



HAL
open science

Evidence of active dinitrogen fixation in surface waters of the eastern tropical South Pacific during El Niño and La Niña events and evaluation of its potential nutrient controls

J. Dekaezemacker, Sophie Bonnet, O. Grosso, T. Moutin, M. Bressac, D.G. Capone

► To cite this version:

J. Dekaezemacker, Sophie Bonnet, O. Grosso, T. Moutin, M. Bressac, et al.. Evidence of active dinitrogen fixation in surface waters of the eastern tropical South Pacific during El Niño and La Niña events and evaluation of its potential nutrient controls. *Global Biogeochemical Cycles*, 2013, 27 (3), pp.768-779. 10.1002/gbc.20063 . hal-02163842

HAL Id: hal-02163842

<https://hal.science/hal-02163842>

Submitted on 15 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Evidence of active dinitrogen fixation in surface waters of the eastern tropical South Pacific during El Niño and La Niña events and evaluation of its potential nutrient controls

J. Dekaezemacker,^{1,2} S. Bonnet,¹ O. Grosso,¹ T. Moutin,¹ M. Bressac,^{3,4} and D.G. Capone⁵

Received 2 November 2012; revised 19 June 2013; accepted 8 July 2013; published 18 August 2013.

[1] Biological N₂ fixation rates were quantified in the Eastern Tropical South Pacific (ETSP) during both El Niño (February 2010) and La Niña (March–April 2011) conditions, and from Low-Nutrient, Low-Chlorophyll (20°S) to High-Nutrient, Low-Chlorophyll (HNLC) (10°S) conditions. N₂ fixation was detected at all stations with rates ranging from 0.01 to 0.88 nmol N L⁻¹ d⁻¹, with higher rates measured during El Niño conditions compared to La Niña. High N₂ fixations rates were reported at northern stations (HNLC conditions) at the oxycline and in the oxygen minimum zone (OMZ), despite nitrate concentrations up to 30 μmol L⁻¹, indicating that inputs of new N can occur in parallel with N loss processes in OMZs. Water-column integrated N₂ fixation rates ranged from 4 to 53 μmol N m⁻² d⁻¹ at northern stations, and from 0 to 148 μmol m⁻² d⁻¹ at southern stations, which are of the same order of magnitude as N₂ fixation rates measured in the oligotrophic ocean. N₂ fixation rates responded significantly to Fe and organic carbon additions in the surface HNLC waters, and surprisingly by concomitant Fe and N additions in surface waters at the edge of the subtropical gyre. Recent studies have highlighted the predominance of heterotrophic diazotrophs in this area, and we hypothesize that N₂ fixation could be directly limited by inorganic nutrient availability, or indirectly through the stimulation of primary production and the subsequent excretion of dissolved organic matter and/or the formation of micro-environments favorable for heterotrophic N₂ fixation.

Citation: Dekaezemacker, J., S. Bonnet, O. Grosso, T. Moutin, M. Bressac, and D. G. Capone (2013), Evidence of active dinitrogen fixation in surface waters of the eastern tropical South Pacific during El Niño and La Niña events and evaluation of its potential nutrient controls, *Global Biogeochem. Cycles*, 27, 768–779, doi:10.1002/gbc.20063.

1. Introduction

[2] Nitrogen (N) is an essential macronutrient for marine productivity [Falkowski *et al.*, 1998], and most of the surface ocean is depleted in dissolved inorganic N (DIN). In these areas, planktonic N₂ fixing organisms referred to as “diazotrophs” may have an ecological advantage because they are able to reduce dissolved N₂ gas to ammonia (NH₃) and assimilate it, alleviating their need for another external source of N. N₂ fixation represents one of the major sources

of new N to the surface oligotrophic ocean [Capone *et al.*, 2005]. N₂ fixation is thought to primarily occur in warm (>24°C) [Breitbarth *et al.*, 2007; Webb *et al.*, 2009] and N-depleted oligotrophic tropical and subtropical areas of the ocean [Karl *et al.*, 1992; Capone *et al.*, 1997; Karl and Letelier, 2008].

[3] The reverse processes that remove N from the ocean, denitrification [Goering, 1968] and anammox [Kuypers *et al.*, 2003], primarily occur in oxygen-deficient sediments and, to a lesser extent, in the water column of oxygen minimum zones (OMZs). Biogeochemical modeling [Deutsch *et al.*, 2007] and remote sensing [Westberry and Siegel, 2006] studies have recently predicted that N₂ fixation might occur at significant rates in surface waters overlying regions of N losses such as the Eastern Tropical South Pacific (ETSP). Biological N₂ fixation has been poorly investigated in OMZs in general and in the Eastern South Pacific [Luo *et al.*, 2012] in particular. The paucity of observations and the few direct measurements of N₂ fixation rates in this region [Raimbault and Garcia, 2008; Moutin *et al.*, 2008; Fernandez *et al.*, 2011] make it difficult to draw conclusions concerning the biogeochemical importance of diazotrophy in the ETSP, which motivated this work.

[4] The N budget for the global ocean is poorly constrained [Codispoti *et al.*, 2001; Brandes and Devol, 2002; Codispoti,

Additional supporting information may be found in the online version of this article.

¹Aix Marseille Université, CNRS/INSU, IRD, Mediterranean Institute of Oceanography, Marseille, France.

²Now at Max Planck Institute for Marine Microbiology, Bremen, Germany.

³ACRI-ST, Sophia-Antipolis, France.

⁴CNRS-INSU, Université Pierre et Marie Curie-Paris 6, Laboratoire d’Océanographie de Villefranche/Mer, Observatoire Océanologique, Villefranche-sur-Mer, France.

⁵Department of Marine and Environmental Biology, University of Southern California, Los Angeles, California, USA.

Corresponding author: J. Dekaezemacker, Max Planck Institute for Marine Microbiology, DE-28195 Bremen, Germany. (jdekaeze@mpi-bremen.de)

©2013. American Geophysical Union. All Rights Reserved.
0886-6236/13/10.1002/gbc.20063

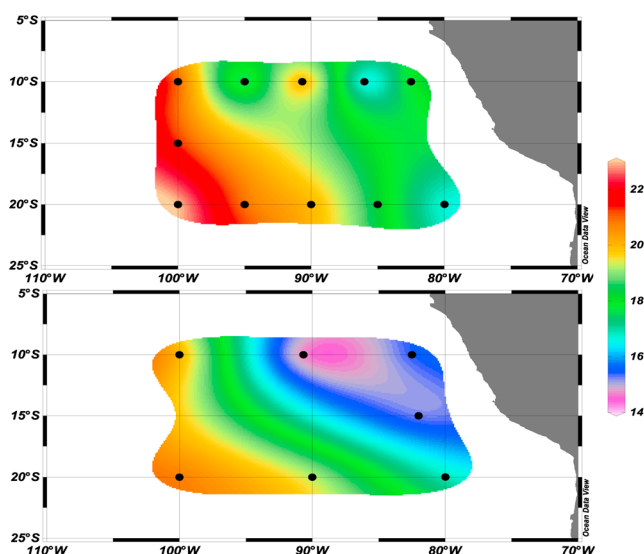


Figure 1. Location of stations during the cruises in (a) February 2010–El Niño conditions and in (b) March–April 2011–La Niña conditions on a map of seawater temperature ($^{\circ}\text{C}$) at 75 m depth.

2007], partly because most in situ studies on N₂ fixation are performed on cyanobacterial diazotrophs in N-depleted warm areas [Capone *et al.*, 1997; Zehr *et al.*, 2001]. Another explanation is that potential N₂ fixation fluxes attributed to other prokaryotes and/or in N-rich waters are not included in global N budgets.

[5] The ETSP is an interesting case study for studying N₂ fixation as it is composed of contrasting biogeochemical provinces. In addition, atmospheric iron deposition to this ocean area is amongst the lowest in the world [Jickells *et al.*, 2005], and Fe availability appears to be limiting for primary production in the region [Bonnet *et al.*, 2008]. Due to the high Fe requirements for nitrogenase [Berman-Frank *et al.*, 2001; Kuska *et al.*, 2003a, 2003b], it is also suspected to control N₂ fixation [Paerl *et al.*, 1994; Mills *et al.*, 2004; Saito *et al.*, 2011], but this process and its controlling factors have been very poorly studied in this area.

[6] Finally, the El Niño–Southern Oscillation (ENSO) subjects the ETSP to interannual climate variability, which impacts the strength of the upwelling and modifies the biogeochemical functioning of this ecosystem. During El Niño events, marine productivity usually decreases [Arntz *et al.*, 1988] compared to “normal” years due to the weaker upwelling of nutrient-rich waters, and waters are warmer than usual. In contrast, during La Niña years, the upwelling is stronger than “normal” years, leading to colder waters and higher primary productivity [Behrenfeld *et al.*, 2001]. The effect of this climatic interannual variability on N₂ fixation has never been studied in the ETSP.

[7] We performed two cruises in the ETSP during both El Niño and La Niña conditions and measured N₂ fixation rates along a 5700 km transect exhibiting strong oxygen and nutrient gradients. The objectives of this study were (1) to quantify N₂ fixation rates across those gradients during contrasted climatic and therefore upwelling conditions, and (2) to determine which nutrients control N₂ fixation rates in surface waters.

2. Material and Methods

[8] Two research cruises took place in the ETSP in February 2010 and in March–April 2011. The 2010 cruise was carried out onboard the R/V Atlantis (Woods Hole Oceanographic Institution) during an El Niño event (Multivariate ENSO index: 1.52, developed at NOAA’s Climate Diagnostics Center and computed by Wolter and Timlin [1993, 1998], taken from the web site <http://www.esrl.noaa.gov/psd/enso/mei.table.html>), and the 2011 cruise was performed onboard the R/V Melville (Scripps Institution of Oceanography) during a La Niña event (Multivariate ENSO index: -1.49). The southern transect (stations 1 to 5) started at 20°S, 80°W and proceeded along 20°S to 100°W (Figure 1) in Low-Nutrient, Low-Chlorophyll waters associated with the South Pacific Gyre [Claustre and Maritorea, 2003]. Surface waters exhibited nitrate (NO_3^-) concentrations close to detection limit, which is known to be favorable for N₂ fixation. The northern transect (including stations 7 to 11, Figure 1) started at 10°S, 100°W and extended along 10°S to 82.5°W, in upwelled rich waters. These conditions create High-Nutrient, Low-Chlorophyll (HNLC) conditions in surface [Martin *et al.*, 1994; Blain *et al.*, 2008] with relatively high phosphate (PO_4^{3-}) and NO_3^- concentrations (Table 1) and an OMZ at depth [Ulloa and Pantoja, 2009]. Experiments were performed at 11 stations in 2010 and at 6 stations in 2011 (Figure 1 and Table 1).

