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Key Points:
- Non-diazotrophic cyanobacteria create an environment of excess phosphate for their diazotrophic phylum group members
- Iron inputs determine the magnitude of nitrogen fixing rates
- Our findings are a step forward in understanding the biological controls on N_2 fixation

Supporting Information:
- Supporting Information S1

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Niche construction by non-diazotrophs for N_2 fixers in the eastern tropical North Atlantic Ocean

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Abstract Diazotrophic dinitrogen (N_2) fixation contributes ~76% to "new" nitrogen inputs to the sunlit open ocean, but environmental factors determining N_2 fixation rates are not well constrained. Excess phosphate (phosphate–nitrate/16 $>$ 0) and iron availability control N_2 fixation rates in the eastern tropical North Atlantic (ETNA), but it remains an open question how excess phosphate is generated within or supplied to the phosphate-depleted sunlit layer. Our observations in the ETNA region (8°N–15°N, 19°W–23°W) suggest that *Prochlorococcus* and *Synechococcus*, the two ubiquitous non-diazotrophic cyanobacteria with cellular N:P ratios higher than the Redfield ratio, create an environment of excess phosphate, which cannot be explained by diapycnal mixing, atmospheric, and riverine inputs. Thus, our results unveil a new biogeochemical niche construction mechanism by non-diazotrophic cyanobacteria for their diazotrophic phylum group members (N_2 fixers). Our observations may help to understand the prevalence of diazotrophy in low-phosphate, oligotrophic regions.

Plain Language Summary Diazotrophic cyanobacteria, only phytoplankton group capable to utilize dinitrogen as N source, flourish in warm oceans when phosphate and iron nutrients are available. However, some of the phosphate-depleted warm waters of the Atlantic Ocean are mysteriously known for their existence of diazotrophs. Upwelling fluxes, atmospheric deposition, and river inputs are depleted in phosphate compared to nitrate in this region. Hence, the reasons behind the source of excess phosphate supply for diazotrophy in this region are not known. Here we have discovered that non-diazotrophic cyanobacteria create an environment of excess phosphate through a virtual biological membrane for their diazotrophic phylum group members. In an environment of sustained phosphate supply in the oligotrophic waters, iron inputs seem to determine the magnitude of nitrogen fixing rates.

1. Introduction

In the open ocean, where bioavailable nitrogen (N) is scarce, the ability to fix dinitrogen (N_2) is a competitive advantage for diazotrophs [*Montoya et al*., 2004] who contribute ~76% to "new" nitrogen inputs to the sunlit open ocean (estimated from Table 3 in *Jickells et al*. [2017]). While numerous studies have shown that mixing, temperature, and organic matter concentration determine N_2 fixation rates [*Howarth et al*., 1988; *Paerl*, 1990; *Zehr and Paerl*, 2008], it is well established that iron and phosphorus are the primary factors which limit these rates [*Mills et al*., 2004; *Moore et al*., 2009]. Based on the idea that N_2 fixation requires phosphate (PO_4^{3-}), and not nitrate (NO_3^-), and that diazotrophs are outcompeted in regimes relatively high in NO_3^-, the global distribution of N_2 fixation rates have been estimated using the concept of $P^* = [PO_4^{3-}] - [NO_3^-]/R$ [*Deutsch et al*., 2007], where R (N:P = 16:1) is the canonical Redfield ratio [*Redfield*, 1958].

The tropical North Atlantic Ocean is a region where low $[PO_4^{3-}]$ limits primary production and N_2 fixation [*Sañudo-Wilhelmy et al*., 2001]. Yet N_2 fixation enigmatically occurs in this region at rates comparable to that at hot spots (e.g., the Arabian Sea) for diazotrophs in the world's oceans [*Capone et al*., 2005; *Luo et al*., 2014]. This enigma has long puzzled biogeochemists [*Landolfi et al*., 2015]. Recent studies have highlighted the role of Ekman advection and upwelling in supplying excess PO_4^{3-} to the euphotic zone of the equatorial Atlantic Ocean [*Palter et al*., 2011; *Subramaniam et al*., 2013; *Reynolds et al*., 2014]. However, since deeper water is NO_3^- rich and surface water is PO_4^{3-} depleted, how excess PO_4^{3-} could be supplied via upwelling in sunlit tropical waters remains debatable. Advective PO_4^{3-} transport could be limited to sustain N_2 fixation in the western Atlantic [*Palter et al*., 2011] and might be consumed on its way to the eastern Atlantic as whole equatorial to subtropical North Atlantic is PO_4^{3-} limited [*Sañudo-Wilhelmy et al*., 2001].

