Global Biogeochemical Cycles

Supporting Information for

[Dinitrogen fixation across physico-chemical gradients of the Eastern Tropical North Pacific oxygen deficient zone]

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Additional Supporting Information (Files uploaded separately)

Introduction

Supplementary information regarding materials and methods is provided (Text S1) alongside an N_2 fixation rate sensitivity analysis (Table S1) and a summary of all N_2 fixation rates measured and hydrographic properties (Table S2). In addition, we present a supplement to Fig. 3 in the text (Fig. S1).

Text S1. Supplemental materials and methods.

2.1 Hydrographic and nutrient measurements

Samples for chlorophyll *a* analysis via the non-acidification method (Welschmeyer, 1994) were filtered onto Whatman GF-75 filters (0.3 μm nominal pore size) and extracted in 90% acetone for 24 hours at -20°C. Filtrate for dissolved nutrient analysis was collected in sterile acid-washed (10% HCl) and sample-rinsed Falcon tubes directly from Niskin bottles through a 0.2 μm Supor cartridge filter. Detection limits for shipboard NO_3^-+NO_2^-, SRP, NO_2^- and NH_4^+ concentrations were 0.14, 0.03, 0.20 and 0.011 μM, respectively (3σ, n=7). The detection limit for chlorophyll *a* was 0.025 μg L^{-1} (3σ, n=7). The uncertainty reported for NO_3^- concentrations was calculated from the standard deviation of the NO_3^-+NO_2^- and NO_2^- concentrations using standard propagation of errors, and that of DIN, defined as the sum of NO_3^-+NO_2^- and NH_4^+ concentration, was calculated similarly from the standard deviation of these measurements. The detection limit for pump profiling system NO_3^-+NO_2^-, NO_2^-, and NH_4^+ concentrations were 0.30, 0.02, and 0.05 μM, respectively.

2.2 N_2 fixation incubation experiments – sample collection and handling

Site water from above and below the OMZ (EUPH and DEEP samples) was collected from Niskin bottles affixed to the CTD rosette. Incubation bottles were rinsed three times with sample water and then filled completely. Bottles were capped, and any remaining air was displaced by injecting site water. After the removal of any remaining air bubbles, highly enriched $^{15}N_2$ gas (~99%, Cambridge Isotopes, Tewksbury, MA) was added to initiate uptake experiments. While contamination of $^{15}N_2$ gas with isotopically-heavy NH_4^+ and NO_3^- has been noted as a concern in measuring N_2 fixation rates, contaminants have only been reported from Cambridge Isotopes stocks at trace levels (Dabundo et al., 2014). Furthermore, we routinely observed undetectable rates of N_2 fixation throughout our study area, in both DIN-deplete and replete waters, which would have been unlikely had there been contamination of our $^{15}N_2$ stock in the present study. To increase the rate of $^{15}N_2$ gas dissolution, sample bottles were gently inverted for 15 minutes on a large see-saw. The remaining gas bubble was then removed with a syringe so that the atom-% enrichment of the source pool was constant in bottles over the remainder of the incubation period.

Samples from within the OMZ were collected at and below the secondary NO_2^- maximum at every station, the depths of which were determined using the high resolution NO_2^- profiles generated from the autoanalyzer interfaced with the PPS. Water was pumped directly from depth into 4 L amber glass bottles. Each bottle was rinsed three times with sample water, filled from the bottom using the PPS hose, and submerged in a 50 L tub of ODZ water while continuing the flow of site water into the bottle. This arrangement allowed for a layer of low-oxygen water to be maintained above the bottle as it displaced the water already in the bottle thereby preventing contamination of the incubation water with air. As the bottles were continuously refilled, water at the surface of the tub was replaced with fresh, low oxygen water being displaced from the incubation bottles. The positive pressure achieved in the bottle further precluded the back-flow of oxygen-contaminated water from the tub into the

sample. Sufficient water was pumped from depth to displace the bottle volume at least three times before the bottle was capped underwater. This sampling process also served to maintain subsurface water temperatures as incubation bottles were being filled. The exposed portion of the PPS sampling tube was wrapped in black electrical tape to prevent light-shock of microbes, and tarps were hung above the tub to shade samples and reduce further warming. Once capped, sample bottles were treated as described above for those collected from above and below the OMZ.