2.1. Sampling Procedures

2.1.1. Vertical Profiles

[9] Seawater was sampled using a CTD-rosette equipped with 12 L Niskin bottles. During the 2010 cruise, individual samples for N₂ fixation rate determination were collected in the euphotic zone at 6 depths between the upper 10 m and 200 m for the southern transect, and within the upper 150 m for the northern transect. During the 2011 cruise, triplicate samples were collected at 4 or 5 depths within and just below the euphotic zone. N₂ fixation rates ($\text{nmol L}^{-1} \text{d}^{-1}$) were determined according to Montoya *et al.* [1996] (further details are given in the supporting information). However, the method to measure N₂ fixation is currently in debate, and it has been noted that the method we used may underestimate rates due to incomplete equilibration of $^{15}\text{N}_2$ gas in the water [Mohr *et al.*, 2010]. Therefore, the results presented in the present study should be considered as minimum rates, and in the context of the unbalanced N budget [Codispoti *et al.*, 2001; Brandes and Devol, 2002; Codispoti, 2007], they prove that N₂ fixation can occur in unexpected areas of the Ocean.

[10] At some stations on the northern transect, the OMZ was shallow. For samples from the hypoxic-anoxic depths, care was made to avoid O₂ contamination and to perform incubations under strict anoxic conditions as described in Hamersley *et al.* [2011]. Bottles were filled with milli-Q water, then flushed with Argon and filled with the seawater sample by tubing into the bottom of the Argon-filled bottles to minimize aeration.

[11] At each depth, samples for DIN ($\text{NO}_3^- + \text{NO}_2^-$) and PO_4^{3-} concentrations determination were collected in acid-washed 20 mL polyethylene flasks, immediately poisoned with HgCl_2 (i.e., final concentration of $20 \mu\text{g mL}^{-1}$) [Kirkwood, 1992] and stored at 4°C until analysis.

Table 1. Initial Characteristics for the Nutrient Enrichment Experiments^a

Latitude Longitude	Station 1		Station 2		Station 3		Station 4		Station 5		Station 6	
	19.99°S	79.98°W	20°S	85°W	20°S	90°W	20°S	95°W	20°S	100°W	15°S	100°W
	2010 (Atlantis)	2011 (Melville)	2010 (Atlantis)		2010 (Atlantis)		2010 (Atlantis)		2010 (Atlantis)		2010 (Atlantis)	
NO ₃ ⁻ (μmol L ⁻¹)	0.10±0.08	0.08±0.05	<0.08		0.88±0.12		1.36±0.22		0.08±0.08			2.00±0.23
PO ₄ ³⁻ (μmol L ⁻¹)	0.44±0.04	0.46±0.07	0.38±0.04		0.42±0.04		0.42±0.04		0.39±0.04			0.60±0.04
DFe (nmol L ⁻¹)	0.16±0.005	1.57±0.09	0.14±0.003		0.16±0.03		0.16±0.01		0.15±0.03			0.18±0.03
P*, Fe/N = 1/16 (μmol L ⁻¹)	0.44	0.45	0.38		0.37		0.33		0.39			0.47
Fe*, Fe/P = 0.47 (nmol L ⁻¹)	-0.05	1.35	-0.04		-0.04		-0.03		-0.03			-0.10
N ₂ Fixation (nmol L ⁻¹ d ⁻¹)	0.74±0.11	0	0		0		0		0.23±0.06			0
	station 7		station 8		station 9		station 10		station 11		station 12	
	10°S	100°W	10°S	95°W	10°S	90.67°W	10°S	86°W	10°S	82.5°W	15°S	82°W
	2010 (Atlantis)	2011 (Melville)	2010 (Atlantis)		2011 (Melville)		2010 (Atlantis)		2011 (Melville)		2011 (La Nina)	
NO ₃ ⁻ (μmol L ⁻¹)	5.60±0.60	6.73±0.69	5.40±0.60		<0.06	6.98±0.556	1.88±0.20		0.36±0.07			0.37±0.07
PO ₄ ³⁻ (μmol L ⁻¹)	0.71±0.05	0.67±0.05	0.65±0.05		0.43±0.04	0.67±0.06	0.75±0.05		0.36±0.04			0.49±0.03
DFe (nmol L ⁻¹)	0.14±0.04	1.56±0.05	0.15±0.05		0.13±0.002	1.81±0.36	0.26±0.11		0.17±0.02			1.95±0.48
P*, Fe/N = 1/16 (μmol L ⁻¹)	0.36	0.25	0.31		0.42	0.23	0.63		0.34			0.47
Fe*, Fe/P = 0.47 (nmol L ⁻¹)	-0.20	1.24	-0.15		-0.07	1.50	-0.09		0.003			1.71
N ₂ Fixation (nmol L ⁻¹ d ⁻¹)	0	0	0		0	0.59±0.48	0		0.13±0.05			0.05±0.01

^an.a., not available.

2.1.2. Nutrient Sensitivity Assays of N₂ Fixation in Euphotic Zone

[12] All N₂ fixation sensitivity assays were performed under strict trace metal clean conditions [Bruland *et al.*, 1979]. Seawater was sampled at ~15 m depth using a trace metal-clean Teflon pump system connected to a PVC tube. The 4.5 L bottles (washed with trace metal grade acid) were rinsed and filled with 200 μm-prefiltered seawater. In a laminar flow hood, the bottles were then amended with individual nutrients or in combination: +Fe (at all the stations of both cruises), +N (or +FeN), +P, and +Glucose (Glc), (at three stations of the 2010 cruise) to reach final concentrations of 4 nmol L⁻¹ FeCl₃, 4 μmol L⁻¹ NaNO₃ (99.99% Suprapur, Merck), 1 μmol L⁻¹ NaH₂PO₄ (99.99% Suprapur, Merck), and 10 μmol L⁻¹ chelexed Glucose (Chelex®100 Molecular Biology Grade Resine 200–400 Mesh, Sodium Form, BioRad, activated using HCl trace metal grade, Fisher Scientific and NaOH; neither HNO₃ nor NH₄OH was used to avoid N contaminations, which could affect N₂ fixation). Each nutrient amendment was performed in triplicates, and triplicate bottles were kept unamended as controls. Bottles were then incubated at 50% ambient light in an on-deck incubator with circulating surface seawater. After 24 h, all bottles were spiked with stable isotopes (¹⁵N₂), and incubated under the same conditions for another 24 h. After incubation, the three replicates of each treatment were used in order to measure N₂ fixation rates and nutrient concentrations. Nutrient concentrations were also measured just after the fertilization in order to confirm the nutrient additions (data not shown).

[13] Samples were also collected at time zero (T0) at the depth of the experiments in order to characterize initial biogeochemical conditions at every station (Table 1). N₂ fixation and macronutrient samples were collected as described above. For dissolved iron (DFe) concentrations, samples were collected in triplicates using the Teflon pump by in-line filtration performed through a 0.2 μm cartridge (Sartorius Sartrobran-P-capsule 0.45 μm prefilter and 0.2 μm final filter) and immediately acidified to pH < 2 with ultrapure HCl (Ultrapur, Merck).

2.2. Analyses

2.2.1. Mass Spectrometry

[14] The isotopic enrichment analyses were performed by continuous flow isotope ratio mass spectrometry using an Integra-CN mass spectrometer using the procedure described in Bonnet *et al.* [2011]. The accuracy of the system was verified regularly using reference material (International Atomic Energy Agency (IAEA), Analytical Quality Control Services). The isotopic enrichment was calibrated using IAEA reference material (IAEA-N-1) every 10–15 samples. The linearity of ¹⁵N atom % as a function of increasing particulate nitrogen mass was verified on both natural and ¹⁵N enriched material since it is critical, especially for samples from ultra-oligotrophic environments. ¹⁵N atom % was linear (Fisher test, p < 0.01) between 0.20 and 39 μmol N, which is within the range of particulate nitrogen measured in all of our 4.5 L incubations (minimum quantities of N per sample varied from 0.21 to 0.66 and maximum varied from 1.68 to 8.68 μmol N, depending on the station). Quantification limits for N₂ fixation rates were 0.01 nmol L⁻¹ d⁻¹. N₂ fixation

Southern transect

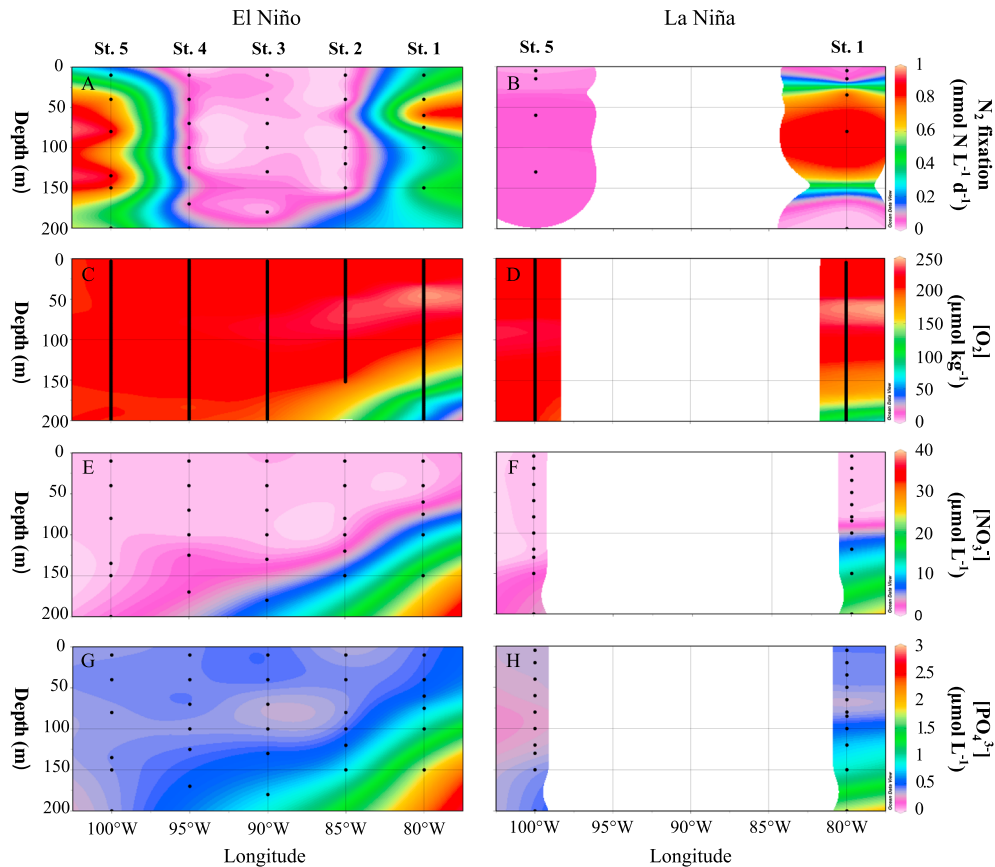


Figure 2. Depth distribution (a and b) N₂ fixation (nmol L⁻¹ d⁻¹), (c and d) O₂ concentrations (µmol kg⁻¹), and (e and f) NO₃⁻ and (g and h) PO₄³⁻ concentrations (µmol L⁻¹) along the southern transect (20°S) between El Niño year (Figures 2a, 2c, 2e, and 2g) and La Niña year (Figures 2b, 2d, 2f, and 2h).

measurements were depth-integrated between 0 and 150 or 200 m in order to determine areal rates (µmol m⁻² d⁻¹).