The eastern tropical North Atlantic (ETNA) is oligotrophic, adjacent to an ample iron source (Sahara desert), and accounts for substantial N_2 fixation globally [*Gruber and Sarmiento*, 1997], thus a suitable site to understand the role of nutrients in N_2 fixation [*Benavides and Voss*, 2015]. In the oligotrophic oceans, slow-growing picophytoplankton that are adapted to low-light conditions occupy the ecological niche at the base of the photic zone, effectively utilizing NO_x (NO_3^- + NO_2^-) at low concentrations [*Johnson and Lin*, 2009; *Martiny et al*., 2009] and leading to the conspicuous deep chlorophyll *a* maximum (DCM). The most prominent members of this group are the non-diazotrophic cyanobacteria *Prochlorococcus* and *Synechococcus*. Due to their N-rich protein machinery required for efficient nutrient uptake, they assimilate nutrients at a N:P ratio greater than the Redfield ratio [*Bertilsson et al*., 2003; *Kretz et al*., 2015], which can hypothetically construct a niche of excess PO_4^{3-} for their diazotrophic phylum group members. Here we tested this hypothesis and assessed the role and regulation of PO_4^{3-} in N_2 fixation rates through concurrent sampling of N_2 fixation rates, primary production, phytoplankton abundance, nutrient concentration, and diapycnal nutrient fluxes in the ETNA.

2. Materials and Methods

Isotopically enriched (99% in heavier isotopes) tracer incubations were performed during R/V *Meteor* cruise M97 in the ETNA at 13 stations (NF-1 to NF-13; Figure 1) during June 2013 to estimate N_2 fixation rates and primary production following *Mohr et al.* [2010]. Incubated filtered samples were analyzed for particulate organic carbon (POC) and nitrogen (PON) and their isotopic composition using an elemental analyzer coupled to an isotope ratio mass spectrometer.

Samples for nutrients [*Hansen and Koroleff*, 2007], pigments [*Mackey et al*., 1996; *Jeffrey et al*., 1999], phytoplankton [*Picheral et al*., 2010], and bacterial counts [*Marie et al*., 1999] were also analyzed. Aerosol optical depth (AOD) was measured using a Sun photometer [*Prasad et al*., 2007]. Microstructure observations combined with simultaneous nutrient profiles at seven sites were used to estimate diapycnal nutrient fluxes entering from below into the DCM (~50 m) and from the DCM into the mixed layer (ML; ~22 m). The uptake ratio in the DCM was estimated from the differences between fluxes into and out of the DCM [*Fischer et al*., 2013; *Schlundt et al*., 2014]. Microstructure profiles were performed with a tethered profiler (Sea&Sun Technology), immediately before or after the corresponding conductivity-temperature-depth and nutrient profile.

We performed a stepwise redundancy analysis (RDA) to assess the variance of N_2 fixation rates using physical, chemical, and biological parameters as explanatory variables [*Legendre and Legendre*, 2012; *Singh and Ramesh*, 2015]. After identifying the parameters that were correlated with N_2 fixation rates in the stepwise RDA, we used stepwise multiple linear regression (MLR) analysis to predict N_2 fixation rates as a function of these variables. A detailed methodology is provided in the supporting information [*Levitus*, 1982; *Montoya et al*., 1996].

3. Results

3.1. Biogeochemical Rates

We observed measurable to high depth-integrated N_2 fixation rates in the ETNA with values ranging from 9 to 77 μmol N m^{-2} d^{-1} (mean ± standard deviation, 32 ± 23 μmol N m^{-2} d^{-1}; Figure 1 and Table S1 in the supporting information). N_2 fixation rates decreased with depth, which was opposite to the primary production profile (Figure S1 in the supporting information). Integrated N_2 fixation rates were higher at NF-7 and NF-13 than at the other stations (Figure 1). Primary production ranged from 21 to 76 mmol C m^{-2} d^{-1} (49 ± 17 mmol C m^{-2} d^{-1}; Table S1).

