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# $N_2$ Fixation in the Eastern Arabian Sea: Probable Role of Heterotrophic Diazotrophs

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Biogeochemical implications of global imbalance between the rates of marine dinitrogen ($N_2$) fixation and denitrification have spurred us to understand the former process in the Arabian Sea, which contributes considerably to the global nitrogen budget. Heterotrophic bacteria have gained recent appreciation for their major role in marine N budget by fixing a significant amount of $N_2$. Accordingly, we hypothesize a probable role of heterotrophic diazotrophs from the $^{15}N_2$ enriched isotope labeling dark incubations that witnessed rates comparable to the light incubations in the eastern Arabian Sea during spring 2010. Maximum areal rates (8 mmol N $m^{-2}$ $d^{-1}$) were the highest ever observed anywhere in world oceans. Our results suggest that the eastern Arabian Sea gains ~92% of its new nitrogen through $N_2$ fixation. Our results are consistent with the observations made in the same region in preceding year, i.e., during the spring of 2009.

**Keywords: dinitrogen fixation, $^{15}$N, $^{13}$C, nitrogen budget, carbon uptake rate, nutrients, biogeochemistry, Arabian Sea**

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## INTRODUCTION

Reactive nitrogen (e.g., $NO_3^-$) is an important substrate for marine primary producers because dinitrogen ($N_2$), though the most abundant gas in the Earth's atmosphere, is unassimilable by most photosynthetic organisms (Middelburg and Nieuwenhuize, 2000). However, marine diazotrophic cyanobacteria (e.g., *Trichodesmium*) have an enzymatic advantage to convert atmospheric $N_2$ gas to a bioavailable form of nitrogen (such as $NH_4^+$). These diazotrophs are distributed over the oligotrophic tropical and sub-tropical marine environments, e.g., in the Arabian Sea located in the northwest Indian Ocean (Capone and Carpenter, 1982; Jickells et al., 2017).

The Arabian Sea is a hotspot for studying $N_2$ fixation. Strong summer monsoonal winds cause intense upwelling over the western Arabian Sea enhancing primary productivity over the Somali coast (Prasannakumar et al., 2001). Further, the northern and central Arabian Seas are known for high productivity during winter, caused by the cooling-driven deep convection (Madhupratap et al., 1996; Singh and Ramesh, 2015). High biological production in the surface layers and its subsequent export leads to oxygen depletion in the subsurface layers (Naqvi and Jayakumar, 2000) that further triggers denitrification process, i.e., the release of $N_2$ and $N_2O$ back to the atmosphere from nitrate ($NO_3^-$) reduction. Denitrification in the oxygen minimum zones would deplete only $NO_3^-$ thereby lowering the nitrogen: phosphorus (N:P) ratio in dissolved nutrient pool (Deutsch et al., 2007). An imbalance between $N_2$ fixation and denitrification rates based on N:P stoichiometry is an imperative problem in the marine nitrogen budget (Codispoti, 2007). $N_2$ fixation and denitrification dominate ocean N sources and sinks processes, respectively (Codispoti et al., 2001).

Global nitrogen loss and gain rates are in imbalance, indicating either an overestimation of the loss processes or an underestimation of the gain processes (Codispoti, 2007). The recent recognition of greater diversity (Zehr et al., 2001, 2003) and wider distribution (Hewson et al., 2007; Mulholland, 2007) of marine diazotrophs than had been appreciated hitherto (Mahaffey et al., 2005) suggested an underestimation of $N_2$ fixation. Contribution of atmospheric deposition and riverine nutrients to primary production is minor in the Arabian Sea (Singh and Ramesh, 2011; Singh et al., 2012) which further supports the fact that $N_2$ fixation is a major process in this region. The Arabian Sea witnesses diverse group of diazotrophs, which may fix $N_2$ at varying rates (Mulholland and Capone, 2009). During spring and autumn seasons, calmer winds, warmer waters, and shallower mixed layers make the Arabian Sea oligotrophic, thus creating a niche for *Trichodesmium* blooms (Gandhi et al., 2011). The seasonal occurrence of $N_2$ fixation over the Arabian Sea makes it an unique region for studying nitrogen budget (Naqvi, 1987; Capone et al., 1998).

*Trichodesmium* is not the only species which fixes $N_2$, as there are some other fixers such as $\gamma$- Proteobacteria, and other small heterotrophs also contribute substantially to $N_2$ fixation rates (Zehr et al., 1995). $N_2$ fixation might be mediated by a variety of auto and heterotrophic bacterial community in the eastern Arabian Sea, a region of rather limited information. Previously estimated rates (Gandhi et al., 2011) were surprisingly high; so in this study, we revisited the $N_2$ fixation and carbon uptake rates over the eastern Arabian Sea to verify the veracity of the reported higher rates, using the $^{15}N_2$ gas tracer technique (Montoya et al., 1996). We report measured $N_2$ fixation rates for dark and light conditions and discuss the possible reasons for this estimated rates.