2.2.2. Macronutrients, Dissolved Fe Analyses, and Biogeochemical Tracers

[15] DIN (NO_x = NO₃⁻ + NO₂⁻) and PO₄³⁻ samples were analyzed using an AutoAnalyzer 3 Digital Colorimeter (Bran Luebbe) according to standard automated colorimetric methods [Aminot and Kerouel, 2007]. The respective lower detection limits were 5 and 9 nmol L⁻¹. For better readability, the sum of the NO₃⁻ and NO₂⁻ will hereafter be referred to as NO₃⁻.

[16] Dissolved Fe analyses were performed in a clean room by flow injection with online preconcentration and chemiluminescence detection (FIA-CL) according to Bonnet and Guieu [2006]. The mean detection limit was 4 pmol L⁻¹, and the mean blank was 0.07 ± 0.01 nmol L⁻¹. The calibration curve has been realized by using 0.2 µm filtered DFe-poor water, enriched with a standard solution of Fe (III), with at least five points. For each run of analyses, the precision and the stability of the measurements have been controlled with an internal standard, but also with SAFe-D1 and SAFe-D2 standards. The reliability of the method was assessed by analyzing the SAFe-D1 (0.676 ± 0.059 nM; consensus value = 0.65 ± 0.01 nM) and D2 (0.937 ± 0.029 nM; consensus value = 0.923 ± 0.029 nM).

[17] Based on these concentrations measurements and on the common stoichiometry of nutrient needs for phytoplankton (N:P ratio = 16:1) [Redfield et al., 1963], two tracers were considered in order to describe the biogeochemical environment before nutrient additions.

[18] The first one was P*, defined as P* = [PO₄³⁻] - [NO₃⁻] / 16 [Deutsch et al., 2007]. P* informs about the relative changes of NO₃⁻ and PO₄³⁻ concentrations in oceanic waters. Decreases in surface ocean PO₄³⁻ that are unaccompanied by concomitant Redfield-ratio decreases in NO₃⁻ (P* decreases) are interpreted as the result of N₂ fixation.

[19] The second one was the Fe*, defined as Fe* = [DFe] - 0.47 mmol mol⁻¹ × [PO₄³⁻] which determines the possible degree of Fe limitation [Parekh et al., 2005] assuming a fixed Fe:P ratio of 0.47 mmol mol⁻¹ during uptake, export and remineralization [Anderson and Sarmiento, 1994].

2.2.3. Uncertainties and Statistics

[20] For nutrient concentrations, uncertainties were calculated using partial derivation as propagation of uncertainties [Hydes et al., 2010]. The expanded measurement uncertainty was used, with a coverage factor k = 2 (i.e., confidence interval of 95%). Uncertainties were calculated as the standard deviation calculated for triplicates assays for N₂ fixation rates from the nutrient enrichment experiments and from the profiles of the 2011 cruise.

Northern transect

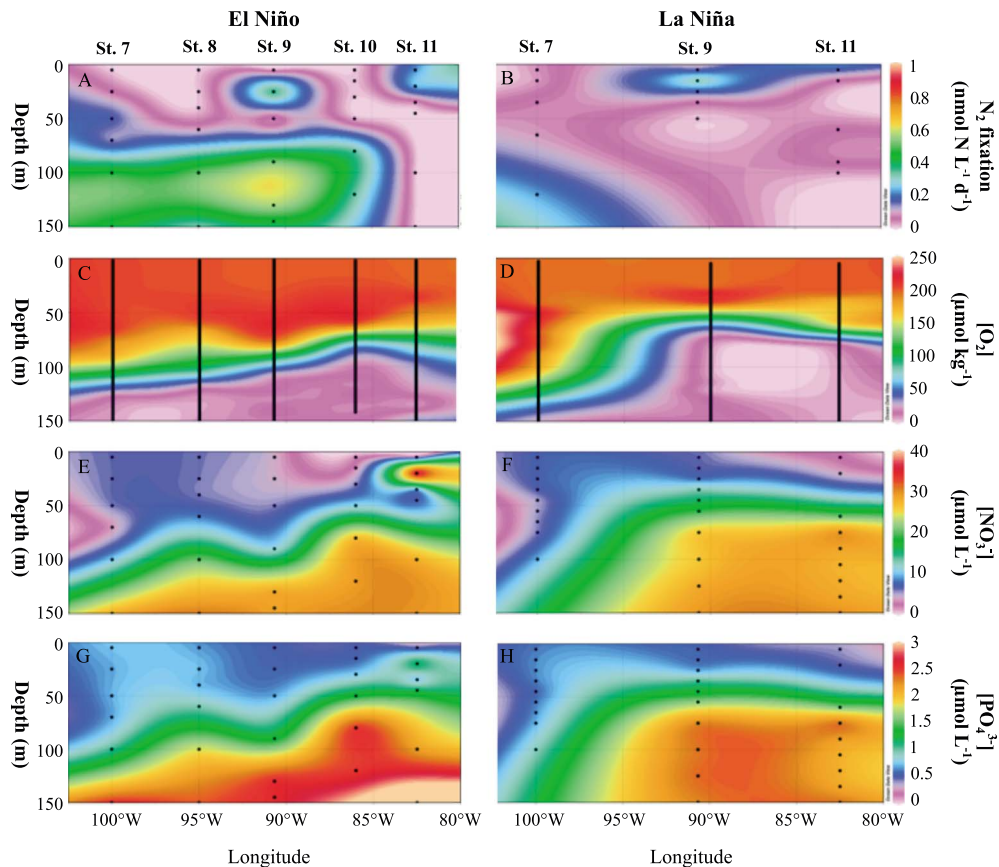


Figure 3. Depth distribution (a and b) N₂ fixation (nmol L⁻¹ d⁻¹), (c and d) O₂ concentrations (μmol kg⁻¹), and (e and f) NO₃⁻ and (g and h) PO₄³⁻ concentrations (μmol L⁻¹) along the northern transect (10°S) between El Niño year (Figures 3a, 3c, 3e, and 3g) and La Niña year (Figures 3b, 3d, 3f, and 3h).

[21] To compare the effect of nutrient addition on N₂ fixation rates, we calculated the relative change (%) for each variable as $100 \times (E - C)/C$, where E and C are the mean value of the variable in the enrichment and the control treatments, respectively. For each variable, we calculated the standard deviation of the relative change by propagating the standard deviation of the measurements in both conditions. The differences between treatments for each variable were analyzed using the non-parametric Mann-Whitney, one-tailed test.

3. Results

3.1. In Situ N₂ Fixation Rates and Biogeochemical Conditions in the ETSP

3.1.1. N₂ Fixation Rates During the 2010 Cruise

[22] N₂ fixation rates across the 20°S transect were highest at the western and eastern ends of the transect (Figure 2a). At station 1 (80°W), a maximum value of 0.80 nmol N L⁻¹ d⁻¹ was measured at 60 m depth, and at station 5, at the edge of the subtropical gyre (100°W), a rate of 0.88 nmol N L⁻¹ d⁻¹ was measured between 80 and 135 m depth. The three stations between 85 and 95°W exhibited rates <0.06 nmol N L⁻¹ d⁻¹ (Figure 2a). The water column of the southern transect was well oxygenated (Figure 2c) with O₂ concentrations > 190 μmol kg⁻¹, except at station 1 below 120 m, where O₂

concentrations decreased with depth to reach a minimum value of 60 μmol O₂ kg⁻¹ at 200 m depth. Surface NO₃⁻ concentrations varied from 0.13 ± 0.06 μmol L⁻¹ at stations 2 and 4 to 0.39 ± 0.07 μmol L⁻¹ at station 3 (Figure 2e). Surface PO₄³⁻ concentrations varied from 0.35 ± 0.04 μmol L⁻¹ at station 2 to 0.44 ± 0.04 μmol L⁻¹ at stations 1 and 3 (Figure 2g). NO₃⁻ and PO₄³⁻ concentrations increased with depth to 20.8 ± 2.1 μmol L⁻¹ and 1.79 ± 0.09 μmol L⁻¹ for NO₃⁻ and PO₄³⁻ concentrations at 150 m at Station 1, respectively. The depth of the nutricline shoaled to the east.