2.3 N_2 fixation rate calculations and error analysis – estimation of uncertainty

Following Montoya et al. (1996) and Gradoville et al. (2017), a sensitivity analysis attributing error among these components, averaged by sample type, is presented in Table S1. When A_{N_2} data was not available, the arithmetic mean and standard deviation of all samples collected either from euphotic (3.78 ±1.05 atom-%) or subeuphotic (3.02 ±0.97 atom-%) waters were used instead. We also used initial PN concentration ([PN]) rather than the average of initial and final [PN] (Montoya et al., 1996) due to the better accuracy of the volume measurement associated with initial PN samples. The uncertainty of the initial PN concentration was calculated by propagating the standard deviation of three replicate PN mass samples and the error associated with volume measurement.

The incubation time is denoted as Δt. Uncertainty in Δt is affected by the length of filtration, which can be time-consuming for large-volume incubations, and biological processes may continue in the sample throughout this duration. For oligotrophic 1 and 4 L samples, we estimated maximum filtration times of 15 min and 1 h, respectively, representing uncertainties of approximately 1, 4, and 2% in the Δt of surface (24 h, 1 L), ODZ (24 h, 4 L), and deep (48 h, 4 L) incubations, respectively. This error was always greater than any variation in the Δt of triplicate incubations. The propagated error of each N_2 fixation rate was calculated as described by Montoya et al. (1996).

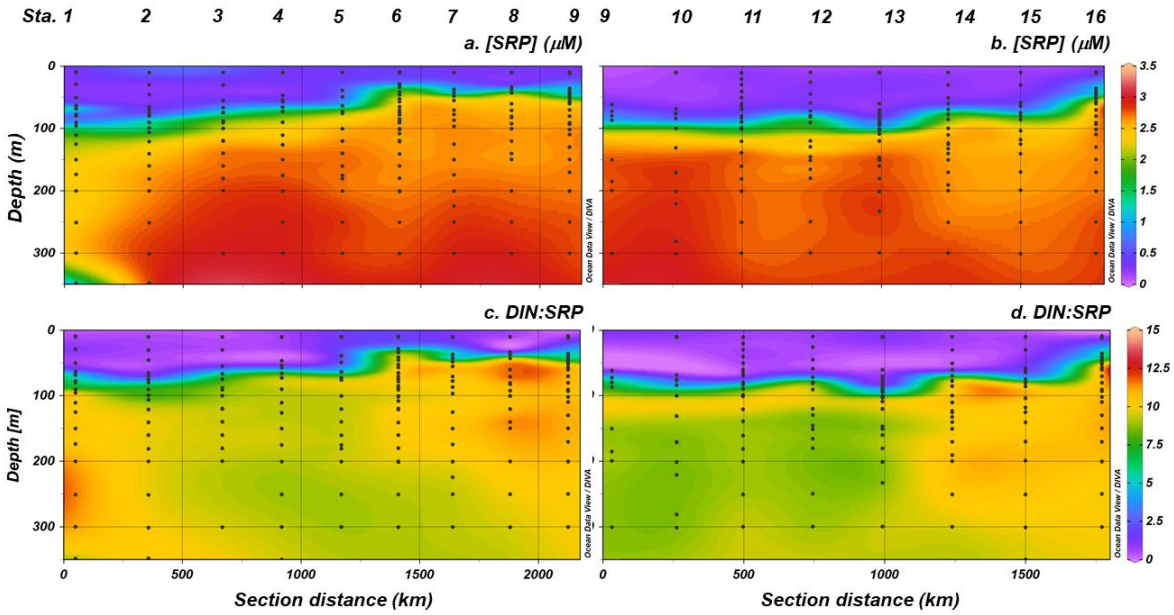

Figure S1. Soluble reactive phosphate concentrations ([SRP], a-b) and the ratio of dissolved inorganic nitrogen (defined as the sum of nitrate, nitrite and ammonium) to SRP in upper 350 m of nearshore (left) and offshore (right) transects. Black dots represent discrete sampling points.