3.2. Hydrography

Since the highest N_2 fixation rates were generally observed near the surface (0–10 m; Figure S1), we present average values of physical and biogeochemical parameters for 0–10 m (Table S1). We observed uniform sea surface temperature (SST; 27.7 ± 0.9°C), sea surface salinity (35.7 ± 0.1), and a shallow mixed layer depth (MLD; Table S1). The shallowest MLD (12 m) was at NF-13, while the deepest (42 m) was at NF-12. Mean nutrient concentrations were low in the ML (Figure 2). NO_x was close to the detection limit (0.02 μM) at most of the stations, while some PO_4^{3-} was present within the ML at all stations. Unlike NO_x, silicate (SiO_4) was always present at all the depths and ranged between 0.99 and 1.54 μM. P* ($[PO_4^{3-}] - [NO_x]/16$) was positive

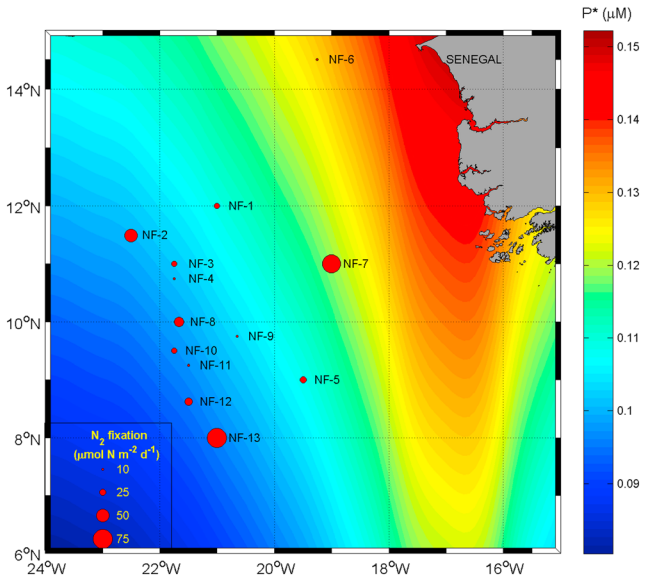

Figure 1. Euphotic depth-integrated N_2 fixation rates (bubble plot) during June 2013 overlaid on surface climatological P* distribution (nutrients data were obtained from World Ocean Atlas [*Garcia et al.*, 2014]).

within the ML, while it was mostly negative below the ML (Figure 2d). Median N:P ratio in inorganic nutrient flux was 19:1 from below toward the DCM, while the median ratio was 12:1 from the DCM toward the ML (Figures 3 and 4). Median N:P uptake ratio was 27:1 in the DCM.

3.3. Microbial Abundances

On average, *Prochlorococcus* (2×10^5 cells mL^{-1}) were most abundant followed by *Synechococcus* (2×10^4 cells mL^{-1}), *Nano-eukaryotes* (3×10^3 cells mL^{-1}), and *Pico-eukaryotes* (53 cells mL^{-1}) at the surface (Table S1). *Pico-eukaryote* abundance was highest at the subsurface depths (>5 m), while other cells were mostly uniformly distributed in the ML (Figure S2). Diatoms were virtually absent at all depths, while dinoflagellates were present in negligible amounts (4 ng Chl *a* L^{-1}; Table S1 and Figure S3). *Trichodesmium* were present in the euphotic zone (346 ± 638 colonies m^{-3}) with highest surface layer values at station NF-7 followed by NF-13 (Table S1 and Figure S2a).

Calculations based on the red fluorescence measured by means of flow cytometry revealed that *Prochlorococcus* and *Synechococcus* contributed 64% and 14%, respectively, to total red fluorescence, which suggests that these two genera dominated the phytoplankton community. This finding is further strengthened by estimates from the high-performance liquid chromatography data where bulk cyanobacteria (mainly *Prochlorococcus* and *Synechococcus*) contributed 58% to chlorophyll.

3.4. Regression Analysis

We observed excess PO_4^{3-} at all sampling stations. There was no correlation between N_2 fixation rates and excess PO_4^{3-}, but the rates were correlated ($r^2 = 0.20$, $p < 0.001$, no of data points $n = 52$) with in situ temperature. Surface rates tend to be anticorrelated ($r^2 = 0.29$, $p = 0.06$, $n = 52$) with MLD. Observed *Trichodesmium* abundance explained 50% variation in the N_2 fixation rates (Tables S1 and S12). While performing stepwise RDA and MLR, four variables (*Trichodesmium* abundance, sampling depth, salinity, and AOD) out of 22 variables (Table 1) could explain up to 66% ($r^2 = 0.66$, $p < 0.05$, $n = 51$) variability in the N_2 fixation rates. *Prochlorococcus* cell abundances were positively correlated with N_2 fixation rates, while other microorganisms were mostly anticorrelated (Table S1). In situ temperature was strongly correlated with volumetric fixation (Table S1) but did not show up as important in the RDA because depth already contains temperature information.

