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

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[1] Biological N_2 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. N_2 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 N_2 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 N_2 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 N_2 fixation rates measured in the oligotrophic ocean. N_2 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 N_2 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 N_2 fixation.

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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 N_2 fixing organisms referred to as "diazotrophs" may have an ecological advantage because they are able to reduce dissolved N_2 gas to ammonia (NH_3) and assimilate it, alleviating their need for another external source of N. N_2 fixation represents one of the major sources of new N to the surface oligotrophic ocean [*Capone et al*., 2005]. N_2 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 N_2 fixation might occur at significant rates in surface waters overlying regions of N losses such as the Eastern Tropical South Pacific (ETSP). Biological N_2 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 N_2 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*,

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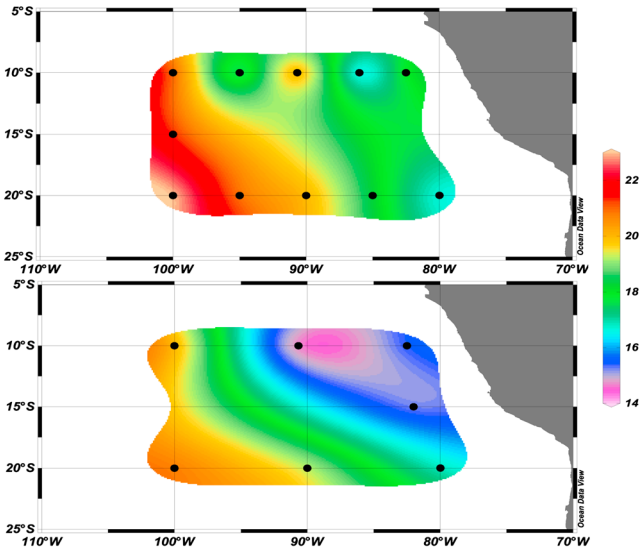

Figure 1. Location of stations during the cruises in (a) February 2010-El Niño conditions and in (b) March–April 2011-La Niña conditions on a map of seawater temperature (°C) at 75 m depth.

2007], partly because most in situ studies on N_2 fixation are performed on cyanobacterial diazotrophs in N-depleted warm areas [*Capone et al.*, 1997; *Zehr et al.*, 2001]. Another explanation is that potential N_2 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 N_2 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; *Kustka et al.*, 2003a, 2003b], it is also suspected to control N_2 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 N_2 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 N_2 fixation rates along a 5700 km transect exhibiting strong oxygen and nutrient gradients. The objectives of this study were (1) to quantify N_2 fixation rates across those gradients during contrasted climatic and therefore upwelling conditions, and (2) to determine which nutrients control N_2 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 Maritorena*, 2003]. Surface waters exhibited nitrate (NO_3^-) concentrations close to detection limit, which is known to be favorable for N_2 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 N_2 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. N_2 fixation rates (nmol L^{-1} d^{-1}) were determined according to *Montoya et al.* [1996] (further details are given in the supporting information). However, the method to measure N_2 fixation is currently in debate, and it has been noted that the method we used may underestimate rates due to incomplete equilibration of $^{15}N_2$ 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 N_2 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 O_2 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 ($NO_3^- + NO_2^-$) 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 µg mL^{-1}) [*Kirkwood*, 1992] and stored at 4°C until analysis.

Table 1. Initial Characteristics for the Nutrient Enrichment Experiments[a]

