

Severe phosphate limitation on nitrogen fixation in the Bay of Bengal

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Abstract

Several anticyclonic (ACE) and cyclonic (CE) eddies constitute the circulation in the Bay of Bengal (BoB) and are associated with the downwelling and upwelling processes leading to oligotrophic and eutrophic conditions respectively. In this study, the nitrogen (N₂) fixation rates and controlling factors are estimated through deck incubation experiments in the BoB using enriched N₂ gas dissolution method. We observed measurable concentrations of dissolved inorganic nitrogen (DIN) and phosphate in the CE and close to detection limits in the ACE in the mixed layer. Photic zone integrated N₂ fixation rates ranged between 53.3 and 194.1 $\mu\text{mol m}^{-2} \text{d}^{-1}$ with lower rates in the ACE ($91 \pm 18 \mu\text{mol m}^{-2} \text{d}^{-1}$) than CE ($162 \pm 28 \mu\text{mol m}^{-2} \text{d}^{-1}$) and no eddy regions (NE; $138 \pm 27 \mu\text{mol m}^{-2} \text{d}^{-1}$). The photic zone integrated N₂ fixation rates are linearly correlated with photic zone integrated chlorophyll *a* and the mean phosphate concentrations in the photic zone suggesting that phosphate is controlling the N₂ fixation in the BoB. The observed high N:P ratio (25 ± 3) also indicate that severe phosphate limitation in the BoB. This is further confirmed from increase in N₂ fixation rates by 1.2 to 8 times due to artificial increase in phosphate from that rates at *in situ* phosphate concentrations. This study suggests that though the conditions are conducive for N₂ fixation in the BoB, the removal of dissolved phosphate within the estuaries opened to the BoB, provide weaker inputs from subsurface due to stratification and less input from atmospheric dust may limit N₂ fixation in the BoB.

1. Introduction

Dissolved inorganic nitrogen (DIN) is one of the essential elements for biological production in the ocean, and it is depleted in most parts of the world ocean in the mixed layer due to strong stratification (Dugdale and Goering, 1967; Montoya et al., 2004). The major source of DIN to the mixed layer comes from subsurface layers through vertical mixing and small fraction is added through atmospheric deposition (Eppley and Peterson, 1979; Platt et al., 1992; Srinivas et al., 2011a). The supply of DIN determines the amount of primary production and its transport to the deeper layers through sinking particles in the tropical ocean (Eppley and Peterson, 1979; Platt et al., 1992; Capone et al., 2005). The warm, stratified waters and depleted nitrogen concentrations are conducive for some groups of phytoplankton (eg: cyanobacteria) to fix atmospheric nitrogen to support primary production (Breitbarth et al., 2007; Bergman et al., 2012). The biological N_2 fixation is found to be a significant external source of nitrogen to the ocean surface and contributes to the oceanic nitrogen budget (Falkowski et al., 1998; Deutsch et al., 2007).

Anticyclonic eddies (ACEs) are reported to be regions of active N_2 fixation (Fong et al., 2008; Loscher et al., 2016) due to lack of DIN and warm water column. In contrast, occurrence of N_2 fixation is reported in the high DIN regions as well (Fernandez et al., 2011; Hamersley et al., 2011; Loescher et al., 2014; Jayakumar et al., 2017). The N_2 fixation is an energy consuming process as dissolved N_2 needs to be converted to ammonium by N_2 fixing organisms, such as *Trichodesmium* spp., before it is assimilated (Karl et al., 2002).

Several cyclonic (CE) and anticyclonic eddies (ACE) constitute the circulation in the Bay of Bengal (BoB) (Chen et al., 2012; Dandapath and Chakraborty, 2016) and they have significant impact on the primary production, oxygen minimum zone and nutrients distribution in the BoB (Prasanna Kumar et al., 2004; Sarma et al., 2018; 2019a; Sridevi and Sarma, 2020). Eddies are generally formed by baroclinic and barotropic instabilities. In the case of BoB, local Ekman pumping, remote forcing from equatorial Indian Ocean and internal instability together play important role to the formation of eddies (Cheng et al., 2018). The equatorial Kelvin waves are a special type of Kelvin wave that balances Coriolis force. The seasonal westerly winds or intraseasonal westerly wind bursts over the equatorial Indian Ocean force the generation of these equatorial Kelvin waves and they always propagate eastward with higher zonal velocity but meridional velocity. Once equatorial Kelvin waves touches the Sumatra coast, they bifurcate into coastal Kelvin waves and propagate poleward along the eastern boundary of the BoB. While coastal Kelvin waves move towards pole, they radiate Rossby waves, also known as planetary waves, naturally occurring in rotating fluids, and forms due to conservation of potential vorticity, and propagating westward into the interior Bay (Rao et al.,

2009;Chatterjee et al., 2017). Due to interaction with the coastal currents, these Rossby waves generate instabilities leading to formation of eddies (Chen et al., 2012; Cheng et al., 2013). It was shown that Rossby wave predominantly generate baroclinic instabilities (Chen et al., 2012; Cheng et al., 2013; Chatterjee et al., 2017; Cheng et al., 2018), however, they are found to be weaker in the BoB. Rossby waves, generated from the coastal Kelvin waves, are significantly altered by the Andaman Nicobar Islands and increase eddy activity in the BoB (Cheng et al., 2018; Mukherjee et al., 2019).