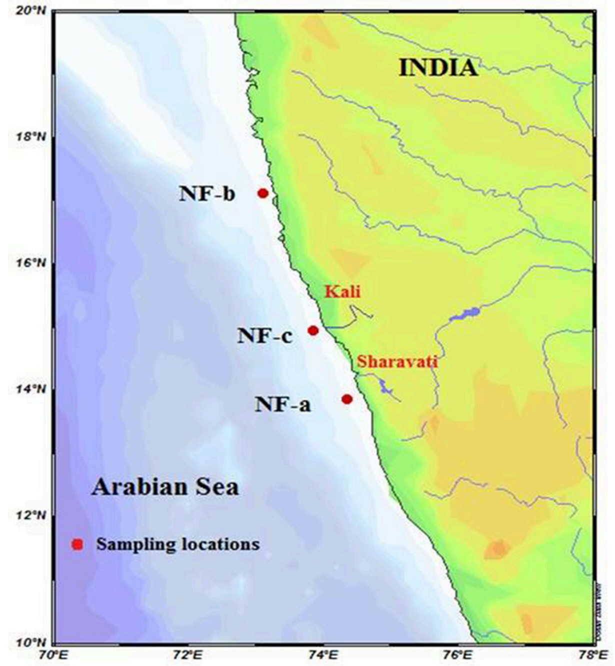


**FIGURE 1 | Sea water sampling locations (filled circles) NF-a, NF-b and NF-c, during *ORV Sagar Manjusha* cruise May-2010.**

# MATERIALS AND METHODS

## Sampling for Incubation Experiments

Water samples were collected using Niskin bottles (bottles were closed by a messenger) from the four different depths (0, 5, 10, and 20 m) within the euphotic zone at three locations (NF-a, NF-b and NF-c that have station depths 42, 37, and 37 m, respectively) over the eastern Arabian Sea, during *ORV Sagar Manjusha* cruise during 10–14 May 2010 (**Figure 1**). Duplicate samples were taken from each depth in 1.225 L polycarbonate Nalgene bottles. All the bottles filled without headspace followed by the addition of $^{15}N_2$ gas (bubble method) with the chromatographic gas tight syringe (Montoya et al., 1996). Two milliliters of $^{15}N_2$ gas (99% $^{15}N$ enriched gas from Cambridge Isotope Laboratories, Inc. USA) and 1 ml of 0.2 mmol ml$^{-1}$ $NaH^{13}CO_3$ (99% $^{13}C$ enriched) tracers were injected to each bottle (final enrichment of 16.6% for $^{15}N$ and 8.5% for $^{13}C$) before the start of the incubations, which were performed during 10:00–14:00 h, i.e., symmetric to local noon. Tracer added bottles were covered with the calibrated neutral density filters to simulate the irradiance at the depths from which the samples were taken. After the incubations, the samples were filtered sequentially through pre-combusted (4 h at 400°C) Whatmann GF/F filters (25 mm diameter and 0.7 μm pore size), washed with filtered sea water, dried in an oven at 50°C overnight and stored for further mass spectrometric analysis. At each station, 2 L surface seawater was collected for measuring the nitrogen isotopic composition of natural particulate organic nitrogen (PON) and carbon (POC).

## Mass Spectrometric Analysis and Calculation of Rates

Elemental analyzer interface with continuous flow mass spectrometer at the Physical Research Laboratory, Ahmedabad was used to measure the PON, POC, atom % $^{15}N$ and atom % $^{13}C$ in the samples. Volumetric rate of $N_2$ fixation were calculated following (Montoya et al., 1996).

$$\text{N uptake rate} = (1/t)[(A_{PNf} - AN_0)/(A_{Nenrich} - A_{PN0})] \times [PON]_f \quad (1)$$

Where, $A_{PN0}$ = $^{15}N$ atom% in PON at the start of experiment, $A_{PNf}$ = $^{15}N$ atom% in PON at the end of experiment, $t$ = time of incubation (4 hrs), $[PON]_f$ = concentration of PON at the end of the experiment and $A_{Nenrich}$ = $^{15}N$ enrichment in the dissolved form after tracer addition at the start of the incubation, which is estimated as:

$$A_{Nenrich} = (^{15}N_{tracer} \times \text{ tracer conc} + {}^{15}N_{natural} \times \text{ ambient conc})/(\text{tracer conc} + \text{natural conc}) \quad (2)$$

Surface water samples (at 0–1 m depth) were also incubated in complete dark condition and these estimates are attributed to the presence of heterotrophic diazotrophs and their contribution to

$N_2$ fixation. It has been discovered that in the "bubble method," only 40% enrichment can be achieved in 4 h incubations, which further results in 40% underestimation in the rates (Mohr et al., 2010). Hence, we multiplied $N_2$ fixation rates by a constant factor of 2.5 to avoid possible underestimation in the bubble gas technique. Later, it was discovered that the underestimation of rates is community dependent-there is less underestimation for *Trichodesmium* compared to that for other diazotrophs. Our sampling area witnesses *Trichodesmium* so underestimation in bubble method was less (Großkopf et al., 2012; White, 2012; Klawonn et al., 2015). We still multiplied our rates by 2.5 because we compared our results with Gandhi et al. (2011), who multiplied by the same factor. Carbon uptake rate is calculated by substituting N and $^{15}$N by C and $^{13}$C, respectively, in Equations (1) and (2) (Slawyk et al., 1977). Areal rates were calculated from the volumetric rates using the trapezoidal rule of integration, i.e., mean of volumetric rates were multiplied by the corresponding depth interval and then summed for all the depth intervals. Hundred milliliters of each sample was separately collected for nutrient measurements using a SKALAR auto analyzer at the offshore laboratory.