	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
Latitude Longitude	2010 (Atlantis)	2011 (Melville)	2010 (Atlantis)		2010 (Atlantis)		2010 (Atlantis)		2010 (Atlantis)	2011 (Melville)	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	< 0.01	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	0.38 ± 0.05	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	1.57 ± 0.16	0.18 ± 0.03	
P*, $r_{P/N}$ = 1/16 (μmol L^{-1})	0.44	0.45	0.38		0.37		0.33		0.39	0.36	0.47	
Fe*, $r_{Fe/P}$ = 0.47 (nmol L^{-1})	−0.05	1.35	−0.04		−0.04		−0.03		−0.03	1.39	−0.10	
N_2 fixation (nmol L^{-1} d^{-1})	0.74 ± 0.11	0	0		0		0		0.23 ± 0.06	0.02 ± 0.002	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
Latitude Longitude	2010 (Atlantis)	2011 (Melville)	2010 (Atlantis)		2010 (Atlantis)	2011 (Melville)	2010 (Atlantis)		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	3.13 ± 0.19	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	0.42 ± 0.08	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	1.89 ± 0.42	1.95 ± 0.48	
P*, $r_{P/N}$ = 1/16 (μmol L^{-1})	0.36	0.25	0.31		0.42	0.23	0.63		0.34	0.23	0.47	
Fe*, $r_{Fe/P}$ = 0.47 (nmol L^{-1})	−0.20	1.24	−0.15		−0.07	1.50	−0.09		0.003	1.69	1.71	
N_2 fixation (nmol L^{-1} d^{-1})	0	0	0		0	0.59 ± 0.48	0		0.13 ± 0.05	0	0.05 ± 0.01	

[a]n.a., not available.

2.1.2. Nutrient Sensitivity Assays of N_2 Fixation in Euphotic Zone

[12] All N_2 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} $FeCl_3$, 4 μmol L^{-1} $NaNO_3$ (99.99% Suprapur, Merck), 1 μmol L^{-1} NaH_2PO_4 (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 HNO_3 nor NH_4OH was used to avoid N contaminations, which could affect N_2 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 ($^{15}N_2$), and incubated under the same conditions for another 24 h. After incubation, the three replicates of each treatment were used in order to measure N_2 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). N_2 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 ^{15}N atom % as a function of increasing particulate nitrogen mass was verified on both natural and ^{15}N enriched material since it is critical, especially for samples from ultra-oligotrophic environments. ^{15}N 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 N_2 fixation rates were 0.01 nmol L^{-1} d^{-1}. N_2 fixation