The Bay of Bengal (BoB) is classically reported as low productive region due to weak nutrient supply caused by strong salinity stratification (Prasanna Kumar et al., 2002; 2004; Gauns et al., 2005). Perennial DIN depletion is normally observed in the surface waters of the BoB due to strong stratification caused by high river discharge that hampers vertical DIN supply (Madhupratap et al., 2003; Gauns et al., 2005; Sarma et al., 2016). The nutrient flux from the major estuaries opened to the BoB revealed that 95% of the riverine inorganic nitrogen and phosphate are removed within the estuaries and the remaining nutrients were utilized within 10 km from the coast (de Sousa et al., 1981; Sarma et al., 2013a,b; Krishna et al., 2015). Coastal upwelling occurs during summer (June to August) associating with winds blow from south-west direction, however, upwelling does not occur in the northern BoB due to strong stratification and it is restricted to the southwestern BoB due to weakening of stratification (Shetye et al., 1991; Sarma et al., 2012; 2015). Despite such low nutrient concentrations in the mixed layer, high f -ratios (new to total production ratio) are reported (0.25 to 0.54) in the BoB and attributed to nutrients supply from subsurface by the CEs. Higher f -ratios were also reported in the region influenced by cyclones where vertical mixing due to strong winds supply nutrients from subsurface layers (Kumar et al., 2004a; Sherin et al., 2018; Sarma et al., 2019). Similarly, the particulate organic carbon fluxes to the deep BoB is higher than in the highly productive Arabian Sea (Ittekkot et al., 1991) and it is attributed to ballasting of organic matter in association with river-borne mineral particles in the former basin. However, the impact of river discharge associating with suspended matter is more noticed in the northern BoB, where discharge occurs, leading to removal of organic matter to the deep water in association with river-borne mineral particles (Ittekkot et al., 1991). Since the impact of river discharge decreases towards southern BoB, the high sinking carbon fluxes may be contributed by high primary production supported by nutrients supply through cyclonic eddies or cyclone (Sarma et al., 2016; Sherin et al., 2018). There are two possible sources of DIN other than vertical mixing. One potential source is atmospheric deposition of DIN and the other is the atmospheric N_2 fixation. The contribution of new production due to atmospheric deposition of DIN is small in the BoB (Srinivas et al., 2011a) and was

estimated to be $\sim 0.5 \text{ Tg yr}^{-1}$ in the BoB (Srinivas et al., 2013). Occurrences of *Trichodesimum* spp. were reported in the BoB (Jyothibabu et al., 2006; Paul et al., 2007; Hegde et al., 2008; Mulholland and Capone, 2009; Shetye et al., 2013; Sahu et al., 2017; Wu et al., 2019). Based on the dry depositional flux of iron (Fe) and DIN, Srinivas and Sarin (2013) estimated that $35\text{-}729 \mu\text{mol m}^{-2} \text{ d}^{-1}$ ($0.4\text{-}8.2 \text{ Tg y}^{-1}$) of N_2 fixation may occur in the BoB, whereas Naqvi et al. (2011) estimated N_2 fixation rates of $80 \mu\text{mol m}^{-2} \text{ d}^{-1}$ (1.0 Tg y^{-1}) based on mass balance approach. Recently, Loscher et al. (2020) reported the occurrence N_2 fixers in the BoB using molecular techniques, however, N_2 fixation is not observed and it is attributed to limitation of micronutrients, other than Fe. Relatively low isotopic composition of nitrogen in particulate organic matter ($\delta^{15}\text{N}_{\text{PON}}$) is observed suggesting that external source of nitrogen or N_2 fixation may be possible in the BoB (Gaye-Haake et al., 2005). In contrast, Sherin et al. (2018) also found low $\delta^{15}\text{N}_{\text{PON}}$ and it is attributed to either N_2 fixation or utilization of dissolved organic nitrogen (DON) by phytoplankton to support primary production. Recently, Sarma et al. (2019b) found that DON supports primary production in the BoB, when DIN concentrations are low. In addition, N_2 fixation may also contribute to new production in the BoB. In order to evaluate the contribution of N_2 fixation to the total primary production, we attempted to measure N_2 fixation rates in the BoB during the pre-southwest monsoon period.