[23] On the northern transect (10°S), N₂ fixation rates were maximum (0.08–0.57 nmol N L⁻¹ d⁻¹) between 50 m and 150 m at all stations (from 85 to 100°W), except the one close to the coast (80°W), where the highest rate over the vertical was measured at 20 m (0.27 nmol N L⁻¹ d⁻¹) (Figure 3a). At stations 9 (90°W) and 11 (82.5°W), measurable rates (0.40 and 0.27 nmol N L⁻¹ d⁻¹) were also detected shallower, at 20 and 25 m depth, respectively (Figure 3a). Surface waters were well oxygenated, and O₂ concentrations decreased with depth, with a shallower oxycline shoaling eastward; suboxic conditions ([O₂] < 20 μmol kg⁻¹, *Paulmier and Ruiz-Pino* [2008]) were reached at 124, 117, 109, 95, and 116 m depth, respectively, for stations 7, 8, 9, 10, and 11 (Figure 3c). Surface nutrients exhibited a strong gradient with NO₃⁻ concentrations varying from 5.89 ± 0.64

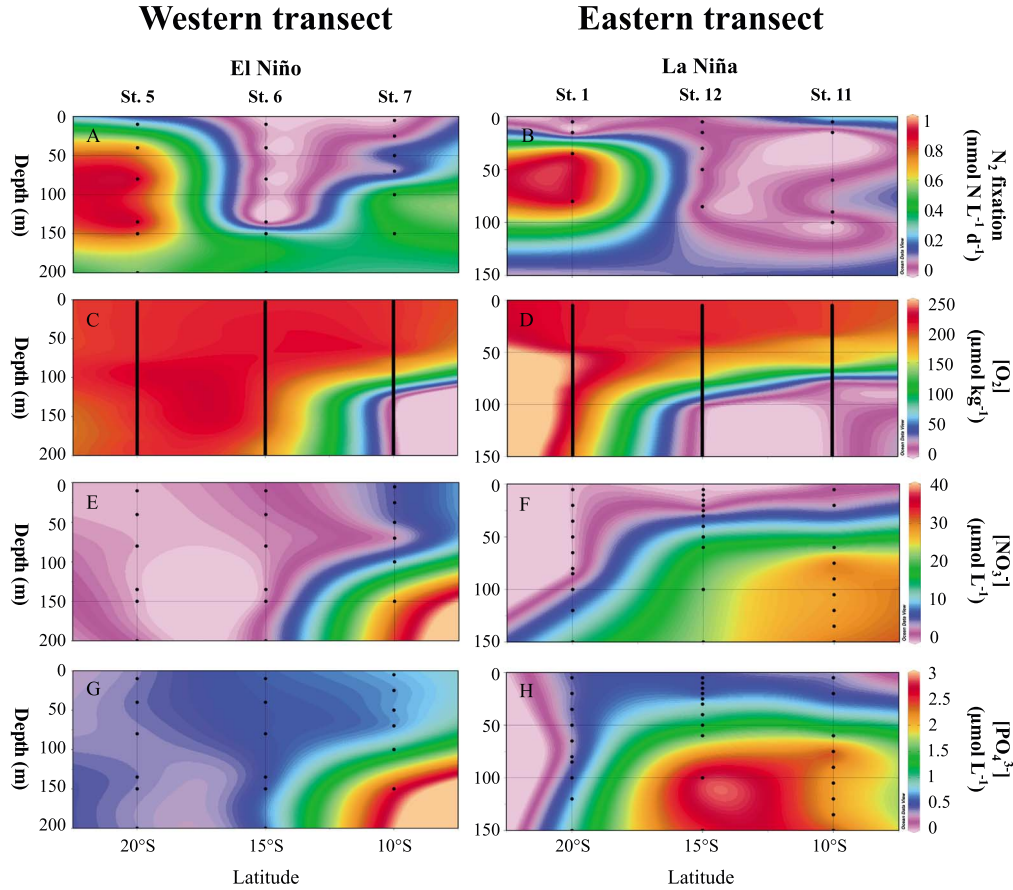


Figure 4. Depth distribution (a and b) N₂ fixation (nmol L⁻¹ d⁻¹), (c and d) O₂ concentrations (μmol kg⁻¹), and (e and f) NO₃⁻ and PO₄³⁻ concentrations (μmol L⁻¹) along the western transect (100°W) in El Niño year (Figures 4a, 4c, 4e, and 4g) and along the eastern transect (82.5°W) in La Niña year (Figures 4b, 4d, 4f, and 4h).

to 0.46 ± 0.08 μmol L⁻¹ and PO₄³⁻ concentrations from 0.72 ± 0.05 to 0.38 ± 0.04 μmol L⁻¹, from the offshore station 7 (100°W) to the most coastal station 11 (82.5°W) (Figures 3e and 3g). NO₃⁻ and PO₄³⁻ concentrations increased with depth to reach values from 28.0 ± 2.8 (station 8) to 3.73 ± 3.1 (station 11) μmol L⁻¹, and from 2.41 ± 0.12 (station 8) to 3.73 ± 0.20 (station 11) μmol L⁻¹ at 150 m, respectively. At station 11, NO₃⁻ concentrations of 29.80 ± 2.97 μmol L⁻¹ and PO₄³⁻ concentrations of 3.73 ± 0.20 μmol L⁻¹ were measured at 150 m. The NO₃⁻ isocline 10 μmol L⁻¹ was at 100 m at station 7, between 50 and 90 m at station 9, and between 20 and 35 m at station 11.

[24] The station 6 (15°S 100°W, Figure 1) was characterized by N₂ fixation rates of 0.32 and 0.37 nmol N L⁻¹ d⁻¹ at 150 and 200 m depth (Figure 4a). O₂ concentrations were homogeneous and ≥ 75 μmol kg⁻¹ from the surface to 200 m (Figure 4c). Surface concentrations of NO₃⁻ and PO₄³⁻ were, respectively, 1.69 ± 0.28 μmol L⁻¹ (Figure 4e) and 0.58 ± 0.05 μmol L⁻¹ (Figure 4g) and increased with depth to reach 2.93 ± 0.35 μmol L⁻¹ and 0.61 ± 0.05 μmol L⁻¹ at 200 m.

3.1.2. N₂ Fixation Rates During the 2011 Cruise

[25] Only stations 1 and 5 were sampled along the southern transect. The highest N₂ fixation rates (0.87 nmol N L⁻¹ d⁻¹, Figure 2b) were measured at station 1 at 80 m. At station 5, rates were low (< 0.06 nmol N L⁻¹ d⁻¹) or undetectable over the vertical profile. The water column was still well

oxygenated (Figure 2d) with O₂ concentrations > 200 μmol kg⁻¹, except at station 1 below 135 m, where O₂ concentrations decreased with depth to reach 90 μmol kg⁻¹ at 200 m depth. Surface NO₃⁻ and PO₄³⁻ concentrations at station 1 were 0.12 ± 0.09 μmol L⁻¹ (Figure 2f) and 0.41 ± 0.04 μmol L⁻¹ (Figure 2h), respectively. Nutrient concentrations increased with depth to reach 22.7 ± 0.6 μmol L⁻¹ for NO₃⁻ and 1.79 ± 0.07 μmol L⁻¹ for PO₄³⁻ at 200 m. At station 5, surface nutrient concentrations were 0.06 ± 0.08 μmol L⁻¹ for NO₃⁻ (Figure 2f), and 0.32 ± 0.05 μmol L⁻¹ for PO₄³⁻ (Figure 2h). NO₃⁻ concentrations increased with depth up to 1.71 ± 0.39 μmol L⁻¹ at 150 m, but a minimum of 0.10 ± 0.06 μmol L⁻¹ was found at 120 m. PO₄³⁻ concentrations reached 0.37 ± 0.05 μmol L⁻¹ at 200 m.

[26] Across the northern transect, N₂ fixation rates varied from below the quantification limit to a maximum value of 0.59 ± 0.48 nmol L⁻¹ d⁻¹ at 15 m depth of station 9 (Figure 3b). The oxycline was shallow at the eastern end of the transect, and suboxic conditions were reached at 76, 38, and 36 m depth, respectively, at stations 7, 9, and 11 (Figure 3d). Surface NO₃⁻ and PO₄³⁻ concentrations varied from 6.77 ± 0.46 to 2.28 ± 0.28 μmol L⁻¹, and from 0.60 ± 0.04 to 0.39 ± 0.08 μmol L⁻¹ from the western to eastern ends of the transect (Figures 3f and 3h), respectively. The nutriclines were also shallower on the eastern part of the transect, and the NO₃⁻ isocline 10 μmol L⁻¹ was between 100

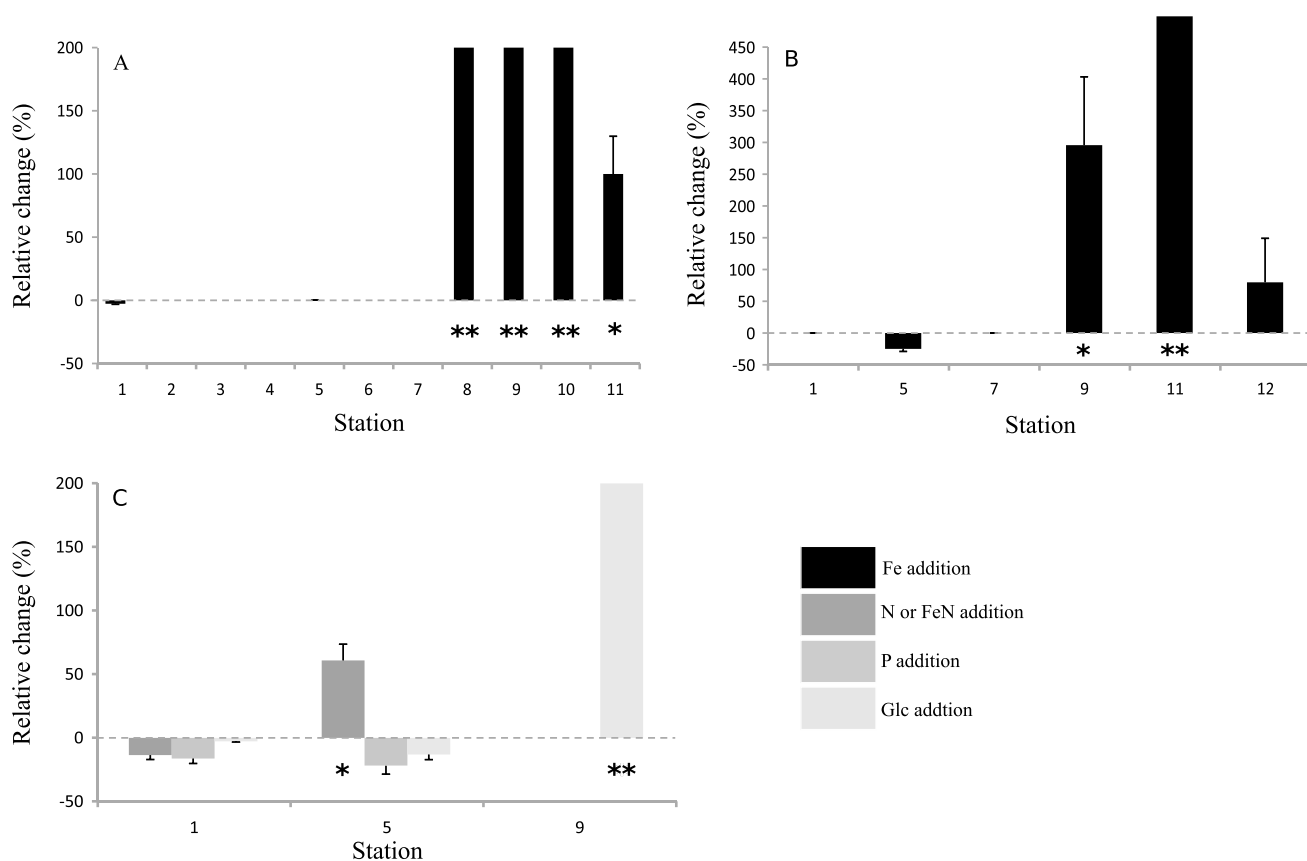


Figure 5. Relative change (%) in N₂ fixation in nutrient-amended bottles as compared with the control bottles for each station, after Fe addition in (a) 2010 and in (b) 2011, and after (c) N addition at station 1 and 9 or FeN addition at station 5 (dark gray), P addition (gray), Glc addition (light gray) in 2010. Relative change was calculated as $100 \times ([\text{mean in treatment} - \text{mean in control}] / \text{mean in control})$. Error bars indicate: 1 SD. Value of 200% (Figures 5a–5c) and 450% (Figure 5b) are arbitrarily chosen and double asterisks are added for showing appearance of N₂ fixation after nutrient addition. Simple or double asterisks denote the existence of significant differences in the mean between the control and the treatment bottles (Mann-Whitney test, one tailed; *: $p < 0.05$).

and 150 m at station 7, between 25 and 35 m at station 9 and between 20 and 60 m at station 11.