Spatial distribution of sea surface temperature (SST) was plotted using the Gridded High Resolution Sea Surface Temperature: Operational sea surface temperature and sea ice analysis (GHRSST: OSTIA) satellite data (Donlon et al., 2012). The mixed layer depth (MLD) was estimated based on the temperature criterion (0.2°C difference from the SST; de Boyer Montégut et al., 2004). Salinity values were obtained from the CTD data, values ranged from 35.23 to 35.56 at the sampling locations (**Figure 2**).

## RESULTS AND DISCUSSION

### Hydrography

Temperature profiles from a portable CTD at sampling locations NF-a, NF-b, and NF-c showed that MLD varied between 17 and 20 m (**Figure 2**). Surface temperature at NF-b was ~1.4°C less than that at the other two stations. Temperature measurements obtained from CTD at surface level were reproduced by the satellite remote sensing data images (**Figure 3**). Salinity values obtained from CTD varied from 35.23 to 35.56 at the surface with maximum at NF-b and minimum at NF-c. NF-b is possibly influenced by Western India Coastal Current (WICC), which brings convecting mixing driven colder water from the north to the south during pre-monsoon (Schott and McCreary, 2001) as evidenced in the temperature profile (**Figure 1**).

Dissolved oxygen from the same CTD casts showed a dip of 2 mg $L^{-1}$ at 3 m depth at sampling location NF-c, whereas there was no vertical gradient in oxygen in the upper 20 m at locations NF-a and NF-b (**Figure 2**). Oxygen showed a sudden decline at all the stations below 20 m depth. Lower temperature at NF-b was associated with the detectable $SiO_4$ values, whereas the dip in the dissolved oxygen was associated with the maximum $NO_x$ ($NO_2^-$ + $NO_3^-$), phosphate ($PO_4^{3-}$) values and higher N:P ratios at NF-c (**Table 1**).

### Nutrients

Nutrient concentrations were higher at the surface and decreased with depth, with maximum surface value at NF-c (**Figure 4**). $NO_3^-$ varied from 0.37 to 2.42 μM while $PO_4^{3-}$ ranged from 0 to 0.71 μM (**Table 1**). At sampling station NF-b, $SiO_4$ values were readily detectable with maximum at surface (1.69 μM) and decreased with depth (**Table 1**). We calculated P* that indicates $PO_4^{3-}$ concentration deviations from the Redfield ratio (N:P = 16):

$$P^* = [PO_4^{3-}] - [NO_3^-]/16 \tag{3}$$

Positive P* indicates $PO_4^{3-}$ concentrations in excess compared to $NO_3^-$ (Deutsch et al., 2007). Surface water P*-values were mostly in excess (except at 20 m at NF-c) and varied from −0.06 to 0.56 μM (**Table 1**). P*-values at surface showed increasing trend from NF-a to NF-c, and decreased with depth at all the three locations. $PO_4^{3-}$ and $NO_3^-$ maxima at NF-c could be due to the upwelling of subsurface water. Low P* at NF-a could also be either due to the consumption of phosphate by diazotrophy or due to the upwelling, which starts during May (Gupta et al., 2016) as evidenced in the temperature profile at NF-a (**Figure 2**).