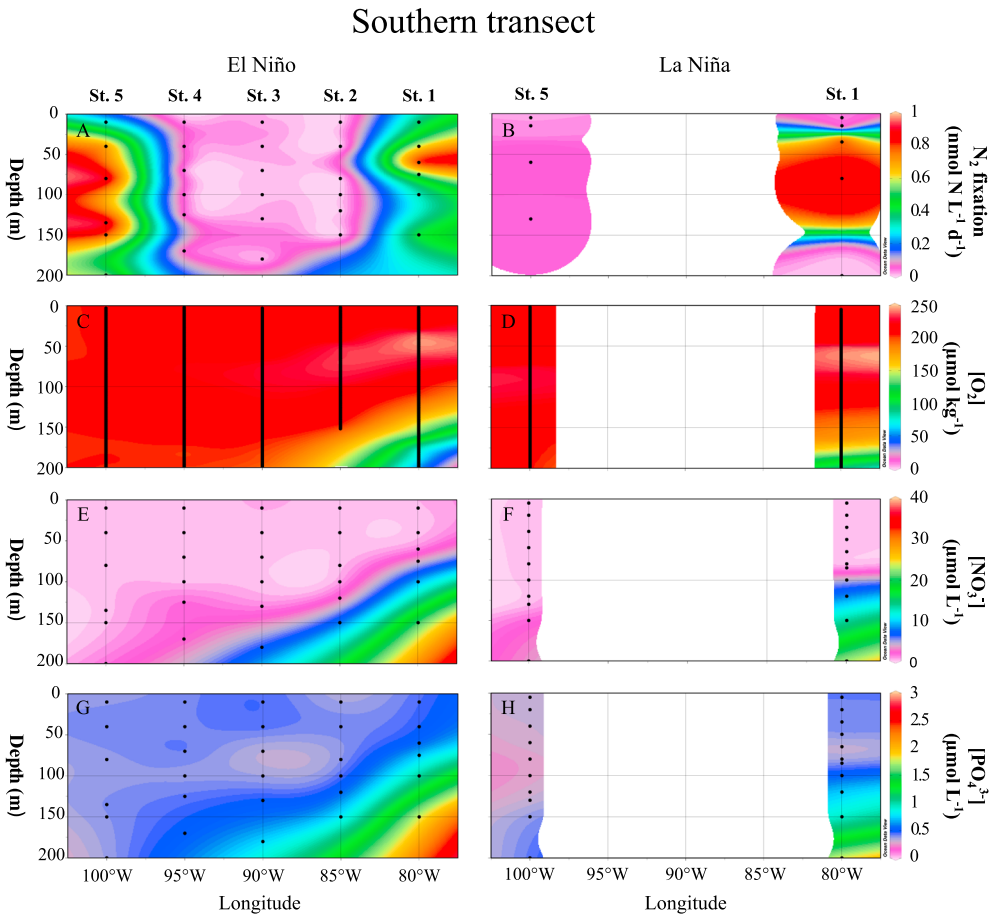

Figure 2. Depth distribution (a and b) N_2 fixation (nmol L^{-1} d^{-1}), (c and d) O_2 concentrations (µmol kg^{-1}), and (e and f) NO_3^- and (g and h) PO_4^{3-} concentrations (µmol L^{-1}) along the southern transect (20°S) between El Niño year (Figures 2a, 2c, 2e, and 2g) and La Niña year (Figures 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 ($NO_x = NO_3^- + NO_2^-$) and PO_4^{3-} 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 NO_3^- and NO_2^- will hereafter be referred to as NO_3^-.

[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 $P^* = [PO_4^{3-}] - [NO_3^-]/16$ [Deutsch et al., 2007]. P* informs about the relative changes of NO_3^- and PO_4^{3-} concentrations in oceanic waters. Decreases in surface ocean PO_4^{3-} that are unaccompanied by concomitant Redfield-ratio decreases in NO_3^- (P* decreases) are interpreted as the result of N_2 fixation.

[19] The second one was the Fe*, defined as Fe* = [DFe] − 0.47 mmol mol^{-1} × $[PO_4^{3-}]$ 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 N_2 fixation rates from the nutrient enrichment experiments and from the profiles of the 2011 cruise.