[27] At Station 12 (15°S 82.5°W, Figure 1), N₂ fixation rates varied from below the quantification limit at 85 m depth to 0.09 ± 0.04 nmol N L⁻¹ d⁻¹ at 30 m depth (Figure 4b). O₂ concentrations decreased below 39 m depth and reached suboxic conditions at 101 m depth (Figure 4d). Surface NO₃⁻ and PO₄³⁻ concentrations were 0.36 ± 0.07 μmol L⁻¹ (Figure 4f) and 0.52 ± 0.05 μmol L⁻¹ (Figure 4h), respectively, and increased to 22.7 ± 0.3 μmol L⁻¹ and 2.51 ± 0.06 μmol L⁻¹ at 150 m. At this station, NO₃⁻ concentrations were intermediate between those of stations 1 (20°S) and 11 (10°S). PO₄³⁻ concentrations were the highest of the transect at this station.

3.2. Nutrient Controls of N₂ Fixation

3.2.1. Initial Biogeochemical Conditions

[28] Experiments performed along the southern transect (stations 1 to 5) were conducted under in situ conditions characterized by low NO₃⁻ (<1.4 μmol L⁻¹) and PO₄³⁻ (<0.4 μmol L⁻¹) concentrations during both cruises (Table 1). In contrast, DFe conditions exhibited a clear temporal variability,

with concentrations 10 times higher during the La Niña conditions experienced on the 2011 cruise (1.57 nmol L⁻¹) compared to the El Niño conditions experienced on the 2010 cruise (0.14 to 0.16 nmol L⁻¹). The P* tracer was constant between the two years with value between 0.33 and 0.45 μmol L⁻¹. The Fe* was negative but close to 0 nmol L⁻¹ during the 2010 cruise and positive during the 2011 cruise.

[29] The biogeochemical conditions on the northern transect (stations 7 to 11) exhibited greater variability between the 2 years (Table 1). For example, surface NO₃⁻ concentrations were lower during the 2010 cruise than the 2011 cruise, i.e., ranging from detection limit to 5.60 μmol L⁻¹ in 2010, and from 0.37 to 6.73 μmol L⁻¹ in 2011, with decreasing concentrations from west to east. The PO₄³⁻ concentrations were high across this transect (from 0.36 ± 0.04 to 0.71 ± 0.05 μmol L⁻¹). DFe concentrations were also higher in 2011 compared to 2010. The P* tracer showed variations between stations and higher values (from 0.31 to 0.63 μmol L⁻¹) in 2010 compared to 2011 (from 0.23 to 0.25 μmol L⁻¹). The Fe* was negative or equal to zero during El Niño conditions (from -0.20 to 0 nmol L⁻¹) and positive during La Niña conditions (from 1.24 to 1.69 nmol L⁻¹).

Table 2. Range of Marine Areal Rates of N₂ Fixation in Contrasting Oceanic Environments

Location	Areal Rates	Integration Depth	Source
	($\mu\text{mol m}^{-2} \text{d}^{-1}$)	(m)	
Eastern North Pacific gyre	520	Mixed layer	<i>Montoya et al.</i> [2004]
North Atlantic	59–898	15 (<i>Trichodesmium</i>)	<i>Capone et al.</i> [2005]
Tropical Atlantic	4–255	100	<i>Voss et al.</i> [2004]
Equatorial Pacific	18–358		<i>Bonnet et al.</i> [2009]
ETSP Coastal OMZ	7–190	120	<i>Fernandez et al.</i> [2011]
ETSP Subtropical gyre	12–190	150–200	<i>Halm et al.</i> [2012]
ETSP 10°S transect 2010	6–53	120–150	This study
ETSP 20°S transect 2010	0–148	150–200	This study
ETSP 10°S transect 2011	4–13	50–120	This study
ETSP 20°S transect 2011	5–99	150–200	This study

3.2.2. N₂ Fixation During Nutrient Sensitivity Assays

[30] In unamended triplicate controls, N₂ fixation rates on the 2010 cruise were measurable at stations 1, 5, and 11 with respective rates of 0.74 ± 0.11 , 0.23 ± 0.06 , and 0.13 ± 0.05 nmol N L⁻¹ d⁻¹ (Table 1). After Fe additions, N₂ fixation was significantly (Mann-Whitney test, one-tailed, $p > 0.05$) stimulated at station 11 (Figure 5a, see also supporting information Table S1), with rates reaching 0.26 ± 0.03 nmol N L⁻¹ d⁻¹, corresponding to a $100 \pm 30\%$ increase. At stations 1 and 5 (Southern transect), N₂ fixation was not stimulated by Fe additions. At stations 2, 3, 4, 6, 7, 8, 9, and 10, N₂ fixation was not detectable (< 0.01 nmol N L⁻¹ d⁻¹) in control (unamended) treatments. After Fe additions, N₂ fixation was detectable at stations 8, 9, and 10, with respective rates of 0.53 ± 0.17 , 0.17 ± 0.01 , and 0.52 ± 0.20 nmol N L⁻¹ d⁻¹ (Figure 5a), and rates remained undetectable at stations 2, 3, 4, 6, and 7 (Figure 5a). Moreover, concomitant Fe and N additions significantly stimulated (Mann-Whitney test, one-tailed, $p > 0.05$) N₂ fixation rates at station 5 by $61 \pm 13\%$ (Figure 5c) with rates reaching 0.37 ± 0.02 nmol N L⁻¹ d⁻¹, while Fe alone did not stimulate N₂ fixation. At station 9, Glc additions resulted in appearance of measurable N₂ fixation with rates of 0.25 ± 0.09 nmol N L⁻¹ d⁻¹ (Figure 5c).

[31] On the 2011 cruise, N₂ fixation was measurable in control treatments at stations 5, 9, and 12 with rates of 0.02 ± 0.002 , 0.59 ± 0.48 and 0.05 ± 0.01 nmol N L⁻¹ d⁻¹ but not detected at stations 1, 7, and 11 (Table 1). Rates were significantly (Mann-Whitney test, $p < 0.05$) stimulated by Fe additions at station 9 by $296 \pm 108\%$ (Figure 5b), corresponding to rates of 2.34 ± 0.23 nmol N L⁻¹ d⁻¹. At station 11 (Figure 5b), N₂ fixation rates were undetectable, but after Fe additions, they reached 1.48 ± 0.32 nmol N L⁻¹ d⁻¹.

4. Discussion

[32] The positive P* tracer (Table 1) indicates that the ETSP ocean is a location of strong N losses with upwelled denitrified waters. However the decrease of P* (Table 1) between some stations (e.g., from station 1 to station 3 in 2010 or from station 9 to station 7 in 2011) indicates that N₂ fixation probably occurs in these waters, which is confirmed by our results. Finally, the high positive Fe* (Table 1) in 2011 indicates that there is enough iron to support the complete consumption of PO₄³⁻. Positive values of Fe* were also observed in the upwelling region of the ETSP [Blain et al., 2008]. Fe* tracer subtracts the contribution of remineralization of organic matter to DFe, and this region is known to receive the lowest aeolian

deposition in the world [Jickells et al., 2005]; we can thus assume that most of the iron came from physical transport.

4.1. N₂ Fixation in the ETSP

4.1.1. N₂ Fixation in Oligotrophic Conditions

[33] During both cruises, N₂ fixation was detected along the southern transect (Figures 2a and 2b). Rates measured at 100°W (station 5) and 80°W (station 1) during the 2010 cruise (Multivariate ENSO Index: 1.52) were in good agreement with those measured during another study in the same area [Raimbault and Garcia, 2008] during El Niño conditions (BIOCOPE cruise, October–November 2004, Multivariate ENSO Index: 0.78).

[34] On the 2011 cruise, which took place during La Niña conditions, surface NO₃⁻ and PO₄³⁻ concentrations were higher, potentially due to the extension and the enhancement of the upwelling [Behrenfeld et al., 2001]. Similarly, euphotic zone DFe concentrations were ~10 times higher in 2011 compared to 2010 across the southern transect (Table 1). In spite of NO₃⁻ depletion, probably caused by phytoplankton consumption, relatively high PO₄³⁻ and Fe concentrations remained (Table 1). These conditions are supposed to be the most favorable conditions for cyanobacterial N₂ fixation [Sañudo-Wilhelmy et al., 2001; Berman-Frank et al., 2001; Mills et al., 2004], but N₂ fixation rates were very low at the edge of the subtropical gyre (Station 5) during the 2011 cruise (Figure 2b), nor was stimulated by Fe additions (Figure 5b). N₂ fixation appears to be highly variable in space and time [Goebel et al., 2007], and controlling factors may actually depend on the diazotroph species considered. A joint study to our measurements (K. A. Turk-Kubo et al., The paradox of marine heterotrophic nitrogen fixation: Abundances of heterotrophic diazotrophs do not account for nitrogen fixation rates in the Eastern Tropical South Pacific, submitted to *Environmental Microbiology*) and recent investigations [Bonnet et al., 2008; Halm et al., 2012] have shown that heterotrophic diazotrophs such as Proteobacteria are dominant in this region and contribute significantly to N₂ fixation in the ETSP. The physiology of these uncultivated heterotrophic diazotrophs remains unknown, but their activity seems to be important. Areal rates (Table 2) of N₂ fixation varied from 0 to 148 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ during the 2010 cruise, and from 5 to 99 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ during the 2011 cruise. These rates are comparable to those measured in other oligotrophic areas of the ocean (Table 2) such as the North Pacific gyre [Montoya et al., 2004] or the tropical Atlantic [Voss et al., 2004; Capone et al., 2005].