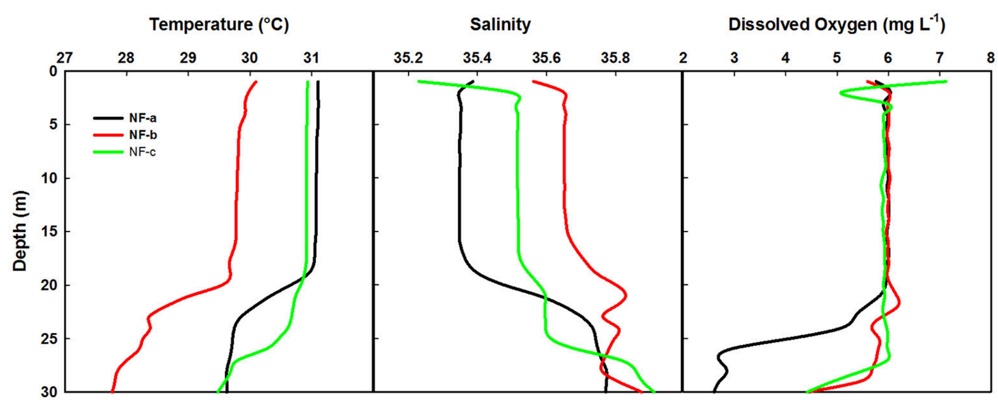


**FIGURE 2 | Temperature, salinity and dissolved $O_2$ profiles at the three sampling locations based on the CTD data.**

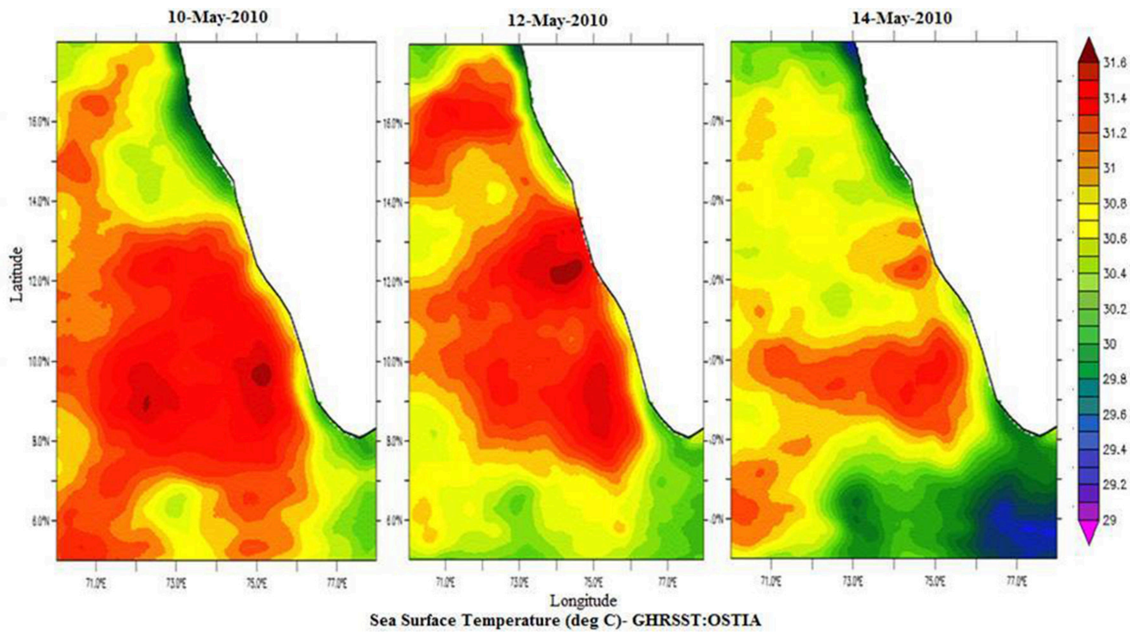


**FIGURE 3 | Spatial distribution of Sea Surface Temperature (°C) for 10th May-2010, 12th May-2010, and 14th May-2010 from GHRSST: OSTIA, http://apdrc.soest.hawaii.edu/las/v6/constrain?var=4928.**

Upwelling would create low oxygen just below MLD (Gupta et al., 2016; Sudheesh et al., 2016), as observed at NF-a (**Figure 2**). However, high P*-values would have been expected in low oxygen water but deeper water, as evidenced from the P* profiles (**Table 1**), has low P*—possibly because of the remineralization of diazotrophy dominated organic matter (high N:P ratio) in the deeper depths. $PO_4^{3-}$ was below the detection limit at 20 m depth at NF-c yet there was $N_2$ fixation. This could again be attributed to the $PO_4^{3-}$ consumption by diazotrophs.

N:P ratio was less than the Redfield ratio (positive P*, **Table 1**) at all stations with maximum at NF- c, which could have resulted in nitrogen limitation and further would have facilitated of $N_2$ fixation. $N_2$ fixation is controlled by iron and $PO_4^{3-}$ availability (Wu et al., 2000; Capone, 2001; Sañudo-Wilhelmy et al., 2001; Mulholland, 2007). At these sampling locations, $N_2$ fixation may be regulated by iron input as $PO_4^{3-}$ is not the limiting nutrient in the northern Indian Ocean as suggested by P*-values (Shiozaki et al., 2014). Iron limitation is likely, as some part of the western Arabian Sea has been highlighted as a high nutrient low chlorophyll (HNLC) region (Naqvi et al., 2010; Moffett et al., 2015). Changes in the response of external forcing (e.g., seasonal monsoon and upwelling events) and inputs (e.g., aeolian and river influx) have specific control on $N_2$ fixation over the basin (Mulholland and Capone, 2009). POC and PON varied between 15 and 73 μM and 3–51 μM, respectively (**Table 1**), but did not show any correlation with C and $N_2$ fixation rates. However, the highest values of POC, PON, C, and $N_2$ fixation rates were observed at the surface at NF-a.

## $N_2$ Fixation Rate and Carbon Uptake

$N_2$ fixation rates varied from 4 to 238 nM N $h^{-1}$, while carbon uptake rate ranged between 16 and 1628 nM C $h^{-1}$ (**Figure 5**). Our $N_2$ fixation rates were higher than those synthesized by Capone et al. (2008) in the world oceans (0–5.4 nM N $h^{-1}$). The highest values for both carbon uptake and $N_2$ fixation rate for light incubation fixation (**Figure 5**) were observed at NF-a lying south of the other two sampling locations (**Figure 1**). $N_2$ fixation rates were higher in dark incubations compared to the light incubations at the surface level, except for NF-a (44.93, 63.32, and 164.7 nM N $h^{-1}$ at NF-a, NF-b, and NF-c, respectively, **Figure 6**). Whereas carbon uptake rates in the light incubations were an order of magnitude higher than in the dark incubations (**Figure 6**). Higher $N_2$ fixation rates for the dark incubations compared to light might be attributable to the presence of heterotrophic species at the surface. Heterotrophs contributed up to 52% to the total $N_2$ fixation (estimated from light and dark incubations, assuming light incubations correspond to phototrophic and dark to heterotrophic). Heterotrophic contribution to $N_2$ fixation could be even higher in the deeper waters due to favorable conditions for them. These higher values associated with dark incubation suggest that heterotrophic $N_2$ fixers might play an important role in fixing $N_2$ over the eastern Arabian Sea. In the surface waters of the Arabian Sea, the DNA and RNA recovered during the southwest monsoon periods were also categorized as those of heterotrophic bacteria (Jayakumar et al., 2012; Bird and Wyman, 2013). Therefore, our results suggest that an active $N_2$ fixation by heterotrophic bacteria could occur in