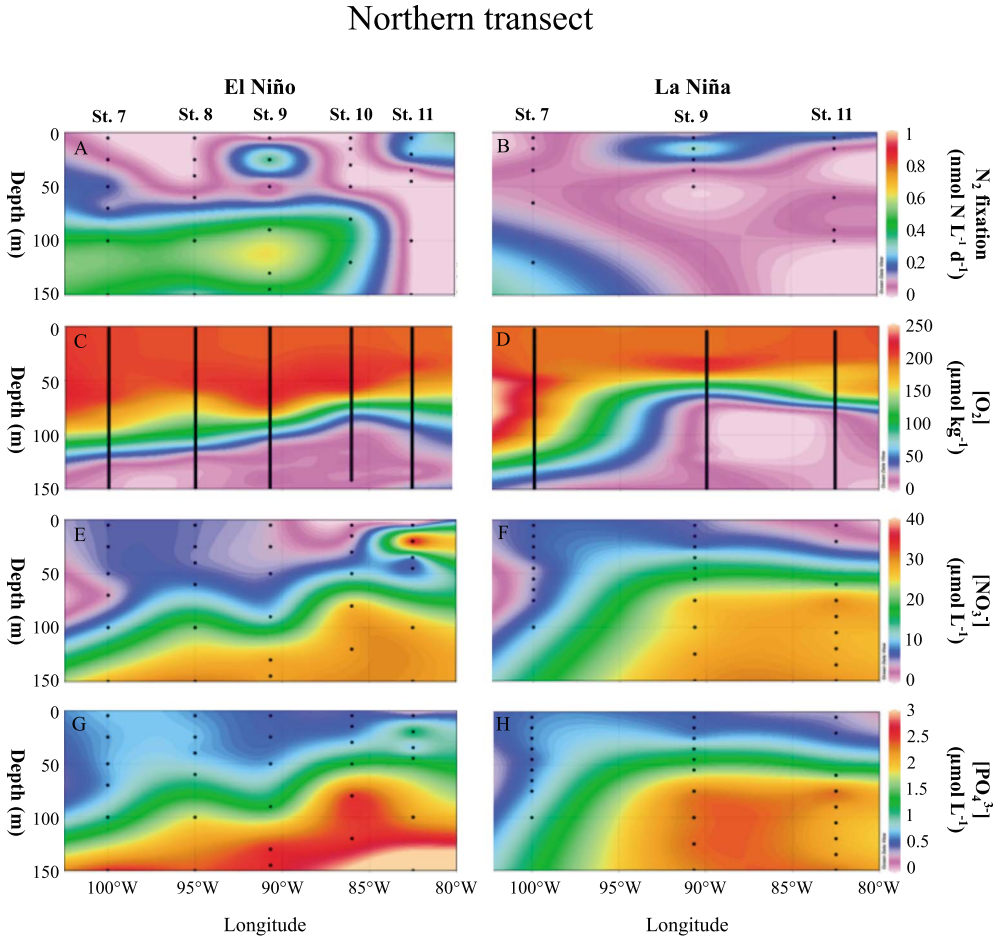

Figure 3. Depth distribution (a and b) N_2 fixation (nmol L^{-1} d^{-1}), (c and d) O_2 concentrations (µmol kg^{-1}), and (e and f) NO_3^- and (g and h) PO_4^{3-} concentrations (µmol L^{-1}) along the northern transect (10°S) between El Niño year (Figures 3a, 3c, 3e, and 3g) and La Niña year (Figures 3b, 3d, 3f, and 3h).

[21] To compare the effect of nutrient addition on N_2 fixation rates, we calculated the relative change (%) for each variable as 100 × (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 N_2 Fixation Rates and Biogeochemical Conditions in the ETSP

3.1.1. N_2 Fixation Rates During the 2010 Cruise

[22] N_2 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 O_2 concentrations > 190 µmol kg^{-1}, except at station 1 below 120 m, where O_2 concentrations decreased with depth to reach a minimum value of 60 µmol O_2 kg^{-1} at 200 m depth. Surface NO_3^- 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 PO_4^{3-} 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). NO_3^- and PO_4^{3-} concentrations increased with depth to 20.8 ± 2.1 µmol L^{-1} and 1.79 ± 0.09 µmol L^{-1} for NO_3^- and PO_4^{3-} concentrations at 150 m at Station 1, respectively. The depth of the nutricline shoaled to the east.

[23] On the northern transect (10°S), N_2 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 O_2 concentrations decreased with depth, with a shallower oxycline shoaling eastward; suboxic conditions ([O_2] < 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 NO_3^- concentrations varying from 5.89 ± 0.64