4.1.2. Changes in the Common Concepts About N₂ Fixation

[35] In addition to N₂ fixation occurring in the euphotic zone of the southern (20°S) transect, significant N₂ fixation rates were also measured in the northern transect (HNLC waters), exhibiting surface NO₃⁻ concentrations of up to 6.98 μmol L⁻¹. HNLC waters are unusual ecosystems for N₂ fixation as these environments are characterized by cold waters, rich in NO₃⁻ and limited by Fe availability. However, few studies have already been reported this process [Moutin *et al.*, 2008; Bonnet *et al.*, 2009] in such environments.

[36] N₂ fixation was also active below the photic zone down to 200 m, in the core of the OMZ, despite NO₃⁻ concentrations are up to 40 μmol L⁻¹ (Figures 3e and 3f). Significant rates have been recently reported in the coastal Peruvian OMZ [Fernandez *et al.*, 2011] or at depth of hypoxic basins [Hamersley *et al.*, 2011] in the Southern California Bight. In the Peru-Chile OMZ, associated with the upwelling [Ulloa and Pantoja, 2009], denitrification and anammox also occur [Castro-González *et al.*, 2005; Hamersley *et al.*, 2007; Lam *et al.*, 2009], creating an N deficit compared to P, which has been predicted to create favorable biogeochemical conditions for N₂ fixation in overlying euphotic zone waters [Deutsch *et al.*, 2007]. Moreover, biological N₂ fixation is a strictly anaerobic process [Falkowski, 1997] due to the sensitivity and the irreversible inactivation of the nitrogenase enzyme by O₂ [Burgess and Lowe, 1996]. It is therefore possible that the low O₂ concentrations in the OMZ contribute to the protection of the enzyme [Fay, 1992] and decrease the energy cost to keep intracellular anaerobiosis [Großkopf and LaRoche, 2012], thus facilitating N₂ fixation in this environment. Finally, redox conditions in the OMZ maintain a higher proportion of Fe in its most available form (Fe(II)) [Moffett *et al.*, 2007], which could help to support the high Fe requirements for the nitrogenase [Berman-Frank *et al.*, 2001; Kustka *et al.*, 2003a, 2003b]. For all these reasons, OMZs could represent a suitable habitat for N₂-fixing organisms despite high NO₃⁻ concentrations. Our results support this hypothesis (Figures 3a and 3b), especially during the 2010 cruise, indicating that prevailing assumptions regarding N₂ fixation in N-depleted areas may be reevaluated.

[37] Active N₂ fixation in ecosystems with appreciable NO₃⁻, including nutrient-enriched estuarine and coastal waters [Short and Zehr, 2007; Rees *et al.*, 2009; Bonnet *et al.*, 2011], hypoxic basins [Hamersley *et al.*, 2011], or at depth in the tropical North Atlantic [Voss *et al.*, 2004], has become progressively better documented. In oxic waters, breaking the triply bound N₂ molecule in N₂ fixation is energetically more costly compared to the assimilation of NO₃⁻ [Falkowski, 1983; Karl *et al.*, 2002; Großkopf and Laroche, 2012], which provides a thermodynamic rationale for community selection for NO₃⁻ utilizers when it is available. Moreover, NO₃⁻ is recognized to inhibit N₂ fixation activity of *Trichodesmium* [Mulholland and Capone, 2001], although even 10 μmol L⁻¹ NO₃⁻ did not fully inhibit nitrogenase activity in *Trichodesmium* [Holl and Montoya, 2005]. Similarly, a recent study performed on *Crocospaera watsonii* [Dekaezemacker and Bonnet, 2011] has shown that this strain is able to fix dinitrogen at high rates under 10 μmol L⁻¹ of NO₃⁻. A comparative study between these two cyanobacterial diazotrophs (*Trichodesmium* and *Crocospaera*) [Knapp *et al.*, 2012] reports that both organisms fixed the same quantity of N

normalized to cell carbon in different culture conditions (i.e., different NO₃⁻: PO₄³⁻ ratios) representative of the nutrient concentrations of the ETSP. Our results in the OMZ as well as culture studies, demonstrate that some species of diazotrophs can actively fix N₂ in the presence of NO₃⁻. Indeed, areal (0–200 m) N₂ fixation rates (Table 2) in the northern transect varied from 6 to 53 μmol N m⁻² d⁻¹ the first year and from 4 to 13 μmol N m⁻² d⁻¹ the second year.

4.1.3. Temporal Variability of N₂ Fixation and Potential Impact on N Budget in the ETSP

[38] This study provides the first data of N₂ fixation across the Peru-Chile upwelling for two consecutive years (in February 2010 and March 2011) marked by different climatic regimes. ENSO variations could have contributed to the variability of N₂ fixation in the upwelling zone between the 2 years, potentially due to the variations of the strength of the upwelling, the availability of the nutrients, and the intensity of O₂ deficiency. The ENSO variations could also modify rates of denitrification and anammox, resulting in an interannual change in N gain and loss processes. Additionally, the possible future expansion of the OMZs [Stramma *et al.*, 2008] could result in a complete change in the oceanic N cycle. If we compare our vertically integrated rates of N₂ fixation from more landward stations (i.e., stations 1, 11, and 12) to the rates of anammox reported in the Peruvian OMZ by Hamersley *et al.* [2007] (Multivariate ENSO index: 0.559) and considering anammox as the only contributor of N losses in the ETSP [Lam *et al.*, 2009], N gains by N₂ fixation could compensate for 6% of the losses during El Niño conditions and up to 8% of the losses during la Niña period. However, at the regional scale, the compensation of N losses by N₂ fixation is probably higher due to the large spatial extension of N₂ fixation (i.e., up to 100°W) compared to the restricted zone (coastal OMZ) where anammox and denitrification occur (i.e., reported by studies in the ETSP from the coast up to 85°W maximum) [Lipschultz *et al.*, 1990; Hamersley *et al.*, 2007; Lam *et al.*, 2009]. Furthermore, the ¹⁵N₂ bubble method [Montoya *et al.*, 1996] used for measuring N₂ fixation in the present study has recently been shown to possibly underestimate rates [Mohr *et al.*, 2010] by a factor of 2 to 6 [Wilson *et al.*, 2012; Großkopf *et al.*, 2012]. Therefore, the N compensation by N₂ fixation would likely be somewhat higher than that estimated above.

4.2. Nutrient Controls of N₂ Fixation

[39] Surface waters of the northern transect are known to be HNLC waters [Martin *et al.*, 1994; Blain *et al.*, 2008], where Fe availability limits NO₃⁻ utilization and primary productivity [Martin *et al.*, 1994; Price *et al.*, 1994]. Our results show that Fe availability can also be limiting for N₂ fixation in these waters (Figures 5a and 5b). At station 11 on the 2010 cruise and station 9 of the 2011 cruise, Fe additions significantly stimulated N₂ fixation rates. At most of the other stations (8, 9, 10 during the 2010 cruise and 11 during the 2011 cruise), inactive diazotrophs were present in these NO₃⁻-rich waters, and Fe addition stimulated their activity (Figures 5a and 5b). In the equatorial Pacific, Fe additions promote the planktonic community to switch from regenerated production based on NH₄⁺ consumption to new production based on NO₃⁻ consumption [Price *et al.*, 1991]. As N₂ fixation is a source of new N to the ocean and considered as new

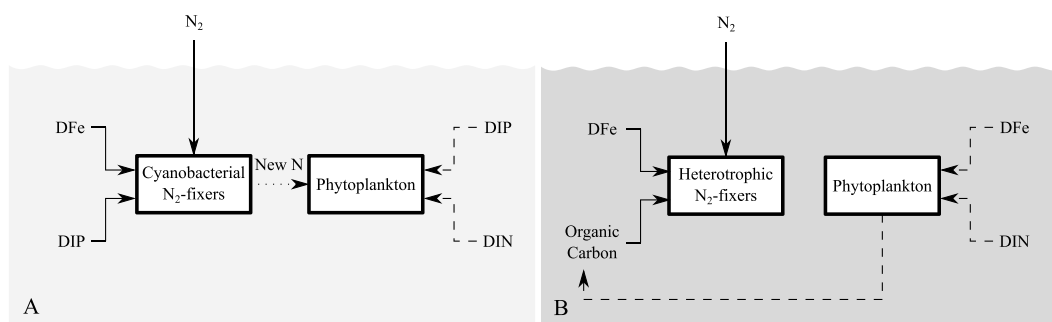


Figure 6. Schematic representations of N₂ fixation in different environments. (a) Conceptual N₂ fixation mainly performed by Cyanobacteria in oligotrophic areas of the oceans and directly controlled by DIP and DFe availabilities (solid arrows) and sustaining possibly the phytoplanktonic primary production by excretion of dissolved nitrogen (dotted arrow). (b) N₂ fixation in the N-rich waters of the ETSP, performed mainly by heterotrophic N₂-fixers, which could be directly controlled by DFe and DOC availabilities (solid arrows) or indirectly controlled through stimulation of phytoplankton by DFe and DIN availabilities (dashed arrows).

production [Dugdale and Goering, 1967], Fe additions may allow utilization of this more energetically expensive pathway of N-nutrition thereby providing access to a larger N pool. This study demonstrates that in the ETSP, as in North Atlantic [Mills *et al.*, 2004] (Figure 6a), Fe limits N₂ fixation rates, but during both El Niño and La Niña conditions and even relatively low or high Fe concentrations (Figure 6b). The degree to which Fe is accessible to these diazotrophs is still unknown due to the uncertainties about chemical and physical Fe speciation in the surface waters of the ETSP [Wells, 2003], to the unknown and probably high Fe requirement of these diazotrophs, and to the probably high competition for Fe with other planktonic organisms.

[40] Primary production in the warm and oligotrophic subtropical gyre is N limited [Bonnet *et al.*, 2008]. Due to the relatively high PO₄³⁻ and low NO₃⁻ concentrations, especially on the 20°S transect, one would predict that these macronutrient concentrations are ideal for N₂ fixation. However, even when Fe was added, no stimulation of N₂ fixation rates was observed (Figure 5a, station 5). When both Fe and NO₃⁻ were added, N₂ fixation was although stimulated (Figure 5c). Our results showed therefore that N additions did not inhibit N₂ fixation and indeed could stimulate the process. We can hypothesize here that primary production was stimulated by NO₃⁻ additions, which could increase dissolved organic carbon (DOC) excretion, enhance heterotrophic production, and in turn stimulate heterotrophic N₂ fixation for sustaining the N demand of Bacteria (Figure 6b). However additions of glucose did not increase N₂ fixation rates at this station (Figure 5c). It is possible that other organic compounds released by phytoplankton, such as DOP or other sources of DOC, stimulated heterotrophic N₂ fixation.