**TABLE 1 | Sampling Date, Latitude (°N), Longitude (°E), water depth (m), Nutrients concentrations (μM), N:P, P* (μM), particulate organic carbon (POC, μM) and nitrogen (PON, μM), $N_2$ fixation (nM N $h^{-1}$) and carbon (C) uptake (nM C $h^{-1}$) at the three locations sampled in the eastern Arabian Sea.**

| Date and Station | Lat | Long | Depth | $NO_2$ | $NO_3$ | $PO_4^{3-}$ | $SiO_4$ | N:P | P* | POC | PON | $N_2$ fixation | C uptake |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10-May-10; NF-a | 13.87 | 74.36 | 0 | 0.04 | 0.37 | 0.22 | 0.00 | 1.66 | 0.20 | 73 | 51 | 238.07 | 1628 |
| | | | 5 | 0.04 | 0.66 | 0.13 | 0.00 | 4.94 | 0.10 | 26 | 5 | 5.05 | 173 |
| | | | 10 | 0.06 | 0.76 | 0.18 | 0.00 | 4.25 | 0.13 | 21 | 7 | 6.68 | 121 |
| | | | 20 | 0.02 | 0.45 | 0.13 | 0.19 | 3.34 | 0.10 | 23 | 5 | 5.98 | 78 |
| 12-May-10; NF-b | 17.12 | 73.11 | 0 | 0.06 | 0.76 | 0.53 | 1.69 | 1.42 | 0.49 | 24 | 6 | 6.63 | 68 |
| | | | 5 | 0.02 | 0.97 | 0.31 | 1.44 | 3.11 | 0.25 | 23 | 5 | 4.88 | 39 |
| | | | 10 | 0.00 | 0.67 | 0.27 | 0.63 | 2.51 | 0.22 | 21 | 4 | 4.01 | 50 |
| | | | 20 | 0.00 | 0.81 | 0.22 | 0.94 | 3.66 | 0.17 | 18 | 4 | 4.78 | 58 |
| 14-May-10; NF-c | 14.96 | 73.84 | 0 | 0.14 | 2.42 | 0.71 | 0.00 | 3.40 | 0.56 | 44 | 25 | 8.70 | 24 |
| | | | 5 | 0.12 | 0.90 | 0.22 | 0.00 | 4.05 | 0.17 | 20 | 4 | 6.19 | 22 |
| | | | 10 | 0.12 | 0.58 | 0.09 | 0.00 | 6.53 | 0.05 | 16 | 4 | 5.38 | 24 |
| | | | 20 | 0.02 | 0.91 | 0.00 | 0.00 | NA | −0.06 | 15 | 3 | 6.15 | 16 |