Materials and methods

2.1. Study area and sampling

For this study water sampling was carried out in the western and central BoB on board *O.R.V. Sagar Nidhi* (Cruise No. SN142) during 11-29 June 2019, the early Southwest monsoon season. Figure 1 shows the location of eddies, their tracks (from where formed to the region of sampling) and sampling locations. A total of 18 stations were occupied for collection of hydrographic variables in the upper 100 m, where water column depth of $>2000\text{m}$ is found, and deck incubation was conducted at 14 stations (Fig. 1). Seawater samples from standard depths were collected with 5L Niskin bottles fixed to a rosette, along with conductivity–temperature–depth (CTD, 19 Plus, Sea Bird Electronics, USA), and fluorescence sensors (Wet Laboratories, USA). The detection limits for temperature, salinity, pressure and fluorescence are 0.001°C , 0.001, 0.1m and $0.01 \mu\text{g/l}$ respectively. Photosynthetic Active Radiation was measured PAR sensor (Chelsea Technologies, UK). Nutrients samples were collected in 100 ml plastic bottles and N_2 fixation incubation samples were collected in $\sim 1\text{L}$ glass crimper bottles.

2.2 Hydrographic measurements

The concentration of nutrients (nitrate, nitrite, ammonium, phosphate and silicate) were measured on-board, within one hour after sampling, using EcoLab autoanalyzer following the methods given in Grasshoff et al. (1983). The detection limits for nitrate+nitrite, nitrite, ammonium, phosphate and silicate were 0.05, 0.04, 0.03, 0.03, 0.03 μM respectively. The accuracy of the nitrate, nitrite, phosphate and silicate were 0.08, 0.04, 0.06 and 0.06 μM respectively. Fluorescence data were calibrated with measured chlorophyll *a* (chl *a*) at discrete depths using High Pressure Liquid Chromatography (HPLC) following Roy et al. (2015). About 3 to 5 litres of the sample was filtered through pre-combusted GF/F filters at 450° C and dried at 50° C for 12 h for measurements of content and isotopic composition of nitrogen on particulate organic matter (PON) retained on the filter using Elemental analyser (Model: Pyrocube, M/s. Elementar, Germany) connected to Isotope Ratio Mass Spectrometer (Isoprime 100, UK). The long-term precision of the PON and $\delta^{15}\text{N}$ was 0.02 μM and 0.2‰ respectively.

2.3 N₂ fixation experiments

The N₂ fixation experiments were conducted following the method in Mohr et al. (2010). The degassed water was prepared by filtering (0.2 μm) nutrients poor aged surface seawater, with nitrate and phosphate concentrations under detection limits, was taken and purging with high purity helium (99.9995%, Med Gases) over 30 minutes following Ahmed et al. (2017). The ¹⁵N₂ gas spike was prepared by addition of 1 ml of ¹⁵N₂ gas (98 atom%; Cambridge Isotope Laboratories, Massachusetts, USA) to 100 ml degassed water and shaken vigorously for 30 minutes until the gas bubble was completely dissolved. We have compared the N₂ fixation rates conducted using two degassing techniques, vacuum versus purging with gas, in the laboratory and both were found similar within the measurement error (Please see supplementary material). N₂ fixation rates were measured by collecting triplicate water samples from surface, 5, 10, 15, 25, and 50 m of water column depths in the photic zone and incubation was initiated by adding ~100 ml of spiked water to the total volume of the sample incubated (varied between 1.15 and 1.25 L) and shaken gently to ensure proper mixing. In this manner, the ¹⁵N₂ spike constitutes to 8% of the total sample volume, which is <10% recommended by Mohr et al. (2010). The incubation bottles were kept in the incubator and surface seawater was continuously passed to maintain the temperature. Neutral density screens (Rosco), calibrated using PAR sensor, were used to maintain different light levels. In addition, triplicate of surface water was taken in 1L glass bottles at all stations between 3 and 17, and ~0.5 μM of dissolved inorganic phosphate (Potassium di hydrogen phosphate; Merck, CAS No. 7778-77-0) was

added in addition to the $^{15}\text{N}_2$ spike to examine the influence of phosphate on N_2 fixation. The phosphate enriched samples were also treated in the same manner of other N_2 fixation experimental samples. All samples were incubated from dawn to dawn (24h) and the incubation is terminated by filtering each sample through pre-combusted GF/F filters (0.7 μm pore size and 25 mm diameter; Advantec) under low vacuum (<10 kPa). All filters were oven dried at 60° C on-board. In the onshore-based laboratory, the entire filter was packed in tin cups and introduced to Elemental analyser (Model: Pyrocube; M/s. Elementar, Germany) coupled to a mass spectrometer (Isoprime 100, UK) to determine content and isotopic ratios of particulate organic nitrogen (PON). A working standard, L-Glutamic acid (LGA) was run for every 5 samples to check reproducibility (Sarma et al., 2012; 2015).

2.4. Calculations

N_2 fixation rate is calculated following Montoya et al. (1996) and Mohr et al. (2010)

$$\text{N}_2 \text{ Fixation rate} = \left[\frac{\text{APN}_{(\text{final})} - \text{APN}_{(t=0)}}{\text{AN}_2 - \text{APN}_{(t=0)}} \right] \times (\text{PN}/\Delta t)$$

Where A=atom% ^{15}N in the particulate nitrogen (PN) at the end (final) or beginning ($t=0$) of the incubation or in the dissolved N_2 pool (N_2), Δt is the incubation period. The solubility of N_2 in seawater was calculated according to Hamme and Emerson (2004). Weighted mean N_2 fixation rates were integrated to the photic zone and expressed as photic zone integrated N_2 fixation.