4. Discussion

4.1. Role of Non-Diazotrophs in N_2 Fixation

There are several theories on physical and chemical controls on N_2 fixation but there is none on the role of biological controls/feedback in regulating N_2 fixation rates. However, in a study that highlighted the positive impact of upwelling on N_2 fixation in the tropical Atlantic, *Subramaniam et al.* [2013] mentioned the idea that non-diazotrophic cyanobacteria may cause changes in nutrients' N:P stoichiometry. The authors did not, however, follow up in their analyses on this, neither has this been rigorously evaluated by others. Organisms, through their metabolic activities, can create their own niche by changing the environmental conditions—a process recognized as niche construction [*Odling-Smee et al.*, 1996]. Furthermore, organisms can create environments that suit other organisms—a phenomenon which is an extension of niche

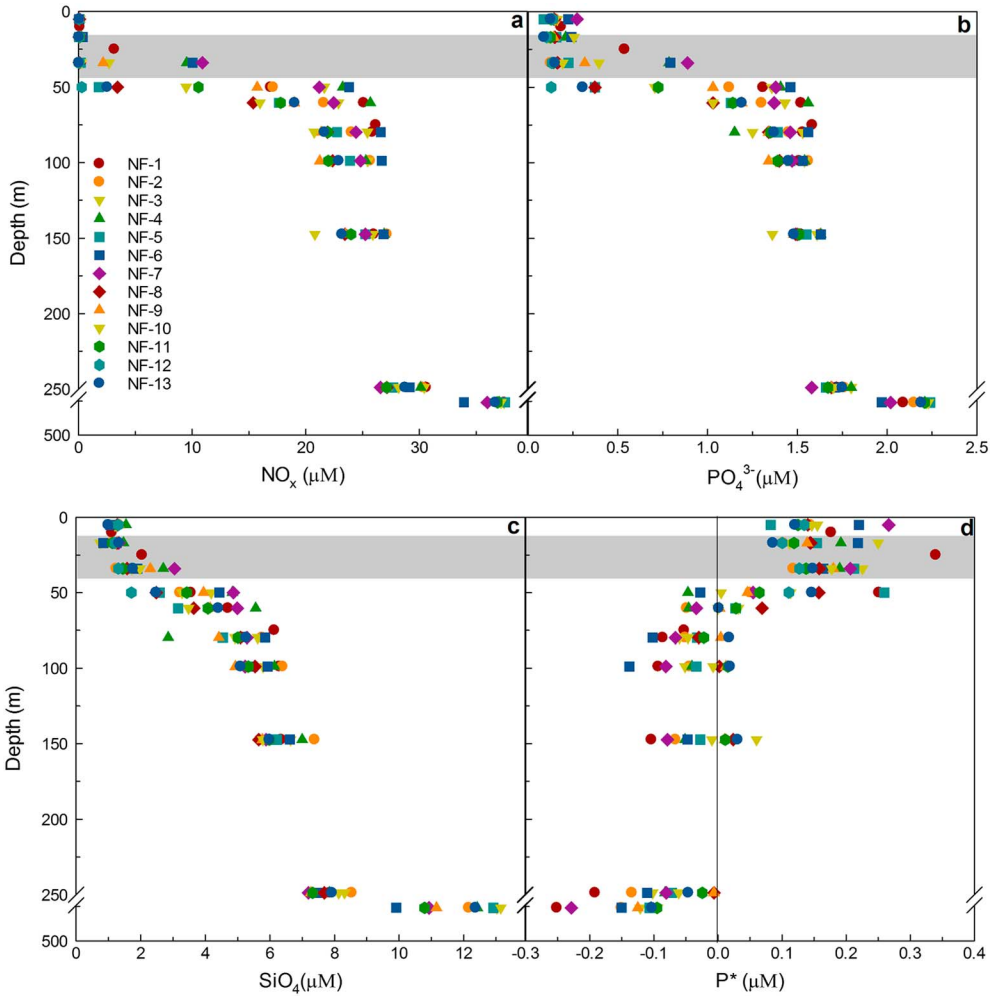

Figure 2. (a) NO_x (NO_3^- + NO_2^-), (b) PO_4^{3-}, (c) SiO_4, and (d) P* (=PO_4^{3-} − NO_x/16) at the stations shown in Figure 1. The top edge of the gray shaded area represents the minimum value (12 m) of MLD while the lower edge is the maximum (42 m). The vertical line in Figure 2d separates positive and negative values of P*. Note the break on the *y* axes at 250–448 m.

construction theory known as ecosystem engineering [*Jones et al.*, 1997]. In this study, we found that non-diazotrophic cyanobacteria (e.g., *Prochlorococcus*) alter the environment to the benefit of diazotrophs by generating excess P.