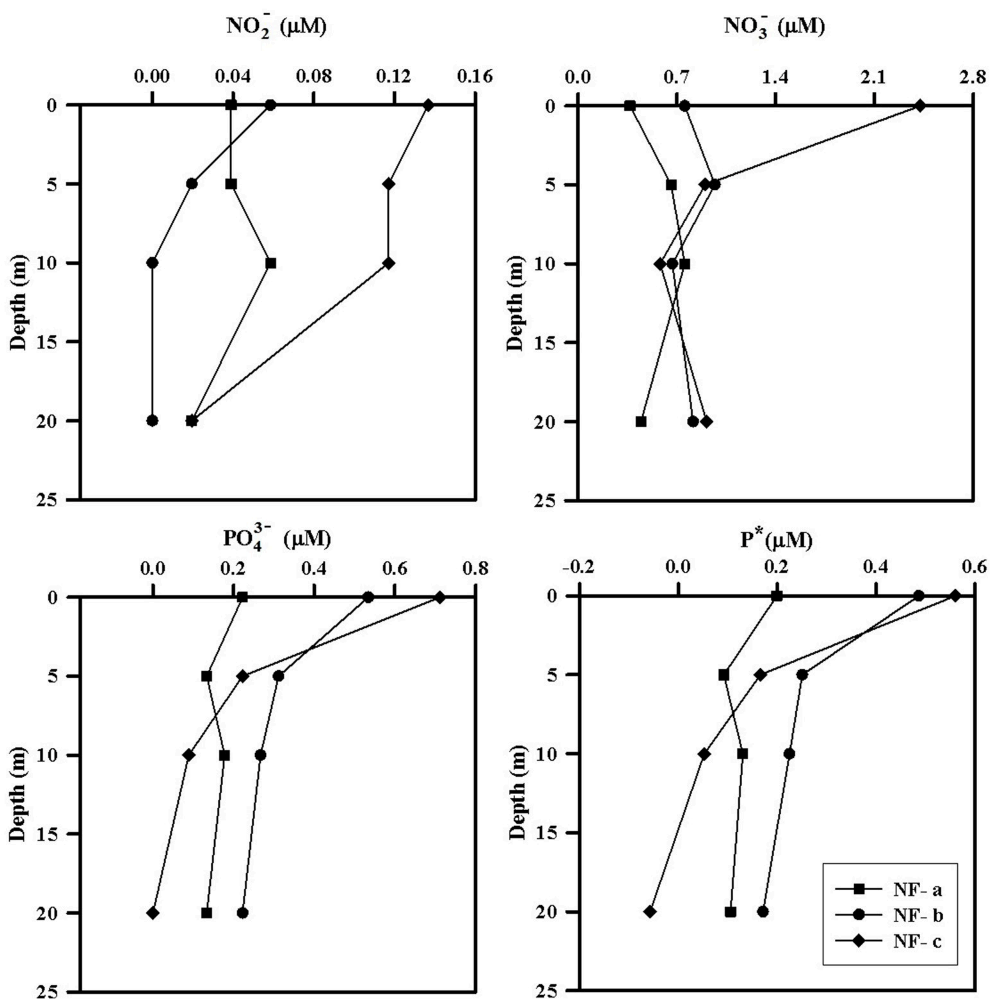


**FIGURE 4 | Depth profiles of measured nutrients ($NO_2^-$, $NO_3^-$, $PO_4^{3-}$) and P*, at sampling locations NF-a, NF-b, and NF-c.**

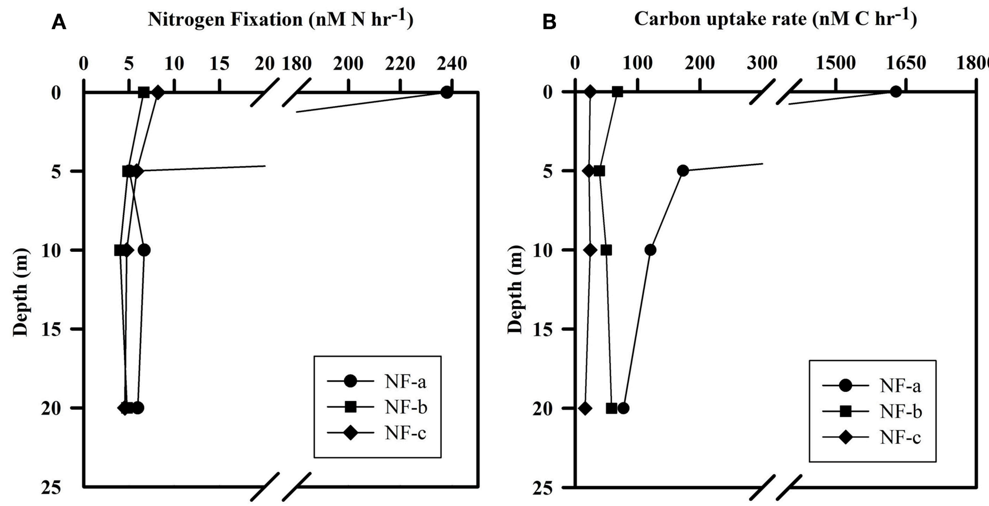


**FIGURE 5 | (A) $N_2$ fixation rate and (B) Carbon uptake rate at the sampling location (NF-a, NF-b, and NF-c) for light (at ambient conditions) incubation for samples at different depths.**

