.*, *37*(3), 286–302, doi:10.1111/j.1574-6976.2012.00352.x.

Berman-Frank, I., J. T. Cullen, Y. Shaked, R. M. Sherrell, and P. G. Falkowski (2001), Iron availability, cellular iron quotas, and nitrogen fixation in Trichodesmium, *Limnol. Oceanogr.*, *46*(6), 1249–1260, doi:10.4319/lo.2001.46.6.1249.

Berthelot, H., S. Bonnet, M. Camps, O. Grosso, and T. Moutin (2015), Assessment of the dinitrogen released as ammonium and dissolved organic nitrogen by unicellular and filamentous marine diazotrophic cyanobacteria grown in culture, *Front. Mar. Sci.*, *2*, doi:10.3389/fmars.2015.00080.

Berthelot, H., S. Bonnet, O. Grosso, V. Cornet, and A. Barani (2016), Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: A comparative study between *Trichodesmium erythraeum*, *Crocosphaera watsonii* and *Cyanothece* sp, *Biogeosciences*, *13*(13), 4005–4021, doi:10.5194/bg-13-4005-2016.

Bird, C., J. Martinez Martinez, A. G. O'Donnell, and M. Wyman (2005), Spatial distribution and transcriptional activity of an uncultured clade of planktonic Diazotrophic -Proteobacteria in the Arabian Sea, *Appl. Environ. Microbiol.*, *71*(4), 2079–2085, doi:10.1128/AEM.71.4.2079-2085.2005.

Bombar, D., R. W. Paerl, and L. Riemann (2016), Marine non-Cyanobacterial Diazotrophs: Moving beyond molecular detection, *Trends Microbiol.*, doi:10.1016/j.tim.2016.07.002.

Bonnet, S., I. C. Biegala, P. Dutrieux, L. O. Slemons, and D. G. Capone (2009), Nitrogen fixation in the western equatorial Pacific: Rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance, *Global Biogeochem. Cycles*, *23*, GB3012, doi:10.1029/2008GB003439.

Bonnet, S., O. Grosso, and T. Moutin (2011), Planktonic dinitrogen fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period (BOUM cruise), *Biogeosciences*, *8*(8), 2257–2267, doi:10.5194/bg-8-2257-2011.

Bonnet, S., et al. (2015), Contrasted geographical distribution of N_2 fixation rates and *nifH* phylotypes in the Coral and Solomon Seas (southwestern Pacific) during austral winter conditions, *Global Biogeochem. Cycles*, *29*, 1874–1892, doi:10.1002/2015GB005117.

Bonnet, S., et al. (2016a), Diazotroph derived nitrogen supports diatom growth in the south West Pacific: A quantitative study using nanoSIMS, *Limnol. Oceanogr.*, *61*(5), 1549–1562, doi:10.1002/lno.10300.

Bonnet, S., H. Berthelot, K. Turk-Kubo, S. Fawcett, E. Rahav, S. L'Helguen, and I. Berman-Frank (2016b), Dynamics of N_2 fixation and fate of diazotroph-derived nitrogen in a low-nutrient, low-chlorophyll ecosystem: Results from the VAHINE mesocosm experiment (New Caledonia), *Biogeosciences*, *13*(9), 2653–2673, doi:10.5194/bg-13-2653-2016.

Bonnet, S., M. Caffin, H. Berthelot, and M. Moutin (2017), Hot Spot of N_2 fixation in the western tropical South Pacific pleads for a spatial decoupling between N_2 fixation and diazotrophy, *PNAS*, E2800-28001, doi:10.1073/pnas.1619514114.

Böttjer, D., J. E. Dore, D. M. Karl, R. M. Letelier, C. Mahaffey, S. T. Wilson, J. Zehr, and M. J. Church (2016), Temporal variability of nitrogen fixation and particulate nitrogen export at station ALOHA, *Limnol. Oceanogr.*, doi:10.1002/lno.10386.

Breitbarth, E., A. Oschlies, and J. LaRoche (2007), Physiological constraints on the global distribution of *Trichodesmium*—Effect of temperature on diazotrophy, *Biogeosciences*, *4*(1), 53–61, doi:10.5194/bg-4-53-2007.

Capone, D. G., M. D. Ferrier, and E. J. Carpenter (1994), Amino acid cycling in colonies of the planktonic marine Cyanobacterium *Trichodesmium thiebautii*, *Appl. Environ. Microbiol.*, *60*(11), 3989–3995.

Capone, D. G., J. P. Zehr, H. W. Paerl, B. Bergman, and E. J. Carpenter (1997), *Trichodesmium*, a Globally Significant Marine Cyanobacterium, *Science*, *27*(5316), 1221–1229, doi:10.1126/science.276.5316.1221.