Depth horizon	Parameter (X)	Average value	Error	δNFR/δX	Error contribution (Error × [δNFR/δX])^2	% Total error	Summary
EUPH	Δt	1.00	1.04×10^{-2}	-3.01×10^{0}	9.81×10^{-4}	0.03	Count……68
	A_{N_2}	3.83	7.06×10^{-1}	-7.32×10^{-1}	2.67×10^{-1}	6.68	Mean……3.05
	A_{PN_0}	0.370	9.26×10^{-4}	-1.86×10^{2}	2.98×10^{-2}	29.3	LOQ……2.60
	A_{PN_f}	0.386	1.09×10^{-2}	1.87×10^{2}	4.20×10^{0}	58.6	LOD……0.78
	PN mass	6.48×10^{2}	7.17×10^{1}	4.71×10^{-3}	1.14×10^{-1}	5.36	
	Volume	0.99	8.87×10^{-3}	0.00×10^{0}	7.27×10^{-4}	0.03	
	$[PN]_i$	6.51×10^{2}					
OMZ	Δt	1.25	4.17×10^{-2}	-9.73×10^{-1}	1.65×10^{-3}	0.29	Count……,,……39
	A_{N_2}	3.06	5.45×10^{-1}	-3.89×10^{-1}	4.50×10^{-2}	7.27	Mean……1.26
	A_{PN_0}	0.370	7.65×10^{-4}	-5.34×10^{1}	1.67×10^{-3}	27.9	LOQ……1.53
	A_{PN_f}	0.393	6.77×10^{-3}	5.39×10^{1}	1.33×10^{-1}	60.8	LOD……0.46
	PN mass	5.61×10^{2}	5.31×10^{1}	2.24×10^{-3}	1.42×10^{-2}	3.65	
	Volume	3.09	2.41×10^{-2}	1.21×10^{1}	9.48×10^{-5}	0.01	
	$[PN]_i$	1.81×10^{2}					
DEEP	Δt	1.92	4.17×10^{-2}	-2.12×10^{-1}	7.78×10^{-5}	0.10	Count……,,……12
	A_{N_2}	3.01	9.03×10^{-1}	-1.17×10^{-1}	1.12×10^{-2}	16.0	Mean……0.41
	A_{PN_0}	0.370	1.32×10^{-3}	-2.15×10^{1}	8.02×10^{-4}	34.8	LOQ……0.27
	A_{PN_f}	0.389	7.01×10^{-3}	2.16×10^{1}	2.30×10^{-2}	40.4	LOD……0.08
	PN mass	3.59×10^{2}	4.92×10^{1}	1.16×10^{-3}	3.23×10^{-3}	8.75	
	Volume	3.28	1.62×10^{-2}	8.08×10^{0}	4.14×10^{-6}	0.01	
	$[PN]_i$	1.09×10^{2}					

Table S1. Sensitivity analysis for N_2 fixation rate (NFR) calculation, performed as in Montoya et al. (1996) and Gradoville et al. (2017) on values averaged by depth horizon. Organic matter additions, bioassays and other measurements subject to extra manipulation are not included here. The parameters Δt, $\boldsymbol{A_{PN_0}}$, $\boldsymbol{A_{PN_f}}$ and $\boldsymbol{A_{N_2}}$ represent incubation length (days), initial and final particulate N (PN) isotopic composition (atom-%), $^{15}N_2$ enrichment (atom-%) and PN concentration ($[PN]_i$, nmol N L^{-1}). Error for these values was determined as described in the text and in Text S1. The partial derivative of NFR with respect to each parameter, calculated using the values in the third and fourth columns, is presented in the fifth column. The sixth and seventh columns show the absolute and relative contribution of each parameter to the total uncertainty in the final NFR. Finally, the count and mean of the total samples averaged, including those below detection and quantification, as well as the mean limit of quantification (LOQ) and limit of detection (LOD), are provided in the rightmost column.

Table S2. Summary of N_2 fixation rates (NFR) from above (EUPH), within (OMZ), and below (DEEP) the ETNP ODZ. Location, temperature (T), salinity (S), dissolved oxygen concentration ([O_2]), chlorophyll *a* concentration ([Chl a]), dissolved inorganic nitrogen ([DIN]) and soluble reactive phosphate ([SRP]) concentrations, and the limits of detection (LOD) and quantification (LOQ) corresponding to each rate measurement are reported. The propagated error for each measurement is given in parentheses; tildas show where samples were not collected or were lost. Rates reported as 'BDL' and 'DNQ' are below the LOD and LOQ, respectively.