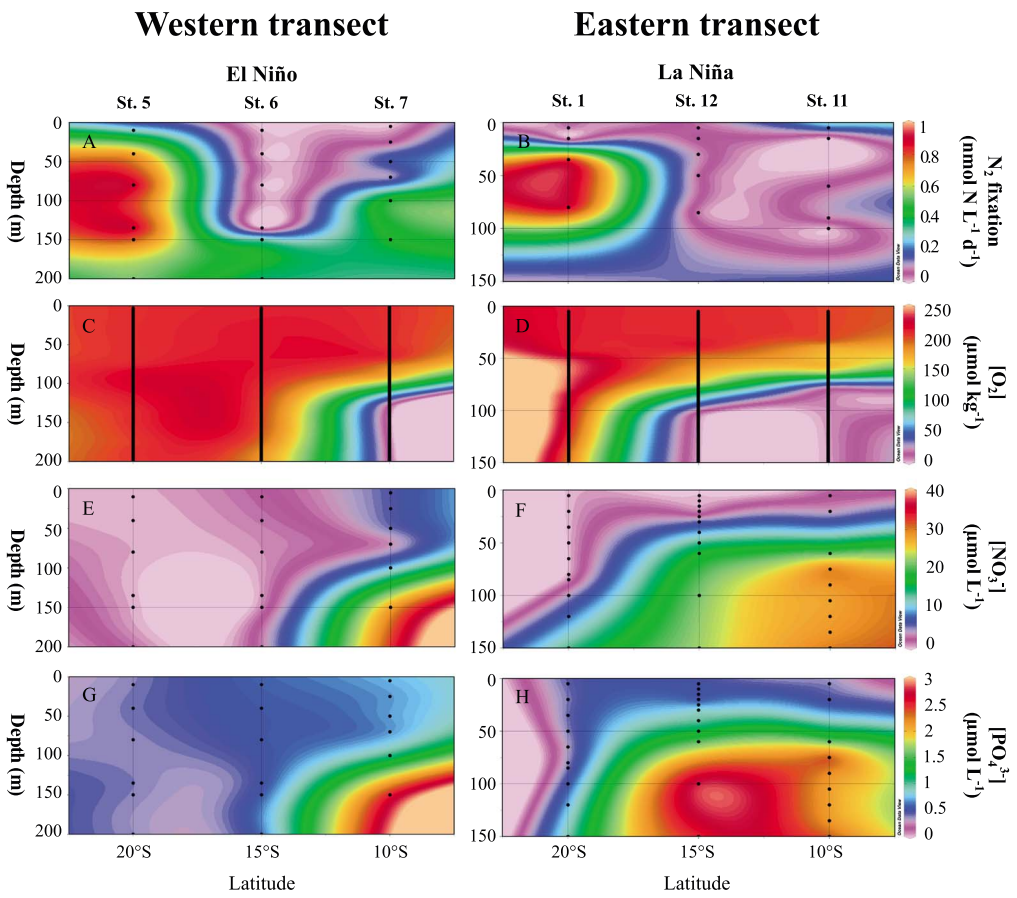

Figure 4. Depth distribution (a and b) N_2 fixation (nmol L^{-1} d^{-1}), (c and d) O_2 concentrations (µmol kg^{-1}), and (e and f) NO_3^- and PO_4^{3-} concentrations (µmol L^{-1}) along the western transect (100°W) in El Niño year (Figures 4a, 4c, 4e, and 4g) and along the eastern transect (82.5°W) in La Niña year (Figures 4b, 4d, 4f, and 4h).

to 0.46 ± 0.08 µmol L^{-1} and PO_4^{3-} 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). NO_3^- and PO_4^{3-} concentrations increased with depth to reach values from 28.0 ± 2.8 (station 7) to 30.8 ± 3.1 (station 8) 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, NO_3^- concentrations of 29.80 ± 2.97 µmol L^{-1} and PO_4^{3-} concentrations of 3.73 ± 0.20 µmol L^{-1} were measured at 150 m. The NO_3^- 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 N_2 fixation rates of 0.32 and 0.37 nmol N L^{-1} d^{-1} at 150 and 200 m depth (Figure 4a). O_2 concentrations were homogeneous and ≥75 µmol kg^{-1} from the surface to 200 m (Figure 4c). Surface concentrations of NO_3^- and PO_4^{3-} 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. N_2 Fixation Rates During the 2011 Cruise

[25] Only stations 1 and 5 were sampled along the southern transect. The highest N_2 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 O_2 concentrations > 200 µmol kg^{-1}, except at station 1 below 135 m, where O_2 concentrations decreased with depth to reach 90 µmol kg^{-1} at 200 m depth. Surface NO_3^- and PO_4^3 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 NO_3^- and 1.79 ± 0.07 µmol L^{-1} for PO_4^{3-} at 200 m. At station 5, surface nutrient concentrations were 0.06 ± 0.08 µmol L^{-1} for NO_3^- (Figure 2f), and 0.32 ± 0.05 µmol L^{-1} for PO_4^{3-} (Figure 2h). NO_3^- 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. PO_4^{3-} concentrations reached 0.37 ± 0.05 µmol L^{-1} at 200 m.