Capone, D. G., J. A. Burns, J. P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A. F. Michaels, and E. J. Carpenter (2005), Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, *Global Biogeochem. Cycles*, *19*, GB2024, doi:10.1029/2004GB002331.

Church, M., B. Jenkins, D. Karl, and J. Zehr (2005a), Vertical distributions of nitrogen-fixing phylotypes at Stn Aloha in the oligotrophic North Pacific Ocean, *Aquat. Microb. Ecol.*, *38*, 3–14, doi:10.3354/ame038003.

Church, M. J., C. M. Short, B. D. Jenkins, D. M. Karl, and J. P. Zehr (2005b), Temporal patterns of nitrogenase gene (nifH) expression in the oligotrophic North Pacific Ocean, *Appl. Environ. Microbiol.*, *71*(9), 5362–5370, doi:10.1128/AEM.71.9.5362.

Dabundo, R., M. F. Lehmann, L. Treibergs, C. R. Tobias, M. A. Altabet, P. H. Moisander, and J. Granger (2014), 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, doi:10.1371/journal.pone.0110335.

Delcroix, T., M.-H. Radenac, S. Cravatte, G. Alory, L. Gourdeau, F. Léger, A. Singh, and D. Varillon (2014), Sea surface temperature and salinity seasonal changes in the western Solomon and Bismarck seas, *J. Geophys. Res. Oceans*, *119*, 2642–2657, doi:10.1002/2013JC009733.

Dore, J. E., R. M. Letelier, M. J. Church, R. Lukas, and D. M. Karl (2008), Summer phytoplankton blooms in the oligotrophic North Pacific subtropical gyre: Historical perspective and recent observations, *Prog. Oceanogr.*, *76*, 2–38, doi:10.1016/j.pocean.2007.10.002.

Dugdale, R. C., and J. J. Goering (1967), Uptake of new and regenerated forms of nitrogen in primary productivity, *Limnol. Oceanogr.*, *12*(2), 196–206, doi:10.4319/lo.1967.12.2.0196.

Dutkiewicz, S., B. A. Ward, F. Monteiro, and M. J. Follows (2012), Interconnection of nitrogen fixers and iron in the Pacific Ocean: Theory and numerical simulations, *Global Biogeochem. Cycles*, *26*, GB1012, doi:10.1029/2011GB004039.

Foster, R. A., A. Subramaniam, C. Mahaffey, E. J. Carpenter, D. G. Capone, and J. P. Zehr (2007), Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical north Atlantic Ocean, *Limnol. Oceanogr.*, *52*(2), 517–532, doi:10.4319/lo.2007.52.2.0517.

Foster, R. A., A. Subramaniam, and J. P. Zehr (2009a), Distribution and activity of diazotrophs in the eastern equatorial Atlantic, *Environ. Microbiol.*, *11*(4), 741–750, doi:10.1111/j.1462-2920.2008.01796.x.

Foster, R. A., A. Paytan, and J. P. Zehr (2009b), Seasonality of N_2 fixation and *nifH* gene diversity in the Gulf of Aqaba (Red Sea), *Limnol. Oceanogr.*, *54*(1), 219–233, doi:10.4319/lo.2009.54.1.0219.

Gallon, J. R. (1992), Reconciling the incompatible: N_2 fixation and O_2, *New Phytol.*, *122*, 571–609.

Glibert, P. M., and D. A. Bronk (1994), Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria *Trichodesmium* spp, *Appl. Environ. Microbiol.*, *60*, 3996–4000.

Großkopf, T., W. Mohr, T. Baustian, H. Schunck, D. Gill, M. M. M. Kuypers, G. Lavik, R. A. Schmitz, D. W. R. Wallace, and J. LaRoche (2012), Doubling of marine dinitrogen-fixation rates based on direct measurements, *Nature*, *488*(7411), 361–364, doi:10.1038/nature11338.

Hasegawa, T., K. Ando, K. Mizuno, R. Lukas, B. Taguchi, and H. Sasaki (2010), Coastal upwelling along the north coast of Papua New Guinea and El Niño events during 1981–2005, *Ocean Dyn.*, *60*(5), 1255–1269.

Herbland, A., A. Le Bouteiller, and P. Raimbault (1985), Size structure of phytoplankton biomass in the equatorial Atlantic Ocean, *Deep Sea Res., Part A*, *32*(7), 819–836, doi:10.1016/0198-0149(85)90118-9.

Holmes, R. M., A. Aminot, R. Kérouel, B. A. Hooker, and B. J. Peterson (1999), A simple and precise method for measuring ammonium in marine and freshwater ecosystems, *Can. J. Fish. Aquat. Sci.*, *56*(10), 1801–1808, doi:10.1139/f99-128.