P* was positive within the ML, but mostly negative in subsurface water at some tens of meters below the ML (Figure 2d) as observed previously [*Gruber and Sarmiento*, 1997; *Singh et al.*, 2013]. Upward transport of nutrients by physical processes alone, like diapycnal mixing, would bring waters of negative P* into the surface waters and not increase surface P*.

We hypothesize that other sources of positive P* could be rivers that debouch into the Senegalese waters or atmospheric deposition (Figure 4). The influence of the Gambia River, which is a major river that debouches into the ETNA, is likely restricted to coastal areas [*Lesack et al.*, 1984]. Furthermore, it has NO_3^-:PO_4^{3-} higher (51:1) than the Redfield ratio and thus cannot contribute to excess PO_4^{3-} in the ETNA [*Lesack et al.*, 1984]. Recently, it has been suggested that atmospheric inputs contain more PO_4^{3-} over the Atlantic Ocean than previously suggested [*Gross et al.*, 2015], but overall N:P (1000:1) in aerosols is much higher than the Redfield ratio [*Baker et al.*, 2010]. Further, atmospheric PO_4^{3-} deposition is insufficient to support observed N_2 fixation rates in the ETNA [*Baker et al.*, 2007]. Hence, riverine and atmospheric sources of positive P* can be ruled out.

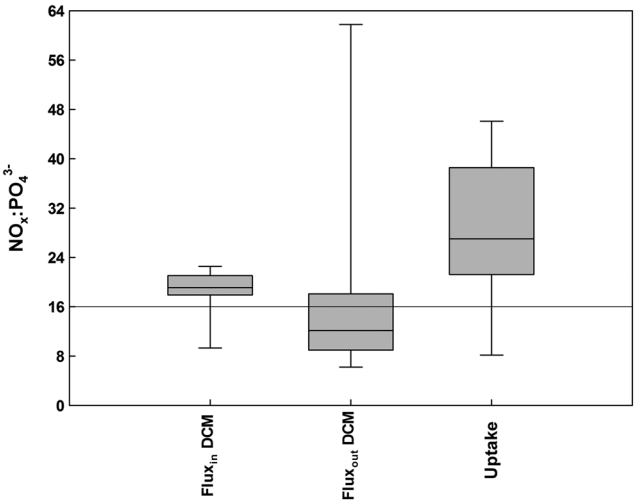

Figure 3. Box-whisker plot showing NO_x:PO_4^{3-} ratio in the upward diapycnal fluxes entering from below into the DCM (~50 m; shown as "$Flux_{in}$ DCM"), leaving the DCM into the MLD (~22 m; shown as "$Flux_{out}$ DCM") and biological uptake of nutrients (or loss of nutrients from the nutrients' pool in the DCM shown as "uptake") in the DCM. These values were estimated at seven locations in the ETNA (8°N–15°N, 19°W–23°W) during 2009–2013 on three different cruises, including M97. Box-whisker plot horizontal line in the boxes shows the median values, boxes are bounded on the top by the third quartile and on the bottom by the first quartile, and short horizontal lines at the extreme represent the minimum and maximum values. The horizontal line separates the ratios from the Redfield ratio (N:P = 16).

Modeled and measured values of dissolved organic matter (DOM) unanimously suggest that the ratio of dissolved organic nitrogen to that of phosphorus (DON:DOP) is higher (20–80) than the Redfield ratio in the ETNA [*Vidal et al*., 1999; *Franz et al*., 2012; *Letscher and Moore*, 2015] because DOP is remineralized about twice as fast as DON [*Letscher and Moore*, 2015]. Since the ETNA is phosphate limited, some fraction of DOP could be utilized by diazotrophs [*Dyhrman et al*., 2006; *Van Mooy et al*., 2012] before its remineralization could enhance P*. Moreover, recent analysis suggests that DOP constitutes only 23% of the total dissolved phosphorus pool in this region, lateral transport of which may not support diazotrophy in the ETNA because it is advected from the northwest African shelf toward the gyre interior [*Reynolds et al*., 2014]. Nonetheless, DOM, due to its remineralization, may partly explain the observed positive P* values.