The mixed layer depth (MLD) was determined following Shankar et al. (2015) by estimating density of water column at *in situ* temperature, salinity and pressure. Then 0.5 °C is subtracted from *in situ* temperature and re-estimated density using in-situ salinity and pressure. The depth of MLD is taken where the density at *in situ* temperature at surface (1 m) is matched with *in situ* temperature minus 0.5 °C. In this manner, the influence of changes in temperature and salinity on MLD is accounted (Shankar et al., 2015).

2.5. Satellite data

The remote sensing sea surface height anomaly (SSHA) and geostrophic currents data was Obtained from <http://marine.copernicus.eu/services-portfolio/access-to-products/>. Based on daily imageries of SSHA the track of each eddy is estimated.

3. Results

3.1. Hydrographic conditions

During this study, we sampled one ACE and two CEs (northern BoB (CE_N) and southern BoB (CE_S)) and all three eddies were originated in the western Bay and advected to the western BoB (Fig. 1). The CE_S is relatively younger (45 days) as it was formed on 30 April 2019 compared to CE_N, which was formed on 05 March 2019 (82 days). The vertical structure of temperature indicates that the upper 60 m of water column is warm (>30°C) and highly stratified in the entire study region (Fig. 2a). Upsloping of cooler waters is seen at stations 1 to 3 (CE_S) and stations 14 to 15 (CE_N) associated with CE and downsloping of warmer waters is noticed at stations 4-7 and stations 17-18 associated with the ACE. The mixed layer mean temperature was warmer in the ACE (30.3±1° C; n=18) than in the CE (29.3±0.2° C; n=15) and No Eddy (NE) (stn. 8-13; 29.5±0.3° C; n=15) regions (see Table 1). Relatively strong winds of >30 knots (15 m/s) prevailed at stations 8-9 due to local weather disturbance (depression) that cooled the upper ocean (Fig 2a,d) up to 50m depth. Though station 9 fall within the CE, we did not consider this station to obtain mean concentrations for CE as this station is influenced by mixing caused by strong local winds. In contrast, the entire 100 m of water column was warm (>29° C) in the ACE (Stns. 4-7 and Stns. 16-18; Fig. 2a). Low salinity water (~33.6) was occupied in the upper 60 m in the ACE. In the CEs, salinity was relatively higher (>33.6). In the NE region of the northern BoB (stns. 9-13), low salinity (~32.0) waters occupied the upper 20 m and high salinity waters at 70-100 m (Fig. 2b). The mixed layer mean salinity was lower in the ACE (33.2±0.1) than in the CE (33.9±0.1) whereas the lowest salinity was observed in the NE region (32.7±0.8) (Table 1). The depth of mixed layer varied between 22 and 66 m with deeper depths in the ACE (58±6 m) than in the CEs (33±4m; Fig. 2a). Low concentration of chl *a* was seen in the upper 40 m with a prominent subsurface chl *a* maximum between 40 and 90m depth (Fig. 2c). Surfacing of subsurface chl *a* maximum was observed between 1000 and 1300 kms distance (Stns 8 and 9) associating with shoaling of mixed layer and higher winds (Fig. 1d). Slight increase in chl *a* was noticed at stations 14-15 associated with CE (Fig. 2c). The mixed layer mean chl *a* was lower in the ACE (0.03±0.05 µg/l) than in the CE (0.05±0.02 µg/l) whereas the highest chl *a* was observed in the NE region (0.12±0.1 µg/l) (Table 1). The depth of photic zone (depth of 1% of surface PAR) was ranged between 48 and 65 m with shallow depths in the NE region associated with low saline waters (Stns. 10-13; Fig. 2b).

Deepening of low DIN concentration contours up to 100 m was noticed in the ACE whereas upsloping of high DIN contours occurred in the CEs (Fig. 3a). The mixed layer mean DIN

concentrations were high in the CE ($1.2\pm 0.7 \mu\text{M}$) than in the ACE ($0.02\pm 0.05 \mu\text{M}$) and NE region ($0.7\pm 0.5 \mu\text{M}$) (Table 1). Similarly, the mean concentrations of phosphate in the mixed layer were low in the ACE ($0.04\pm 0.05 \mu\text{M}$) than in the NE region ($0.06\pm 0.05 \mu\text{M}$) and in the CE ($0.10\pm 0.04 \mu\text{M}$) (Table 1). Dissolved inorganic silicate in the mixed layer was low ($<2 \mu\text{M}$) in the entire transect and increased with depth. Unlike DIN and phosphate, no significant increase in silicate concentration was noticed in the CE ($2.0\pm 0.8 \mu\text{M}$) compared to that in the ACE ($1.9\pm 0.3 \mu\text{M}$) (Fig. 3c; Table 1).