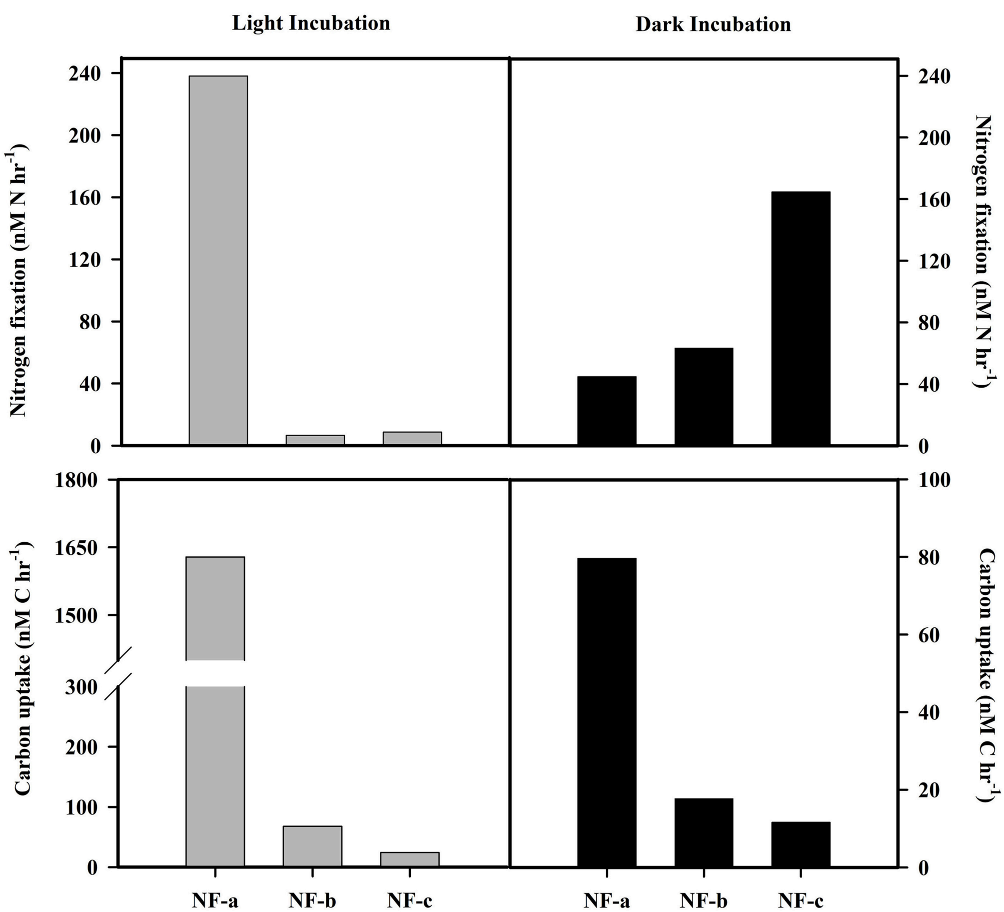


**FIGURE 6 | $N_2$ fixation and carbon uptake rate at surface for light (gray bars) and dark (black bars) incubation.**

the surface water of the eastern Arabian Sea (Shiozaki et al., 2014).

Euphotic-depth integrated autotrophic $N_2$ fixation and carbon uptake rates were highest at NF-a, where $N_2$ fixation rate was $8.4 \pm 2.8$ mmol N $m^{-2}$ $d^{-1}$ (standard deviation of duplicate samples) and carbon uptake rate was $74.7 \pm 17.7$ mmol C $m^{-2}$ $d^{-1}$. Our areal rates (8 mmol N $m^{-2}$ $d^{-1}$) were the highest ever observed in anywhere in world oceans (**Table 2**) and comparable to those measured by Gandhi et al. (2011) in the similar region-suggesting that the high rates during the spring 2009 were not episodic rather it could be a regular phenomenon during the spring in the Arabian Sea. High rates in the eastern Arabian Sea are probably due to the availability of both the essential nutrients for diazotrophs, i.e., iron and $PO_4^{3-}$. Iron is available because this region unlike the western Arabian Sea is close to the *Thar* desert, whereas the western Arabian Sea is recognized to be an HNLC region (Naqvi et al., 2010). $PO_4^{3-}$ is available because upwelled water brings $PO_4^{3-}$ rich (compared to $NO_3^-$) water as $NO_3^-$ is lost in denitrification. So the Arabian Sea is unique basin for sustaining diazotrophy and the higher rates of $N_2$ fixation. On

**TABLE 2 | Summary of Photic $N_2$ fixation rates in the world oceans (Table updated after Singh et al., 2013; Benavides and Voss, 2015).**

| Methodology | Areal Rates (μmol N $m^{-2}$ $d^{-1}$) | Region | References |
|---|---|---|---|
| Acetylene Reduction Assay | 239 | Western tropical North Atlantic | Capone et al., 2005 |
| $^{15}N_2$ tracer-bubble method | 850 | Western tropical North Atlantic | Capone et al., 2005 |
| $^{15}N_2$ tracer-bubble method | 2.4–532 | Western North Atlantic | Mulholland et al., 2012 |
| Extrapolation | 714–3,571 | North Atlantic Ocean | Carpenter and Romans, 1991 |
| Extrapolation | 160–430 | North Atlantic Ocean | Lipschultz and Owens, 1996 |
| $N^* = [NO_3^-] - 16 \times [PO_4^{3-}] + 2.72$ | 500–2,500 | North Atlantic Ocean | Michaels et al., 1996 |
| $N^* = ([NO_3^-] - 16 \times [PO_4^{3-}] + 2.90) \times 0.87$ | 197 | North Atlantic Ocean | Gruber and Sarmiento, 1997 |
| $DIN_{XS} = [NO_3^-] - 16 \times [PO_4^{3-}]$ | 70–208 | North Atlantic Ocean | Hansell et al., 2004 |
| $P^* = [PO_4^{3-}] - [NO_3^-]/16$ | 63 | North Atlantic Ocean | Deutsch et al., 2007 |
| $P^* = [PO_4^{3-}] - [NO_3^-]/16$ | 151–178 | North Atlantic Ocean | Palter et al., 2011 |
| $^{15}N_2$ tracer-bubble method | 1.2–298 | North Atlantic Ocean | FernÁndez et al., 2010 |
| $^{15}N_2$ tracer-bubble method | 1.8–182 | North Atlantic Ocean | Moore et al., 2009 |
| $^{15}N_2$ tracer-dissolution method | 91 ± 4 | Atlantic Ocean | Großkopf et al., 2012 |
| $^{15}N$-nitrate and ammonium | 4.5–68.1 | Tropical Northwest Atlantic | Goering et al., 1966 |
| $^{15}N_2$- bubble and Acetylene Reduction Assay | 73–90 | Tropical Northwest Atlantic | Falcón et al., 2004 |
| $^{15}N_2$ tracer-bubble method | 41–93 | Sargasso Sea | Orcutt et al., 2001 |
| $DIN_{XS} = [NO_3^-] - 16 \times [PO_4^{3-}]$ | 45–259 | Sargasso Sea | Bates and Hansell, 2004 |
| $N^* = [NO_3^-] - 14.63 \times [PO_4^{3-}]$ | 120 ± 9 | Sargasso Sea | Singh et al., 2013 |
| Acetylene Reduction Assay | 0.05–540 | Subtropical Northeast Atlantic | Carpenter and Price, 1977 |
| Acetylene Reduction Assay | 0.001–0.09 | Subtropical Northeast Atlantic | Benavides et al., 2011 |
| $^{15}N_2$ tracer-bubble method | 28–142 | Tropical Northeast Atlantic | Turk et al., 2011 |
| $^{15}N_2$ tracer-bubble method | 56–60 | Tropical Northeast Atlantic | Turk-Kubo et al., 2012 |
| $^{15}N_2$ tracer-bubble method | 15–424 | Eastern equatorial Atlantic | Subramaniam et al., 2013 |
| $^{15}N_2$ tracer-bubble method | 0–148 | Eastern tropical south Pacific tropical South Pacific | Dekaezemacker et al., 2013 |
| $^{15}N_2$ tracer-bubble method | up to 800 | Off Peru-South Pacific | Loescher et al., 2014 |
| $^{15}N_2$ tracer-bubble method | 0–23 | Eastern tropical South Pacific | Knapp et al., 2016 |
| $^{15}N_2$ tracer-bubble method | 520 ± 160 | Eastern North Pacific gyre | Montoya et al., 2004 |
| $^{15}N_2$ tracer-bubble method | 126 ± 47 | Timor–Arafura–Coral seas | Montoya et al., 2004 |
| $^{15}N_2$ tracer-bubble method | 3,995 | Arafura Sea | Montoya et al., 2004 |
| Acetylene Reduction Assay | 85 | Subtropical North Pacific Ocean | Karl et al., 1997 |
| $^{15}N_2$ tracer-bubble method | 1–13 | South China Sea | Chen et al., 2008 |
| $^{15}N_2$ tracer-bubble method | 0–90 | North Pacific Ocean | Shiozaki et al., 2010 |
| $^{15}N_2$ tracer-bubble method | 20–310 | North Pacific Ocean | Church et al., 2009 |
| Acetylene Reduction Assay | 170 | Central Arabian Sea | Capone et al., 1998 |
| $^{15}N_2$ tracer-bubble method | 24.6–47.1 | Arabian Sea | Shiozaki et al., 2014 |
| $^{15}N_2$ tracer-bubble method | 6.27–16.6 | Equatorial and southern Indian Ocean | Shiozaki et al., 2014 |
| $^{15}N_2$ tracer-bubble method* | 100–34,000 | Eastern Arabian Sea | Gandhi et al., 2011 |
| $^{15}N_2$ tracer-bubble method | 174–238 | Southeastern Arabian Sea | Bhavya et al., 2016 |
| $^{15}N_2$ tracer-bubble method* | 1,140–8,405 | Eastern Arabian Sea | This study |