[26] Across the northern transect, N_2 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 NO_3^- and PO_4^{3-} 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 NO_3^- isocline 10 µmol L^{-1} was between 100

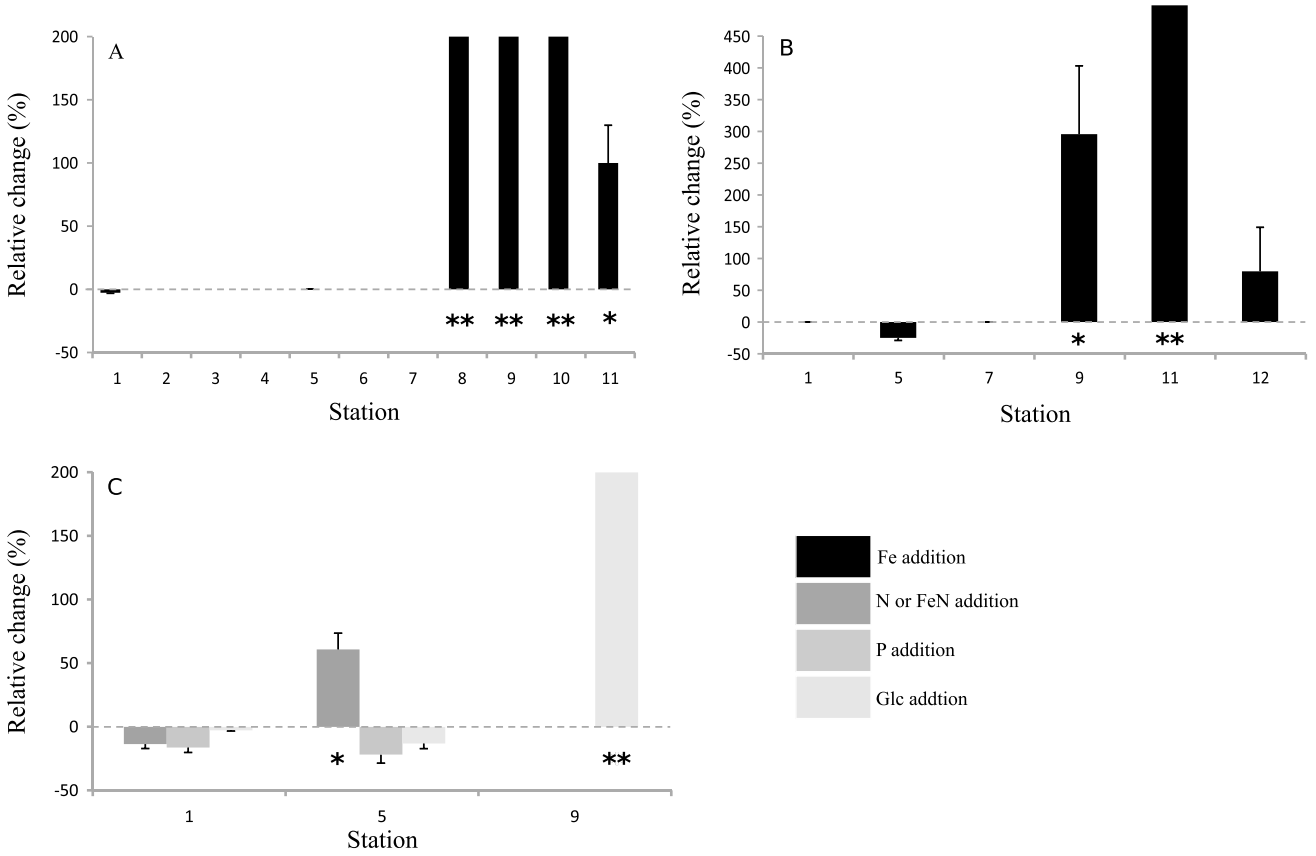

Figure 5. Relative change (%) in N_2 fixation in nutrient-amended bottles as compared with the control bottles for each station, after Fe addition in (a) 2010 and in (b) 2011, and after (c) N addition at station 1 and 9 or FeN addition at station 5 (dark gray), P addition (gray), Glc addition (light gray) in 2010. Relative change was calculated as 100 × ([mean in treatment − mean in control] / 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 N_2 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), N_2 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). O_2 concentrations decreased below 39 m depth and reached suboxic conditions at 101 m depth (Figure 4d). Surface NO_3^- and PO_4^{3-} 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, NO_3^- concentrations were intermediate between those of stations 1 (20°S) and 11 (10°S). PO_4^{3-} concentrations were the highest of the transect at this station.