Karl, D., A. Michaels, B. Bergman, D. Capone, E. Carpenter, R. Letelier, F. Lipschultz, H. Paerl, D. Sigman, and L. Stal (2002), Dinitrogen fixation in the world's oceans, *Biogeochemistry*, *57*(1), 47–98, doi:10.1023/A:1015798105851.

Karl, D. M., M. J. Church, J. E. Dore, R. M. Letelier, and C. Mahaffey (2012), Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation, *Proc. Natl. Acad. Sci. U.S.A.*, *109*(6), 1842–1849, doi:10.1073/pnas.1120312109.

Konno, U., U. Tsunogai, D. D. Komatsu, S. Daita, F. Nakagawa, A. Tsuda, T. Matsui, Y.-J. Eum, and K. Suzuki (2010), Determination of total N_2 fixation rates in the ocean taking into account both the particulate and filtrate fractions, *Biogeosciences*, *7*(8), 2369–2377, doi:10.5194/bg-7-2369-2010.

Labatut, M., F. Lacan, C. Pradoux, J. Chmeleff, A. Radic, J. W. Murray, F. Poitrasson, A. M. Johansen, and F. Thil (2014), Iron sources and dissolved-particulate interactions in the seawater of the western equatorial Pacific, iron isotope perspectives, *Global Biogeochem. Cycles*, *28*, 1044–1065, doi:10.1002/2014GB004928.

Langlois, R., T. Großkopf, M. Mills, S. Takeda, and J. LaRoche (2015), Widespread distribution and expression of Gamma A (UMB), an uncultured, diazotrophic, gamma-proteobacterial *nifH* phylotype, *PLoS One*, *10*(6), doi:10.1371/journal.pone.0128912.

Langlois, R. J., J. LaRoche, and P. A. Raab (2005), Diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean, *Appl. Environ. Microbiol.*, *71*(12), 7910–7919, doi:10.1128/AEM.71.12.7910.

LaRoche, J., and E. Breitbarth (2005), Importance of the diazotrophs as a source of new nitrogen in the ocean, *J. Sea Res.*, *53*(1–2), 67–91, doi:10.1016/j.seares.2004.05.005.

Luo, Y.-W., et al. (2012), Database of diazotrophs in global ocean: Abundance, biomass and nitrogen fixation rates, *Earth Syst. Sci. Data*, *4*(1), 47–73, doi:10.5194/essd-4-47-2012.

Messer, L. F., C. Mahaffey, C. M. Robinson, T. C. Jeffries, K. G. Baker, J. Bibiloni Isaksson, M. Ostrowski, M. A. Doblin, M. V. Brown, and J. R. Seymour (2015), High levels of heterogeneity in diazotroph diversity and activity within a putative hotspot for marine nitrogen fixation, *ISME J.*, *10*(6), 1–15, doi:10.1038/ismej.2015.205.

Mohr, W., T. Großkopf, D. W. R. Wallace, J. LaRoche, and T. Grosskopf (2010), Methodological underestimation of oceanic nitrogen fixation rates, *PLoS One*, *5*(9), e12583, doi:10.1371/journal.pone.0012583.

Moisander, P. H., R. A. Beinart, M. Voss, and J. P. Zehr (2008), Diversity and abundance of diazotrophic microorganisms in the South China Sea during intermonsoon, *ISME J.*, *2*, 954–967, doi:10.1038/ismej.2008.84.

Moisander, P. H., R. A. Beinart, I. Hewson, A. E. White, K. S. Johnson, C. A. Carlson, J. P. Montoya, and J. P. Zehr (2010), Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain, *Science*, *327*(5972), 1512–1514, doi:10.1126/science.1185468.

Moisander, P. H., T. Serros, R. W. Paerl, R. A. Beinart, and J. P. Zehr (2014), Gammaproteobacterial diazotrophs and *nifH* gene expression in surface waters of the South Pacific Ocean, *ISME J.*, *8*(10), 1962–1973, doi:10.1038/ismej.2014.49.

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

Moore, J. K., et al. (2013), Processes and patterns of nutrient limitation, *Nat. Geosci.*, *6*, 1–10, doi:10.1038/NGEO1765.

Mulholland, M., D. Bronk, and D. Capone (2004), Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101, *Aquat. Microb. Ecol.*, *37*, 85–94, doi:10.3354/ame037085.

Mulholland, M. R., P. W. Bernhardt, C. A. Heil, D. A. Bronk, and J. M. O'Neil (2006), Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico, *Limnol. Oceanogr.*, *51*(4), 1762–1776, doi:10.4319/lo.2006.51.4.1762.