3.2 Nitrogen fixation rates

N_2 fixation rates were higher at surface and decreased with depth. N_2 fixation rates in the surface waters ranged between 0.02 and $1.1 \mu\text{mol m}^{-3} \text{d}^{-1}$ (Fig. 4a). Mixed layer mean N_2 fixation rates were low in the ACE ($0.17\pm 0.1 \mu\text{mol m}^{-3} \text{d}^{-1}$) than in the NE ($0.22\pm 0.1 \mu\text{mol m}^{-3} \text{d}^{-1}$) and CE regions ($0.37\pm 0.2 \mu\text{mol m}^{-3} \text{d}^{-1}$; Table 1) (Fig. 1d, 3b). Relatively higher N_2 fixation rates were observed in the upper 10 m of water column in the CEs ($0.51\pm 0.2 \mu\text{mol m}^{-3} \text{d}^{-1}$) compared to CE_N ($0.28\pm 0.05 \mu\text{mol m}^{-3} \text{d}^{-1}$; Fig. 4a). Photic zone integrated N_2 fixation rates ranged between 3.8 and $13.8 \mu\text{mol m}^{-2} \text{d}^{-1}$ with lower mean rates in the ACE region ($6.5\pm 1 \mu\text{mol m}^{-2} \text{d}^{-1}$) than in the CE ($10.5\pm 2 \mu\text{mol m}^{-2} \text{d}^{-1}$) and NE regions ($8.8\pm 1 \mu\text{mol m}^{-2} \text{d}^{-1}$) (Fig. 4a). Photic zone integrated N_2 fixation rates displayed significant linear (positive) relation with photic zone mean phosphate (Fig. 5a; $r^2=0.75$; $p<0.001$) and photic zone integrated chl *a*, except at station 8 where high chl *a* was observed at the surface due to local depression (Fig 5b; $r^2=0.69$; $p<0.001$). The $\delta^{15}\text{N}$ values of PON ($\delta^{15}\text{N}_{\text{PON}}$) varied between 3.7 and 8.3‰ in the upper 50 m with lower values associated with high N_2 fixation (Fig. 4b). Higher $\delta^{15}\text{N}_{\text{PON}}$ values were seen in the NE region by up to 2-3‰ (Fig. 4b). The mixed layer mean N:P ratios varied between 0 and 36 and very low values (0.5 ± 0.3) occur in the ACE where concentration of both N and P were close to the detection limits of the method used, in contrast, higher than Redfield (16) values were seen in CE regions (26.2 ± 6) (Fig. 6a). Increase in N_2 fixation rates, by 1.2 to 8 times, was resulted in the phosphate added samples (enriched phosphate) than *in situ* phosphate concentrations (Fig. 6b).

Discussion

4.1. Influence of eddies and river discharge on nutrients distribution in the BoB

The concentration of DIN and phosphate were low in the ACE (0.02 ± 0.05 and $0.04\pm 0.05 \mu\text{M}$ respectively) compared to those in the CE region (1.26 ± 0.7 and $0.10\pm 0.04 \mu\text{M}$ respectively) under the influence of downwelling and upwelling processes associated with the ACE and CE respectively (Figs. 3a,b). Enhanced phytoplankton biomass, primary and export productions were reported earlier

in the CE region in the BoB associated with increase in nutrients (Prasanna Kumar et al., 2004; Sarma et al., 2016; 2019a,b; Vidya and Prasanna Kumar, 2013). The N:P ratio in the mixed layer was higher than Redfield ratio (16) in the CE (26.2 ± 6) and NE (20.1 ± 3) indicating that phosphate is severely limiting the primary production in the BoB. In contrast, N:P ratio in the ACE was very low (0.5 ± 0.3) as the concentrations of DIN and phosphate reached close to the detection limits (Table 1). This suggests that both these nutrients seem to be limiting primary production in the ACE region.

Low concentrations of DIN ($\sim 0.1\pm 0.06 \mu\text{M}$) and phosphate ($\sim 0.03\pm 0.01 \mu\text{M}$) and high silicate ($2.3\pm 0.7 \mu\text{M}$) were observed in the northern BoB associated with low salinity waters (Stn. 11-12). Though BoB receives significant amount of river discharge from major rivers, such as Ganges, Brahmaputra, Godavari etc., however, >95% of DIN and phosphate in the river water is removed within their estuaries and the remaining concentrations are removed in the coastal waters within 10-20 kms from the coast (Sarma et al., 2013a,b; Krishna et al., 2015). Enhanced concentrations of dissolved silicate associated with low concentrations of DIN and phosphate is reported in the regions influenced by river discharge in the northern Bay (Sarma et al., 2016; 2018) suggesting that river waters are not the significant source of nutrients to the BoB.