3.2. Nutrient Controls of N_2 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 NO_3^- (<1.4 µmol L^{-1}) and PO_4^{3-} (<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 NO_3^- 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 PO_4^{3-} 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 N_2 Fixation in Contrasting Oceanic Environments

Location	Areal Rates ($\mu mol\, m^{-2}\, d^{-1}$)	Integration Depth (m)	Source
Eastern North Pacific gyre	520	Mixed layer	*Montoya et al*. [2004]
North Atlantic	59–898	15 (*Trichodesmium*)	*Capone et al*. [2005]
Tropical Atlantic	4–255	100	*Voss et al*. [2004]
Equatorial Pacific	18–358		*Bonnet et al*. [2009]
ETSP Coastal OMZ	7–190	120	*Fernandez et al*. [2011]
ETSP Subtropical gyre	12–190	150–200	*Halm et al*. [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. N_2 Fixation During Nutrient Sensitivity Assays

[30] In unamended triplicate controls, N_2 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, N_2 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), N_2 fixation was not stimulated by Fe additions. At stations 2, 3, 4, 6, 7, 8, 9, and 10, N_2 fixation was not detectable (<0.01 nmol N L^{-1} d^{-1}) in control (unamended) treatments. After Fe additions, N_2 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, $p > 0.05$) N_2 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 N_2 fixation. At station 9, Glc additions resulted in appearance of measurable N_2 fixation with rates of 0.25 ± 0.09 nmol N L^{-1} d^{-1} (Figure 5c).

[31] On the 2011 cruise, N_2 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 L^{-1} d^{-1}. At station 11 (Figure 5b), N_2 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 N_2 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 PO_4^{3-}. 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. N_2 Fixation in the ETSP

4.1.1. N_2 Fixation in Oligotrophic Conditions

[33] During both cruises, N_2 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 (BIOSOPE cruise, October–November 2004, Multivariate ENSO Index: 0.78).

[34] On the 2011 cruise, which took place during La Niña conditions, surface NO_3^- and PO_4^{3-} 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 NO_3^- depletion, probably caused by phytoplankton consumption, relatively high PO_4^{3-} and Fe concentrations remained (Table 1). These conditions are supposed to be the most favorable conditions for cyanobacterial N_2 fixation [*Sañudo-Wilhelmy et al*., 2001; *Berman-Frank et al*., 2001; *Mills et al*., 2004], but N_2 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). N_2 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 N_2 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 N_2 fixation varied from 0 to 148 μmol N m^{-2} d^{-1} during the 2010 cruise, and from 5 to 99 μmol N m^{-2} 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 N_2 Fixation

[35] In addition to N_2 fixation occurring in the euphotic zone of the southern (20°S) transect, significant N_2 fixation rates were also measured in the northern transect (HNLC waters), exhibiting surface NO_3^- concentrations of up to 6.98 μmol L^{-1}. HNLC waters are unusual ecosystems for N_2 fixation as these environments are characterized by cold waters, rich in NO_3^- 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] N_2 fixation was also active below the photic zone down to 200 m, in the core of the OMZ, despite NO_3^- 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 N_2 fixation in overlying euphotic zone waters [*Deutsch et al*., 2007]. Moreover, biological N_2 fixation is a strictly anaerobic process [*Falkowski*, 1997] due to the sensitivity and the irreversible inactivation of the nitrogenase enzyme by O_2 [*Burgess and Lowe*, 1996]. It is therefore possible that the low O_2 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 N_2 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 N_2-fixing organisms despite high NO_3^- concentrations. Our results support this hypothesis (Figures 3a and 3b), especially during the 2010 cruise, indicating that prevailing assumptions regarding N_2 fixation in N-depleted areas may be reevaluated.