Mulholland, M. R., P. W. Bernhardt, I. Ozmon, L. A. Procise, M. Garrett, J. M. O'Neil, C. A. Heil, and D. A. Bronk (2014), Contribution of diazotrophy to nitrogen inputs supporting *Karenia brevis* blooms in the Gulf of Mexico, *Harmful Algae*, *38*, 20–29, doi:10.1016/j.hal.2014.04.004.

Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Quéguiner (1995), Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation, *Global Biogeochem. Cycles*, *9*, 359–372, doi:10.1029/95GB01070.

Pujo-Pay, M., and P. Raimbault (1994), Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters, *Mar. Ecol. Prog. Ser.*, *105*, 203–207, doi:10.3354/meps105203.

Raimbault, P., and N. Garcia (2008), Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: Impact on new and export production, *Biogeosciences*, *5*, 323–338.

Ridame, C., C. Guieu, and S. L'Helguen (2013), Strong stimulation of N_2 fixation in oligotrophic Mediterranean Sea: Results from dust addition in large in situ mesocosms, *Biogeosciences*, *10*(11), 7333–7346, doi:10.5194/bg-10-7333-2013.

Riemann, L., H. Farnelid, and G. Steward (2010), Nitrogenase genes in non-cyanobacterial plankton: Prevalence, diversity and regulation in marine waters, *Aquat. Microb. Ecol.*, *61*(3), 235–247, doi:10.3354/ame01431.

Shiozaki, T., T. Kodama, S. Kitajima, M. Sato, and K. Furuya (2013), Advective transport of diazotrophs and importance of their nitrogen fixation on new and primary production in the western Pacific warm pool, *Limnol. Oceanogr.*, *58*(1), 49–60, doi:10.4319/lo.2013.58.1.0049.

Shiozaki, T., T. Kodama, and K. Furuya (2014), Large-scale impact of the island mass effect through nitrogen fixation in the western South Pacific Ocean, *Geophys. Res. Lett.*, *41*, 2907–2913, doi:10.1002/2014GL059835.

Shiozaki, T., T. Nagata, M. Ijichi, and K. Furuya (2015), Nitrogen fixation and the diazotroph community in the temperate coastal region of the northwestern North Pacific, *Biogeosciences*, *12*(15), 4751–4764, doi:10.5194/bg-12-4751-2015.

Slawyk, G., and P. Raimbault (1995), Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ^{15}N-tracer experiments and improving the isotopic mass balance, *Mar. Ecol. Prog. Ser.*, *124*, 289–299, doi:10.3354/meps124289.

Sohm, J. A., E. A. Webb, and D. G. Capone (2011), Emerging patterns of marine nitrogen fixation, *Nat. Rev. Microbiol.*, *9*(7), 499–508, doi:10.1038/nrmicro2594.

Subramaniam, A., et al. (2008), Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean, *Proc. Natl. Acad. Sci.*, *105*, 10,460–10,465, doi:10.1073/pnas.0710279105.

Thompson, A., B. J. Carter, K. Turk-Kubo, F. Malfatti, F. Azam, and J. P. Zehr (2014), Genetic diversity of the unicellular nitrogen-fixing cyanobacteria UCYN-A and its prymnesiophyte host, *Environ. Microbiol.*, doi:10.1111/1462-2920.12490, 10.1111/1462-2920.12490.

Tyrrell, T. (1999), The relative influences of nitrogen and phosphorus on oceanic primary production, *Nature*, *400*(6744), 525–531, doi:10.1038/22941.

Ward, B. B., and M. M. Jensen (2014), The microbial nitrogen cycle, *Front. Microbiol.*, *5*, doi:10.3389/fmicb.2014.00553.

Wilson, S. T., D. Böttjer, M. J. Church, and D. M. Karl (2012), Comparative assessment of nitrogen fixation methodologies, conducted in the oligotrophic North Pacific Ocean, *Appl. Environ. Microbiol.*, *78*(18), 6516–6523, doi:10.1128/AEM.01146-12.

Yeung, L. Y., et al. (2012), Impact of diatom-diazotroph associations on carbon export in the Amazon River plume, *Geophys. Res. Lett.*, *39*, L18609, doi:10.1029/2012GL053356.

Yool, A., A. P. Martin, C. Fernández, and D. R. Clark (2007), The significance of nitrification for oceanic new production, *Nature*, *447*(7147), 999–1002, doi:10.1038/nature05885.

Zehr, J. P., and R. M. Kudela (2011), Nitrogen cycle of the open ocean: From genes to ecosystems, *Annu. Rev. Mar. Sci.*, *3*(1), 197–225, doi:10.1146/annurev-marine-120709-142819.