4.2. Variations in N₂ fixation in the eddies

Our observations show warm temperature ($>28^\circ\text{C}$), low salinity (33.5) and under detection limits of DIN ($<0.1 \mu\text{M}$) in the mixed layer indicating prevalence of conducive conditions for N₂ fixation. Earlier investigators reported perennial occurrence of *Trichodesmium* spp. in the BoB during spring intermonsoon (Jyothibabu et al., 2006; Shetye et al., 2013), postmonsoon (Hedge et al., 2008) and summer monsoon (Paul et al., 2007; Wu et al., 2019) in the open ocean region and in the coastal waters during winter (Sahu et al., 2017) indicating possible occurrence of N₂ fixation. The estimated mean N₂ fixation rates in the mixed layer varied between 0.02 and $1.07 \mu\text{mol m}^{-3} \text{d}^{-1}$ in the study region with higher rates in the CE region ($0.35\pm 0.2 \mu\text{mol m}^{-3} \text{d}^{-1}$) and low values in the ACE region ($0.17\pm 0.1 \mu\text{mol m}^{-3} \text{d}^{-1}$) and NE regions ($0.22\pm 0.2 \mu\text{mol m}^{-3} \text{d}^{-1}$) (Fig. 4a; Table 1). Higher rates of N₂ fixation in the CE region are associated with high phosphate concentrations ($0.1\pm 0.04 \mu\text{M}$) than in the ACE region ($0.04\pm 0.05 \mu\text{M}$; Fig. 3b and Table 1) in the mixed layer indicating upwelling under CE enhanced phosphate concentration leading to high N₂ fixation. Significantly higher N₂ fixation rates were noticed in the CE_S in the upper 10m of water column ($0.50\pm 0.2 \mu\text{mol m}^{-3} \text{d}^{-1}$) than in the CE_N ($0.28\pm 0.05 \mu\text{mol m}^{-3} \text{d}^{-1}$; $t_{\text{stat}}=2.27$; $p<0.05$), with relatively higher phosphate and lower N:P ratios in the former region ($0.11\pm 0.02 \mu\text{M}$ and 19 ± 3 respectively) than latter region ($0.08\pm 0.02 \mu\text{M}$ and 25 ± 4 respectively) however, this difference is statistically insignificant. The CE_S is younger

(45 days) than CE_N (82 days; Fig. 1). The mean suspended particulate matter (SPM) was relatively higher in the CE_N (22±2 mg/l) than in the CE_S (12±4 mg/l) due to higher impact of river discharge in the north compared to south (Sarma et al., 2020). In addition to this, the variations in abundance of N₂ fixers in both the eddies may also contributed to the difference in N₂ fixation. Due to lack of such data, we could not able to evaluate the difference in N₂ fixation between CE_S and CE_N are caused by age of eddy, nutrients, SPM or abundance of N₂ fixers in the BoB. On the other hand, significant mixing was noticed in the station 9 (Fig. 2d) associating with increase in phosphate concentrations (Fig. 3b), increase in N₂ fixation was not evidenced (Fig. 4a). This could possibly due to lag in the biological response as we had sampled when mixing is being occurred resulting in observation of chemical response in nutrients. But it may take some time for biological response to increase in nutrients. Significant impact of river discharge is noticed at station 12 where N₂ fixation was low compared to north (stations 11 and 13) associating with low phosphate (0.03 μM) in the former than latter stations (0.08 μM).

Nevertheless, N₂ fixation in the BoB is much lower than the ranges reported elsewhere in the world ocean (Table 2). The N₂ fixation rates in the literature showed wide variability in the Arabian Sea, the adjacent basin that might be due to methodological differences, such as direct injection of enriched N₂ gas and injection of enriched N₂ gas dissolved water. Recently Ahmed et al. (2017) estimated N₂ fixation rates, following the similar method of the present work, and found higher rates of N₂ fixation in the Arabian Sea (0.8 to 1739 μmol m⁻² d⁻¹) than present study in the BoB (2 to 23 μmol m⁻² d⁻¹; Table 2). The present estimates of N₂ fixation is higher than N₂ fixation rates measured during winter in the BoB (0 μmol m⁻² d⁻¹; Loscher et al., 2020) (Table 2). Loscher et al. (2020) collected samples in the ACE region (Sridevi and Sarma, 2020; see supplementary Figure S1) where concentration of phosphate was reported to be under detection limits in the upper ocean resulting in insignificant N₂ fixation. The difference in N₂ fixation during winter and summer (present study) could be due to strong seasonal variability in hydrographic conditions, nutrients availability, mixing, eddies and atmospheric deposition of nutrients. For instance, river discharge peaks during summer when some amount of nutrients are brought to the BoB and eddy activity is more during summer than winter period (Chen et al., 2012), due to generation of more instabilities by high freshwater flux during former than latter period, resulting more nutrients input to the surface during former than latter. In addition to this, the mechanism of nutrients inputs, i.e., river-borne, wind-driven mixing, atmospheric deposition, divergence and convergence, brings variable ecosystem responses. In case of Arabian Sea, mostly nutrients are brought to the surface through vertical mixing (upwelling or convective mixing), whereas rivers, eddies and atmospheric deposition are the major sources of