[41] The same indirect control of N₂ fixation by the phytoplanktonic activities may have occurred after Fe additions (Figure 6b). On the northern (10°S) transect, the biologically available Fe in the photic layer is mainly upwelled [Gordon *et al.*, 1997], and during El Niño, the Fe fluxes from below usually decrease [Barber *et al.*, 1996; Friedrichs and Hofmann, 2001], which is consistent with our Fe concentration measurements (Table 1). In the nutrient sensitivity assays performed on samples collected from the northern transect, N₂ fixation was stimulated by Fe additions

(Figures 5a and 5b). We can hypothesize that Fe stimulated nitrogenase synthesis. However, Fe additions also stimulated primary production (data not shown), resulting in a possible excretion of labile dissolved organic matter, like DOC, which was limiting for N₂ fixation in the HNLC surface waters (Figure 5c). The potential stimulation of the bacterial productivity supported by this new DOC [Van Wambeke *et al.*, 2008] could have created low oxygen conditions and increased the bacterial N demand, fostering N₂ fixation. The hypothesis about the mutualistic link between heterotrophic diazotrophs and photoautotrophs (Figure 6b) was also proposed in the South Pacific gyre [Halm *et al.*, 2012].

[42] The generally accepted optimum conditions for N₂ fixation (i.e., low N, high availability of P and Fe and warm temperature, Figure 6a) based on our knowledge of cyanobacterial diazotrophs physiology [Sañudo-Wilhelmy *et al.*, 2001; Mills *et al.*, 2004] probably need to be re-evaluated in order to take into account heterotrophic diazotrophs and the possible linkage between phototrophs and heterotrophs. The details about the N physiology of these organisms need to be investigated in order to better define their ecological niches and significance for the global N budget.

5. Conclusion

[43] This study reports for the first time that N₂ fixation occurs all across the ETSP at rates comparable to those documented elsewhere in the oligotrophic ocean and with a temporal variability, which can be linked with biogeochemical variations related with the ENSO phenomenon. Surprisingly, El Niño provided preferential conditions for N₂ fixation than La Niña, especially in the HNLC waters and at the border of the gyre. Therefore, the provisional scenario about the increase of the frequency of El Niño events [Timmermann *et al.*, 1999] and the expansion of low oxygenated waters in the Pacific ocean [Keeling and Garcia, 2002; Stramma *et al.*, 2008] may modify the N cycle in the ETSP to the benefit of N₂ fixation [Großkopf and LaRoche, 2012]. However, doing this expensive process in these N-rich waters and at such depths in the OMZ is still an enigma. Finally, nutrient limitation of N₂ fixation in the surface is closely related to the nutrient limitation of primary

production and unlike the common thought, it could be possible that phytoplankton sustains heterotrophic N₂ fixation.

[44] **Acknowledgments.** This work was performed in the framework of the “Documenting N₂ fixation in N-deficient waters of the Eastern Tropical South Pacific” supported by NSF Chemical Oceanographic (OCE 0850801) and the French Government (CNRS-INSU and IRD) supported the LEFE project “Humboldt-fix.” The authors thank the captains and crews from the R/V Atlantis and R/V Melville for outstanding shipboard operations. A PhD scholarship for Julien Dekaezemacker came from the French Ministry of Research and Education.

References

- Aminot, A., and R. Kerouel (2007), *Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu*, Méthodes d’analyse en milieu marin, Ifremer, Plouzané.
- Anderson, L., and J. Sarmiento (1994), Redfield ratios of remineralization determined by nutrient data analysis, *Global Biogeochem. Cycles*, **8**, 65–80.
- Arntz, W. E., E. Valdivia, and J. Zeballos (1988), Impact of El Niño 1982–83 on the commercially exploited invertebrates (mariscos) of the Peruvian shore, *Meeresforsch.*, **32**, 3–22.
- Barber, R. T., M. P. Sanderson, S. T. Lindley, F. Chai, J. Newton, C. C. Trees, D. G. Foley, and F. P. Chavez (1996), Primary productivity and its regulation in the equatorial Pacific during and following the 1991–92 El Niño, *Deep Sea Res. Part II*, **43**, 933–969.
- Behrenfeld, M. J., et al. (2001), Biospheric primary production during an ENSO transition, *Science*, **291**, 2594–2597.
- 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.
- Blain, S., S. Bonnet, and C. Guieu (2008), Dissolved iron distribution in the tropical and subtropical South Eastern Pacific, *Biogeochemistry*, **5**, 269–280.
- Bonnet, S., and C. Guieu (2006), Atmospheric forcing on the annual in the Mediterranean Sea. A one year survey, *J. Geophys. Res.*, **111**, C09010, doi:10.1029/2005JC003213.
- Bonnet, S., et al. (2008), Nutrient limitation of primary productivity in the southeast Pacific (BIOPEPE cruise), *Biogeochemistry*, **5**, 215–225.
- Bonnet, S., I. C. Biegala, P. Dutrieux, L. O. Slemmons, 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), *Biogeochemistry*, **8**, 2257–2267, doi:10.5194/bg-8-2257-2011.
- Brandes, J. A., and A. H. Devol (2002), A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling, *Global Biogeochem. Cycles*, **16**, 1120, doi:10.1029/2001GB001856.
- Breitbart, E., A. Oschlies, and J. LaRoche (2007), Physiological constraints on the global distribution of *Trichodesmium*: Effect of temperature on diazotrophy, *Biogeochemistry*, **4**, 53–61.
- Bruland, K. W., R. P. Franks, G. Knauer, and J. H. Martin (1979), Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel in seawater, *Anal. Chim. Acta*, **105**, 233–245.
- Burgess, B. K., and D. J. Lowe (1996), Mechanism of molybdenum nitrogenase, *Chem. Rev.*, **96**, 2983–3011.
- Capone, D. G., J. P. Zehr, H. Paerl, B. Bergman, and E. J. Carpenter (1997), *Trichodesmium*: A globally significant marine cyanobacterium, *Science*, **276**, 1221–1229.
- 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.
- Castro-González, M., G. Braker, L. Fariás, and O. Ulloa (2005), Communities of nirS-type denitrifiers in the water column of the oxygen minimum zone in the eastern South Pacific, *Environ. Microbiol.*, **7**, 1298–1306.
- Claustre, H., and S. Maritorena (2003), The many shades of ocean blue, *Science*, **302**, 1514–1515.
- Codispoti, L. A. (2007), An oceanic fixed nitrogen sink exceeding 400 Tg N a⁻¹ vs. the concept of homeostasis in the fixed-nitrogen inventory, *Biogeochemistry*, **4**, 233–253.
- Codispoti, L. A., J. A. Brandes, J. P. Christensen, A. H. Devol, S. W. A. Naqvi, H. W. Paerl, and T. Yoshinari (2001), The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene?, *Sci. Mar.*, **65**, 85–105.
- Dekaezemacker, J., and S. Bonnet (2011), Sensitivity of N₂ fixation to combined nitrogen forms (NO₃⁻ and NH₄⁺) in two strains of the marine diazotroph *Crocospaera watsonii* (Cyanobacteria), *Mar. Ecol. Prog. Ser.*, **438**, 33–46.
- Deutsch, C., J. L. Sarmiento, D. M. Sigman, N. Gruber, and J. P. Dunne (2007), Spatial coupling of nitrogen inputs and losses in the ocean, *Nature*, **445**, 163–167, doi:10.1038/nature05392.
- Dugdale, R. C., and J. J. Goering (1967), Uptake of new and regenerated forms of nitrogen in primary productivity, *Limnol. Oceanogr.*, **12**, 196–206.
- Falkowski, P. G. (1983), Enzymology of nitrogen assimilation, In *Nitrogen in the marine environment*, edited by Carpenter E. J., and Capone D. G., p. 839–868, Academic Press, New York, NY.
- Falkowski, P. G. (1997), Evolution of the nitrogen cycle and its influence on the biological sequestration of CO₂ in the ocean, *Nature*, **387**, 272–275.
- Falkowski, P. G., R. T. Barber, and V. Smetacek (1998), Biogeochemical controls and feedbacks on ocean primary production, *Science*, **281**, 200–206.
- Fay, P. (1992), Oxygen relations of nitrogen fixation in cyanobacteria, *Microbiol. Rev.*, **56**, 340–373.
- Fernandez, C., L. Fariás, and O. Ulloa (2011), Nitrogen fixation in denitrified marine waters, *PLoS One*, **6**, e20539, doi:10.1371/journal.pone.0020539.
- Friedrichs, M. A. M., and E. E. Hofmann (2001), Physical control of biological processes in the central equatorial Pacific Ocean, *Deep Sea Res. Part I*, **48**, 1023–1069.
- Goebel, N. L., C. A. Edwards, M. J. Church, and J. P. Zehr (2007), Modeled contributions of three diazotrophs to nitrogen fixation at Station ALOHA, *ISME J.*, **1**, 606–619.
- Goering, J. J. (1968), Denitrification in oxygen minimum layer of Eastern Tropical Pacific Ocean, *Deep Sea Res.*, **15**, 157–164.
- Gordon, M. R., K. H. Coale, and K. Johnson (1997), Iron distributions in the equatorial Pacific: Implications for new production, *Limnol. Oceanogr.*, **42**, 419–431.
- Großkopf, T., and J. LaRoche (2012), Direct and indirect costs of dinitrogen fixation in *Crocospaera watsonii* WH8501 and possible implications for the nitrogen cycle, *Front. Microbiol.*, **3**, 236.
- 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 N₂ fixation rates based on direct measurements, *Nature*, **488**, 361–364.
- Halm, H., P. Lam, T. G. Ferdelman, G. Lavik, T. Dittmar, J. LaRoche, S. D’Hondt, and M. M. M. Kuypers (2012), Heterotrophic organisms dominate nitrogen fixation in the South Pacific Gyre, *ISME J.*, **6**, 1238–1249.
- Hamersley, M. R., et al. (2007), Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, *Limnol. Oceanogr.*, **52**, 923–934.
- Hamersley, M. R., K. A. Turk, A. Leinweber, N. Gruber, J. P. Zehr, T. Gunderson, and D. G. Capone (2011), Nitrogen fixation within the water column associated with two hypoxic basins in the Southern California Bight, *Aquat. Microb. Ecol.*, **63**, 193–205.
- Holl, C. M., and J. P. Montoya (2005), Interactions between nitrate uptake and N₂ fixation in *Trichodesmium*, *J. Phycol.*, **41**, 1178–1183, doi:10.1111/j.1529-8817.2005.00146.x.
- Hydes D., et al. (2010), Determination of dissolved nutrients (N, P, Si) in seawater with high precision and inter-comparability using gas segmented continuous flow analysers. In *The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and guidelines*. IOCCP Report No 14, ICPO Publication Series No. 134, version 1 (UNESCO/IOC).
- Jickells, T. D., et al. (2005), Global iron connections between desert dust, ocean biogeochemistry, and climate, *Science*, **308**, 67–71.
- Karl, D. M., and R. M. Letelier (2008), Nitrogen fixation-enhanced carbon sequestration in low nitrate, low chlorophyll seascapes, *Mar. Ecol. Prog. Ser.*, **364**, 257–268.
- Karl, D. M., R. Letelier, D. V. Hebel, D. F. Bird, and C. D. Winn (1992), *Trichodesmium* blooms and new nitrogen in the North Pacific Gyre, in *Marine pelagic cyanobacteria: Trichodesmium* and other diazotrophs, edited by Carpenter E. J., Capone D. G., Rueter J. G., p. 219–237, Kluwer Academic, Dordrecht.
- Karl, D. M., A. Michaels, B. Bergman, D. G. Capone, E. Carpenter, R. Letelier, F. Lipschultz, H. Paerl, D. Sigman, and L. Stal (2002), Dinitrogen fixation in the world’s oceans, *Biogeochemistry*, **57**, 47–98.
- Keeling, R. F., and H. Garcia (2002), The change in oceanic O₂ inventory associated with recent global warming, *Proc. Nat. Acad. Sci. U.S.A.*, **99**, 7848–7853.
- Kirkwood, D. S. (1992), Stability of solutions of nutrient salts during storage, *Mar. Chem.*, **38**, 151–164.
- Knapp, A. N., J. Dekaezemacker, S. Bonnet, J. A. Sohm, and D. G. Capone (2012), Sensitivity of *Trichodesmium* and *Crocospaera* growth and N₂ fixation rates to varying NO₃⁻ and PO₄³⁻ concentrations in batch cultures, *Aquat. Microb. Ecol.*, **66**, 223–236.