So what else contributes to positive P* values? After exhausting all the probable factors that could contribute to positive P*, we found that positive P* values were driven by biology of the ETNA. Relatively PO_4^{3-}-rich water can be generated through above-Redfieldian uptake of NO_3^- associated with phytoplankton stoichiometry [*Mills and Arrigo*, 2010]. Indeed, slow-growing cyanobacteria, such as *Prochlorococcus* and *Synechococcus*, have higher cellular requirements of N than P [*Bertilsson et al*., 2003; *Kretz et al*., 2015]. In fact, these cyanobacteria synthesize a sugar membrane lipid rather than a lipid that contains PO_4^{3-} [*Van Mooy et al*., 2006], and as a result their average cellular N:P = 33:1 is higher than the Redfield ratio [*Singh et al*., 2015]. Accordingly, it does not come as a surprise that these cyanobacteria preferentially uptake NO_3^- over PO_4^{3-} [*Yin et al*., 2017].

PON:POP in the surface waters (30:1) and uptake ratio of NO_x and PO_4^{3-} (27:1) in the DCM (Figure 3) reflect *Prochlorococcus* and *Synechococcus* stoichiometry (Table S1 and Figure 3). *Prochlorococcus* and *Synechococcus* were the most abundant cells throughout the ML and dominated in terms of autotrophic biomass (Table S1 and Figures S2 and S3). Other phytoplankton with N:P ratios lower than the Redfield ratio, such as diatoms [*Klausmeier et al*., 2004], were virtually absent in our sampling area (Table S1 and Figures S2 and S3). Furthermore, the biomass of other microorganisms (e.g., Cryptophytes) was negligible compared to *Prochlorococcus* (Table S1 and Figure S3). Even if *Pico-* and *Nano-eukaryotes* were abundant, they would not have caused a great change in NO_3^-:PO_4^{3-} stoichiometry as their N:P ratio is similar to the Redfield ratio [*Singh et al*., 2015]. The low NO_x:PO_4^{3-} (12:1) in the water leaving the DCM toward the ML suggests that the positive P* in the ML is generated by *Prochlorococcus* and *Synechococcus* while the water crossed the DCM (Figures 3 and 4). In other words, the picocyanobacteria-rich DCM layer functions like a biological membrane that filters out part of the NO_x from the upwelled water before it reaches to the surface (Figure 4).

Median N supply into the DCM was 2.5 nmol m^{-2} s^{-1}, which was less than the estimated mean N demand by *Prochlorococcus* and *Synechococcus* (4.2 nmol m^{-2} s^{-1}; calculated by assuming that *Prochlorococcus* and *Synechococcus* complete their N uptake quota within 24 h [*Partensky et al*., 1999]). It suggests that regenerated nutrients fulfill 40% of the N demand of *Prochlorococcus* and *Synechococcus*. Thus, our analysis allows