nutrients in the BoB. Due to the differences in the source of nutrients, caused by regional oceanographic conditions and climate, it is difficult to compare N_2 fixation rates in the BoB with other parts of the world ocean. However, photic zone integrated N_2 fixation rates in the BoB (2 and 23 $\mu\text{mol m}^{-2} \text{d}^{-1}$) are lower than the Western Equatorial Pacific (18 to 358 $\mu\text{mol m}^{-2} \text{d}^{-1}$; Bonnet et al., 2009), Atlantic Ocean along 10°N (3.7-267 $\mu\text{mol m}^{-2} \text{d}^{-1}$; Voss et al., 2004), North Atlantic Ocean (200 ± 64 $\mu\text{mol m}^{-2} \text{d}^{-1}$; Moore et al., 2009) and Mediterranean Sea (0-76 $\mu\text{mol m}^{-2} \text{d}^{-1}$; Ibello et al., 2010) (Table 2).

Photic zone integrated N_2 fixation rates displayed significant linear relation with photic zone integrated chl *a* (Fig. 5b) and photic zone mean phosphate concentrations suggesting that phosphate may be a limiting nutrient in the BoB (Fig. 5a). Micro-nutrient, iron (Fe), also limits the N_2 fixation in the ocean (Kustka et al., 2002; Jiang et al., 2018). Mills et al. (2004) suggested that Fe and phosphorus co-limit N_2 fixation in the eastern tropical North Atlantic and it is possible in the BoB as well. Chinni et al. (2019) reported that concentrations of Fe in the photic zone varied between 0.4 and 0.6 nM in the BoB during non-monsoon period. Such high concentrations of Fe suggests it may not limit phytoplankton production in the aquatic environment (Morel and Hudson, 1985; Anderson and Morel, 1982; Martin, 1992). Loscher et al. (2020) also suggested that Fe may not be limiting N_2 fixation in the BoB. The BoB receives significant amount of dust from Indo-Gangetic Plain (IGP) and it is one of the highest dust receiving regions in the world (Zhang and Reid, 2010; Kumar et al., 2008). Srinivas et al. (2011b) noticed that solubility of aerosol Fe is more in the BoB than Arabian Sea that enhances primary production and estimated that 30-729 $\mu\text{mol m}^{-2} \text{d}^{-1}$ atmospheric N_2 is fixed in the former basin based on DIN and Fe deposition. N_2 fixation in the Northeastern Atlantic Ocean is supported by Fe from the dust from Saharan desert (Sohm et al., 2011) and off the Florida coast (Lenes et al., 2001; Mills et al., 2004). It has been reported that phosphate limits rather than Fe in the eastern basin of the tropical North Atlantic (Turk-Kubo et al., 2012) and central Atlantic Ocean (Sanudo-Wilhelmy et al., 2001). However, phosphate supply through atmospheric sources are rather limited over BoB (Yadav et al., 2016; Sarma et al., 2019a) and weak vertical mixing, due to stratification, further lowers phosphate supplies from subsurface layers leading to controlling N_2 fixation in the BoB. It is further confirmed by linear relationship between photic zone integrated N_2 fixation and photic zone mean concentration of phosphate (Fig. 5a). The higher N:P ratios in the CE (26.2 ± 6) and NE region (20.1 ± 3) than Redfield ratio of 16 indicate that phosphate is severely limiting N_2 fixation in the BoB during the study period.

In order to further confirm phosphate limitation on N_2 fixation in the BoB, phosphate enrichment experiment was conducted by addition of ~ 0.5 μM of dissolved inorganic phosphate to the surface

water at different stations along with enriched N₂ gas solubilized water and compared the same with N₂ fixation with that of *in situ* phosphate levels. It is observed that N₂ fixation rates were increased by 2 to 7 times due to addition of artificial phosphate in the ACE region where both DIN and phosphate concentrations were close to detection limits than in the CE and NE regions, where the observed increase was 1.2 to 1.8 times, with N:P ratio of 26.2±6 and 20.1±3 respectively. Within the CEs, the higher N:P ratios were observed in the CE_N (25±4) compared to CE_S (19±3) and higher response to N₂ fixation for addition of phosphate was observed in the former (55%) than latter region (13%). Since phosphate concentration was low in the CE_N (0.08±0.02 μM), the response for N₂ fixation was higher to addition of phosphate. This experiment strongly suggests that phosphate is severely limiting N₂ fixation in the BoB, especially more in the ACE region. Dyhrman et al. (2002; 2006) found that *Trichodesmium* spp. can utilize dissolved organic phosphorus (DOP) in the absence of inorganic phosphate in the Northwestern and Southwestern Pacific. Dyhrman et al. (2006) found that organic phosphorus can be taken up through hydrolysis of phosphonate compounds by a carbon-phosphorus lyase pathway in the Sargasso Sea. Benavides et al. (2017) found uptake of dissolved organic matter by *Trichodesmium* spp. in the Southwest Pacific. Though concentrations of DOP in the BoB during southwest monsoon is unknown, however river discharge is expected to bring significant amount to the Bay. Since primary production in the BoB is low, most of the DOP may be contributed by terrestrial sources but its composition and nature (lability) of DOP is unknown in the BoB. It is unclear that whether *Trichodesmium* spp. in the BoB can able to utilize terrestrial sourced DOP in the BoB. It is interesting to evaluate the role of DOP in N₂ fixation in the future. Nevertheless, our incubation experiments suggest that significant increase in N₂ fixation occurred due to increase in inorganic phosphate.