**These rates were corrected for bubble in-equilibrium.*

the other hand, the other major oceans, like the Atlantic does not have enough phosphate (Wu et al., 2000), while the Pacific does not have enough iron (Behrenfeld and Kolber, 1999).

Gandhi et al. (2011) showed an overall increasing trend in the $N_2$ fixation rate from south to north, whereas in this present study, it was in the opposite direction. $N_2$ fixation supplies a large portion of the new N that supports marine productivity (Capone, 1997; Karl et al., 2002; LaRoche and Breitbarth, 2005; Mahaffey et al., 2005). Estimated contribution of new nitrogen to $N_2$ fixation in the present study is about 92% of new nitrogen, which is the same as that reported by the previous study (Gandhi et al., 2011).

Most observations around the world oceans are indirectly derived from geochemical estimates that are based on sub-surface nutrient distribution (**Table 2**). We have directly measured $N_2$ fixation using $^{15}N_2$ tracer, which is still the best available method despite its inherent problems of incomplete dissolution of the bubble (Großkopf et al., 2012). Our findings of high $N_2$ fixation in this region have large implications in understanding marine C, N, and cycles. If we extrapolate these high rates over the Arabian Sea, then we might find the missing nitrogen inputs (Codispoti, 2007). But we require to conduct more $N_2$ fixation experiments using the dissolution method in this region for extrapolation. In addition to seasonal upwelling, $N_2$ fixation might play a role in making the Arabian Sea perennially productive (Singh and Ramesh, 2015). Higher $N_2$ fixation and its degradation in the subsurface waters may also be useful in understating the phosphorous cycle-low P* at the deeper waters and higher P* at the surface-contrary to what is expected from the denitrified waters.

## CONCLUSION

Results from the three sampling locations in the eastern Arabian Sea suggest that the Arabian Sea witnessed the highest ever rates of the $N_2$ fixation among the world ocean for the two consecutive springs. Out of all nitrogen gain processes, about 92% of new nitrogen is gained through $N_2$ fixation only, with highest areal rates (8 mmol N $m^{-2}$ $d^{-1}$). $N_2$ fixation rate for the dark was higher than the light incubation at the surface except for NF-a, which alluded to the presence of heterotrophic species. Based on the higher $N_2$ fixation values at the surface for dark incubation, we hypothesize that heterotrophic fixers dominantly (about 52% of total $N_2$ fixation is by heterotrophs) play an important role in fixing $N_2$.

## AUTHOR CONTRIBUTIONS

AS and RR designed research; AS, RR, and NT performed research; KK and AS analyzed data; and KK and AS wrote the manuscript with major inputs from all the co-authors.

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