- Kustka, A., S. Sanudo-Wilhelmy, E. J. Carpenter, D. G. Capone, and J. A. Raven (2003a), A revised estimate of the Fe use efficiency of nitrogen fixation, with special reference to the marine N₂ fixing cyanobacterium, *Trichodesmium* spp. (Cyanophyta), *J. Phycol.*, *39*, 12–25.
- Kustka, A. B., S. A. Sanudo-Wilhelmy, E. J. Carpenter, D. Capone, J. Burns, and W. G. Sunda (2003b), Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium* (IMS 101): Comparison with nitrogen fixation rates and iron: Carbon ratios of field populations, *Limnol. Oceanogr.*, *48*(5), 1869–1884.
- Kuypers, M. M. M., A. O. Slikkers, G. Lavik, M. Schmid, B. B. Jørgensen, J. G. Kuenen, J. S. Sinninghe Damsté, M. Strous, and M. S. M. Jetten (2003), Anaerobic ammonium oxidation by Anammox bacteria in the Black Sea, *Nature*, *422*, 608–611.
- Lam, P., et al. (2009), Revising the nitrogen cycle in the Peruvian oxygen minimum zone, *Proc. Nat. Acad. Sci. U.S.A.*, *106*, 4752–57.
- Lipschultz, F., S. C. Wofsy, B. B. Ward, L. A. Codispoti, G. Friedrich, and J. W. Elkins (1990), Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical South Pacific Ocean, *Deep Sea Res. Part A*, *37*, 1513–1541.
- Luo, Y.-W., et al. (2012), Database of diazotrophs in global ocean: abundances, biomass and nitrogen fixation rates, *Earth Syst. Sci. Data Discuss.*, *5*, 47–106, doi:10.5194/essd-5-47-2012.
- Martin, J. H. K. H., et al. (1994), Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean, *Nature*, *371*, 123–129, doi:10.1038/371123a0.
- Mills, M. M., C. Ridame, M. Davey, J. La Roche, and R. J. Geider (2004), Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic, *Nature*, *429*, 292–294, doi:10.1038/nature02550.
- Moffett, J. W., T. J. Goepfert, and S. W. A. Naqvi (2007), Reduced iron associated with secondary nitrite maxima in the Arabian Sea, *Deep Sea Res. Part I*, *54*, 1341–49.
- Mohr, W., T. Großkopf, D. W. R. Wallace, and J. LaRoche (2010), Methodological underestimation of oceanic nitrogen fixation rates, *PLoS One*, *5*(9), e12583, doi:10.1371/journal.pone.0012583.
- Montoya, J. P., M. Voss, P. Kaehler, and D. G. Capone (1996), A simple, high precision tracer assay for dinitrogen fixation, *Appl. Environ. Microbiol.*, *62*, 986–993.
- Montoya, J. P., C. Holl, J. P. Zehr, A. Hansen, T. Villareal, and D. G. Capone (2004), High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, *Nature*, *430*, 1027–1032, doi:10.1038/nature02824.
- Moutin, T., D. M. Karl, S. Duhamel, P. Rimmelin, P. Raimbault, B. A. S. Van Mooy, and H. Claustre (2008), Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, *Biogeosciences*, *5*, 95–109, doi:10.5194/bg-5-95-2008.
- Mulholland, M. R., and D. G. Capone (2001), The stoichiometry of N and C utilization in cultured populations of *Trichodesmium* IMS101, *Limnol. Oceanogr.*, *46*, 436–443.
- Paerl, H. W., L. E. Prufert-Bebout, and C. Gao (1994), Iron-stimulated N₂ fixation and growth in natural and cultures populations of the planktonic marine cyanobacteria *Trichodesmium* spp., *Appl. Environ. Microbiol.*, *60*(3), 1044–1047.
- Parekh, P., M. Follows, and E. A. Boyle (2005) Decoupling of iron and phosphate in the global ocean, *Global Biogeochem. Cycles*, *19*, GB2020, doi:10.1029/2004GB002280.
- Paulmier, A., and D. Ruiz-Pino (2008), Oxygen Minimum Zones (OMZs) in the modern ocean, *Prog. Oceanogr.*, *80*(3–4), 113–128.
- Price, N. M., L. F. Andersen, and F. M. M. Morel (1991), Iron and nitrogen nutrition of equatorial Pacific plankton, *Deep Sea Res.*, *38*, 1361–1378.
- Price, N. M., B. A. Ahner, and F. M. M. Morel (1994), The equatorial Pacific ocean: Grazer-controlled phytoplankton populations in an iron-limited ecosystem, *Limnol. Oceanogr.*, *39*, 520–534.
- 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 estimates, *Biogeosciences*, *5*, 323–338.
- Redfield, A. C., B. H. Ketchum, and F. A. Richards (1963), The influence of organisms on the composition of seawater, in *The composition of seawater. comparative and descriptive oceanography. The Sea: Ideas and Observations on Progress in the Study of the Seas*, edited by Hill, M. N., vol. 2, pp. 26–77, Wiley-Interscience, New York, USA.
- Rees, A. P., J. A. Gilbert, and B. A. Kelly-Gerrey (2009), Nitrogen fixation in the western English Channel (NE Atlantic Ocean), *Mar. Ecol. Prog. Ser.*, *374*, 7–12.
- Saito, M. A., et al. (2011), Iron conservation by reduction of metallo enzyme inventories in the marine diazotroph *Crocospaera watsonii*, *Proc. Nat. Acad. Sci. U.S.A.*, *108*, 2184–2189.
- Sañudo-Wilhelmy, S. A., A. B. Kustka, C. J. Gobler, D. A. Hutchins, M. Yang, K. Lwiza, J. Burns, D. G. Capone, J. A. Raven, and E. J. Carpenter (2001), Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean, *Nature*, *411*, 66–69, doi:10.1038/35075041.
- Short, S. M., and J. P. Zehr (2007), Nitrogenase gene expression in the Chesapeake Bay Estuary, *Environ. Microbiol.*, *9*, 1591–1596.
- Stramma, L., G. C. Johnson, J. Sprintall, and V. Mohrholz (2008), Expanding oxygen minimum zones in the tropical oceans, *Science*, *320*, 655–658.
- Timmermann, A., J. Oberhuber, A. Bacher, M. Esch, M. Latif, and E. Roeckner (1999), Increased El Niño frequency in a climate model forced by future greenhouse warming, *Nature*, *357*, 230.
- Ulloa, O., and S. Pantoja (2009), The oxygen minimum zone of the eastern South Pacific, *Deep Sea Res. Part II*, *56*, 987–991.
- Van Wambeke, F., S. Bonnet, T. Moutin, P. Raimbault, G. Alarçon, and C. Guieu (2008), Factors limiting heterotrophic bacterial production in the southern Pacific Ocean, *Biogeosciences*, *5*, 833–845.
- Voss, M., P. Croot, K. Lochte, M. Mills, and I. Peeken (2004), Patterns of nitrogen fixation along 10°N in the tropical Atlantic, *Geophys. Res. Lett.*, *31*, L23S09, doi:10.1029/2004GL020127.
- Webb, E. A., I. M. Ehrenreich, S. L. Brown, F. W. Valois, and J. B. Waterbury (2009), Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocospaera watsonii*, isolated from the open ocean, *Environ. Microbiol.*, *11*, 338–348, doi:10.1111/j.1462-2920.2008.01771.x.
- Wells, M. L. (2003), The level of iron enrichment required to initiate diatom blooms in HNLC waters, *Mar. Chem.*, *82*, 101–114.
- Westberry, T. K., and D. A. Siegel (2006), Spatial and temporal distribution of *Trichodesmium* blooms in the world's oceans, *Global Biogeochem. Cycles*, *20*, GB4016, doi:10.1029/2005GB002673.
- 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.*, doi:10.1128/AEM.01146-12.
- Wolter, K., and M. S. Timlin (1993), Monitoring ENSO in COADS with a seasonally adjusted principal component index. Proc. of the 17th Climate Diagnostics Workshop, Norman, OK, NOAA/NMC/CAC, NSSL, Oklahoma Clim. Survey, CIMMS and the School of Meteor., Univ. of Oklahoma, 52–57.
- Wolter, K., and M. S. Timlin (1998), Measuring the strength of ENSO events - how does 1997/98 rank?, *Weather*, *53*, 315–324.
- Zehr, J. P., J. B. Waterbury, P. J. Turner, J. P. Montoya, E. Omereg, G. F. Steward, A. Hansen, and D. M. Karl (2001), Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean, *Nature*, *412*, 635–638, doi:10.1038/35088063.