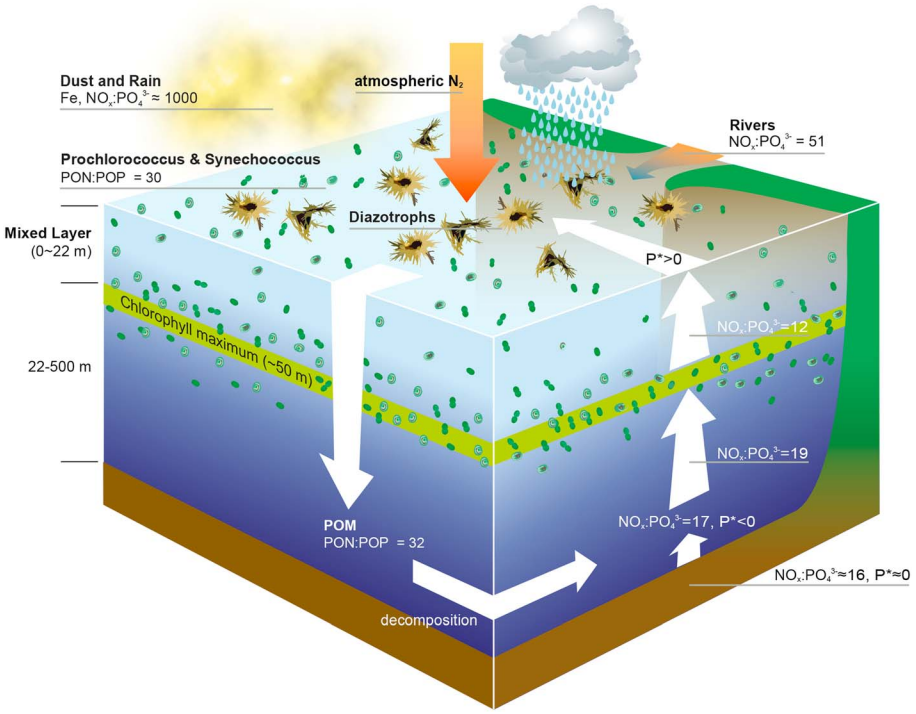

Figure 4. Diagram shows the evidences in support of our main conclusion that excess PO_4^{3-} is generated by biogeochemical processes rather than by upwelling/diapycnal mixing. The brown color in the bottom represents the water below 500 m depth. All the estimates mentioned in the diagram are from our data, except for the NO_x:PO_4^{3-} ratios in fluxes from waters below 500 m [*Gruber and Sarmiento*, 1997; *Singh et al*., 2015], rivers [*Lesack et al*., 1984], and atmospheric deposition [*Baker et al*., 2010].

the conclusion that positive P* in the ML is generated by non-diazotrophic cyanobacteria in and above the DCM (Figure 4).

4.2. Role of SST, MLD, and Macronutrients in N_2 Fixation

Our sampling area is characterized by low NO_x, a shallow MLD, and high SST (Table S1); hence, our observations are in agreement with earlier studies, which suggested that N_2 fixation occurs in oligotrophic, warm, and stratified waters [*Staal et al*., 2003; *Langlois et al*., 2008; *Ratten et al*., 2015].

Diazotrophs fix atmospheric N_2 but their growth is constrained by [PO_4^{3-}] [*Wu et al*., 2000; *Sohm et al*., 2008]. Combining a global biogeochemical ocean model with observed climatology of [PO_4^{3-}] and [NO_3^-] suggested that denitrification and N_2 fixation occurs in proximity to each other because denitrification creates an environment of excess PO_4^{3-} [*Deutsch et al*., 2007]. However, we found no correlation of N_2 fixation rates with [PO_4^{3-}] (Table S1). Furthermore, to our surprise, PO_4^{3-} and P* were the least correlated parameters with N_2 fixation rates in the stepwise MLR (Table 1). This is in line with earlier observations where higher [PO_4^{3-}] was not associated with higher N_2 fixation rates in the ETNA [*Staal et al*., 2007] and elsewhere (e.g., the Indian Ocean [*Shiozaki et al*., 2014]). However, no correlation of N_2 fixation rates with [PO_4^{3-}] must be interpreted with caution. No correlation does not mean that N_2 fixation is independent of PO_4^{3-}: instead, we would rather conclude that at the time of our sampling, all phosphorous requirements to sustain N_2 fixation were fulfilled, and other factors (such as Fe) constrained the rates. [PO_4^{3-}] was above the detection limit (0.02 μM), unlike in previous observations [*Wu et al*., 2000]. Furthermore, P* was always positive within the ML at all the stations (Figures 2a and 2d). Positive P* distribution from the World Ocean Atlas surface climatological data also matches with our observations (Figure 1), although the subeuphotic water in the North Atlantic Ocean is slightly PO_4^{3-} deficient [*Gruber and Sarmiento*, 1997].

Table 1. Statistical Analysis of the 22 Environmental Parameters With Volumetric N_2 Fixation Rates of All the Data

Stepwise Redundancy Analysis (RDA)[a]			
Variable	Partial Explained	Cumulative Explained	*p*
***Trichodesmium* (colonies m^{-3})**	0.4969	0.4969	0.01
Depth (m)	0.2071	0.6011	0.01
Aerosol optical depth at 500 nm	0.0767	0.6317	0.01
Salinity	0.0844	0.6628	0.01
Nano-eukaryotes (cells mL^{-1})	0.0152	0.6800	0.46
Synechococcus (cells mL^{-1})	0.0387	0.7154	0.90
Dinoflagellates (ng L^{-1})	0.0100	0.7182	0.54
Prochlorococcus (cells mL^{-1})	0.0096	0.7209	0.29
NO_x (μM)	0.0187	0.7261	0.38
Mixed layer depth (m)	0.0142	0.7300	0.40
Chrysophytes (ng L^{-1})	0.0061	0.7317	0.72
Cryptophytes (ng L^{-1})	0.0055	0.7331	1
Chlorophytes (ng L^{-1})	0.0069	0.7350	0.56
Chlorophyll *a* (ng L^{-1})	0.0193	0.7401	0.60
Prymnesiophytes (ng L^{-1})	0.0377	0.7499	0.54
Primary productivity (nM N h^{-1})	0.0192	0.7547	0.29
SiO_4 (μM)	0.0220	0.7601	0.29
Temperature (°C)	0.0135	0.7633	0.40
PO_4^{3-} (μM)	0.0024	0.7639	0.85
Prasinophytes (ng L^{-1})	0.0014	0.7642	0.99
Pico-eukaryotes (cells mL^{-1})	10^{-3}	0.7644	0.72
P* (μM)	10^{-29}	0.7644	0.52
Stepwise Linear Regression			
Variable	Estimate	Standard Error	*p*
Intercept	1.647	0.779	0.04
Trichodesmium (colonies m^{-3})	4.875×10^{-5}	0.738×10^{-5}	10^{-8}
Depth (m)	-9.981×10^{-4}	2.520×10^{-4}	10^{-4}
Salinity	-4.523×10^{-2}	2.178×10^{-2}	0.04