[37] Active N_2 fixation in ecosystems with appreciable NO_3^-, 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 N_2 molecule in N_2 fixation is energetically more costly compared to the assimilation of NO_3^- [*Falkowski*, 1983; *Karl et al*., 2002, *Großkopf and Laroche*, 2012], which provides a thermodynamic rationale for community selection for NO_3^- utilizers when it is available. Moreover, NO_3^- is recognized to inhibit N_2 fixation activity of *Trichodesmium* [*Mulholland and Capone*, 2001], although even 10 μmol L^{-1} NO_3^- did not fully inhibit nitrogenase activity in *Trichodesmium* [*Holl and Montoya*, 2005]. Similarly, a recent study performed on *Crocosphaera watsonii* [*Dekaezemacker and Bonnet*, 2011] has shown that this strain is able to fix dinitrogen at high rates under 10 μmol L^{-1} of NO_3^-. A comparative study between these two cyanobacterial diazotrophs (*Trichodesmium* and *Crocosphaera*) [*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 NO_3^-: PO_4^{3-} 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 N_2 in the presence of NO_3^-. Indeed, areal (0–200 m) N_2 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 N_2 Fixation and Potential Impact on N Budget in the ETSP

[38] This study provides the first data of N_2 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 N_2 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 O_2 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 N_2 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 N_2 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 N_2 fixation is probably higher due to the large spatial extension of N_2 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 $^{15}N_2$ bubble method [*Montoya et al*., 1996] used for measuring N_2 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 N_2 fixation would likely be somewhat higher than that estimated above.

4.2. Nutrient Controls of N_2 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 NO_3^- utilization and primary productivity [*Martin et al*., 1994; *Price et al*., 1994]. Our results show that Fe availability can also be limiting for N_2 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 N_2 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 NO_3-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 NH_4^+ consumption to new production based on NO_3^- consumption [*Price et al*., 1991]. As N_2 fixation is a source of new N to the ocean and considered as new

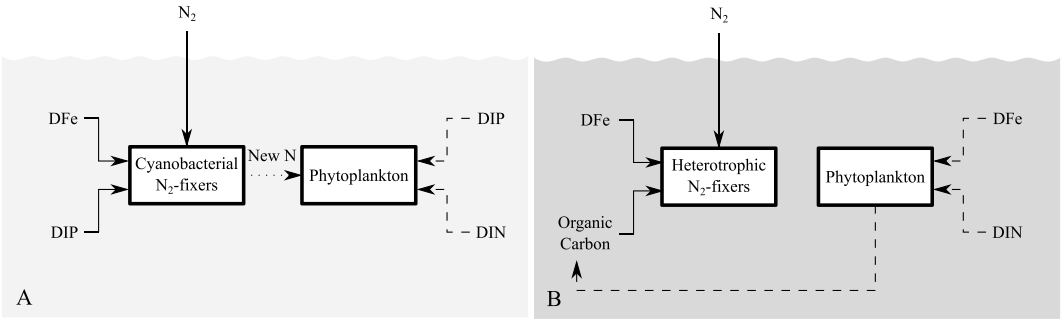

Figure 6. Schematic representations of N_2 fixation in different environments. (a) Conceptual N_2 fixation mainly performed by Cyanobacteria in oligotrophic areas of the oceans and directly controlled by DIP and DFe availabilities (solid arrows) and sustaining possibly the phytoplanktonic primary production by excretion of dissolved nitrogen (dotted arrow). (b) N_2 fixation in the N-rich waters of the ETSP, performed mainly by heterotrophic N_2-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 N_2 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 PO_4^{3-} and low NO_3^- concentrations, especially on the 20°S transect, one would predict that these macronutrient concentrations are ideal for N_2 fixation. However, even when Fe was added, no stimulation of N_2 fixation rates was observed (Figure 5a, station 5). When both Fe and NO_3^- were added, N_2 fixation was although stimulated (Figure 5c). Our results showed therefore that N additions did not inhibit N_2 fixation and indeed could stimulate the process. We can hypothesize here that primary production was stimulated by NO_3^- additions, which could increase dissolved organic carbon (DOC) excretion, enhance heterotrophic production, and in turn stimulate heterotrophic N_2 fixation for sustaining the N demand of Bacteria (Figure 6b). However additions of glucose did not increase N_2 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 N_2 fixation.

[41] The same indirect control of N_2 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, N_2 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 N_2 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 N_2 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 N_2 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 reevaluated in order to take into account heterotrophic diazotrophs and the possible linkage between phototrophs and heterotophs. 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 N_2 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 N_2 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 N_2 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 N_2 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 N_2 fixation.

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