[a]Variables significant in RDA (in bold) were used in multiple regression.

4.3. Role of Fe in N_2 Fixation

Fe is an essential nutrient for nitrogenase enzymes, which are responsible for N_2 fixation [*Raven*, 1988]. Although we did not measure Fe on the cruise, there are parameters in our observations that can be used to understand the role of Fe in N_2 fixation. The ETNA, being in the proximity of the Saharan desert, receives Fe through dust deposition [*Jickells et al*., 2005]. Dust is deposited into the ocean through rain (wet deposition) and gravitational settling (dry deposition) [*Duce et al*., 1991]. Since surface salinity is decreased by rain, it can be used as an indicator for wet deposition; while AOD can be used as an indicator of dry deposition. Salinity has been used as an indicator of the migration of the Intertropical Convergence Zone (a phenomenon related to the rainfall patterns [*Capone*, 2014]), which was located in the north of equator, i.e., in our sampling area during June 2013 (Figure S4) [*Kalnay et al*., 1996]. Increase in rainfall results in an increase in the Fe deposition in the Atlantic Ocean, which eventually enhances N_2 fixation [*Schlosser et al*., 2014]. Stepwise RDA revealed that salinity and AOD are the only two parameters (other than *Trichodesmium*, depth and, thus, light) that significantly ($p < 0.05$) explain a part of the variation in N_2 fixation rates (Table 1). A negative salinity coefficient in the stepwise linear regression implies increase in N_2 fixation due to increase in freshwater input, while the positive AOD coefficient suggests increase in N_2 fixation rates at higher amounts of suspended dust in the atmosphere. Increase in dust deposition due to increase in rainfall would be inevitable in the ETNA as the troposphere in this region contains dust perennially [*Jickells et al*., 2005]. Hence, we infer that Fe deposition determines the magnitude of N_2 fixation rates in the ETNA, as suggested previously for ETNA and other oceans [*Langlois et al*., 2008; *Shiozaki et al*., 2014]. Bioassay experiments have also shown that both Fe and Saharan dust addition can increase N_2 fixation rates in the ETNA [*Langlois et al*., 2012; *Krupke et al*., 2014]. Having identified Fe as a limiting nutrient, PO_4^{3-} might be not be limiting since only one element limits the growth of organisms at any given time as proposed by the Liebig's law of the minimum [*Odum et al*., 1971].

5. Conclusions

Concurrent data on microorganisms' abundance, nutrients, and N_2 fixation rates from the ETNA suggest a major role that non-diazotrophic cyanobacteria play in generating excess PO_4^{3-} for N_2 fixation. *Prochlorococcus and Synechococcus* seem to establish an environment of low NO_3^- and excess PO_4^{3-} in the ecosystem as schematically summarized in the Figure 4. PO_4^{3-} is prerequisite allowing N_2 fixation to occur as it strengthens competitive advantage of diazotrophs over faster growing microorganisms, such as diatoms. DOM stoichiometry may partly explain excess PO_4^{3-} but it is a complex pool of nutrients and requires a better understanding of its uptake from the field measurements. The role of atmospheric and river inputs in developing excess PO_4^{3-} can be ruled out as both of these inputs have N:P ratios much higher than the Redfield ratio in this region. Statistical analysis suggests that the magnitude of N_2 fixation rates is influenced by Fe deposition. Our findings are a step forward in understanding the biological controls—in addition to the role of physical and chemical controls—on N_2 fixation. Our analysis could be used to improve the parameterization of N_2 fixation rates in biogeochemical models since current models do not consider the niche construction by non-diazotrophs to facilitate N_2 fixation.

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