The source of nitrogen is reflected in the isotopic composition of nitrogen in PON ($\delta^{15}\text{N}$) as it is close to zero (between -2 and +2‰), if the PON is produced through N₂ fixation (Altabet and McCarthy, 1985; Gandhi et al., 2011). Nevertheless, the $\delta^{15}\text{N}_{\text{PON}}$ in the surface waters varied between 3.6 and 8.6‰ (Fig. 4b) with lower values (<4‰) in the CE region associated with high N₂ fixation and higher values (7 to 8.6‰) were noticed in the ACE and NE regions, where low N₂ fixation occurred. The lower $\delta^{15}\text{N}_{\text{PON}}$ values were noticed in the surface at stations 1-3 where mean N₂ fixation of ~1 μmol m⁻³d⁻¹ was observed. Such low N₂ fixation did not contribute significantly to the POM pool, the low $\delta^{15}\text{N}_{\text{PON}}$ may be caused by higher N₂ fixation before we occupied the stations. Due to cloud cover we could not able to obtain satellite data during and before study period. Decrease in $\delta^{15}\text{N}_{\text{PON}}$ in the surface water was observed in association with high N₂ fixation rates at stations 1-3 (3.8±0.5‰), 10 (5.4±0.2‰), 14 (5.2±0.2‰) compared to other stations (6.4±0.5‰).

Insignificant relationship observed between $\delta^{15}\text{N}_{\text{PON}}$ and N_2 fixation in the photic zone as the former gives an averaged signal over the residence time of PON in the water and the latter represents conditions at the time of incubation. The lower $\delta^{15}\text{N}_{\text{PON}}$ in the CE region may be caused by continuous supply of nitrate from deeper layer as the $\delta^{15}\text{N}_{\text{PON}}$ derived from nitrate would be lower. In addition to this, Sarma et al. (2019) noticed that DON acts as an alternative source of nitrogen when dissolved inorganic nitrogen is not available. Therefore, the contribution PON through DON uptake may have significant influence on $\delta^{15}\text{N}_{\text{PON}}$. Due to lack of $\delta^{15}\text{N}_{\text{DON}}$, it is not possible to evaluate its contribution. ACEs are reported to be regions of active N_2 fixation in the world ocean (eg: Fong et al., 2008; Loscher et al., 2016) and lower $\delta^{15}\text{N}$ in PON values were reported associated with *Trichodesmium* spp. bloom (Liu et al. 1996; Maya et al., 2011; Ahmed et al., 2019) due to intensive atmospheric N_2 uptake. In contrast, the mean $\delta^{15}\text{N}_{\text{PON}}$ in the ACE (5-7 ‰; Fig. 4b) is close to that of mean $\delta^{15}\text{N}_{\text{NO}_3}$ in the depth below mixed layer is $7.5 \pm 0.8\text{‰}$ (Bristow et al., 2017) indicating that PON contribution by N_2 fixation may not be significant in the BoB. Gaye-Haake et al. (2005) noticed lower $\delta^{15}\text{N}_{\text{PON}}$ in the sediment traps and suggested possible N_2 fixation in the BoB indicating that sporadic events of high N_2 fixation rates may be possible.

4.3. Contribution of N_2 fixation to the new production

Singh et al. (2014) found that primary production in the cyclonic eddy is more than twice higher than that in the non-eddy region. Similarly, high f -ratio of 0.4 to 0.7 was observed in the BoB and it was attributed to CEs (Kumar et al., 2004; Singh et al., 2014; Sarma et al., 2019). Based on the mean new production in the BoB of $\sim 282 \pm 18 \text{ mgC m}^{-2} \text{ d}^{-1}$ (Kumar et al., 2004; Singh et al., 2014), the contribution of N_2 fixation in the photic zone ($9.8 \mu\text{molN m}^{-2} \text{ d}^{-1} = 0.8 \text{ mgC m}^{-2} \text{ d}^{-1}$) contributes to $<0.3\%$ during study period. Srinivas et al. (2013) estimated that about $114 \text{ mgC m}^{-2} \text{ d}^{-1}$ of new production occurs due to atmospheric deposition of nitrogen. The N_2 fixation in the BoB is contributing to $\sim 0.7\%$ of the new production contributed by atmospheric deposition. This study suggests that N_2 fixation did not contribute significantly to the new production in the BoB during study period due to severe phosphate limitation.

Summary and Conclusions

The N_2 fixation rates were measured in the BoB during pre-southwest monsoon using $^{15}\text{N}_2$ gas dissolution method. The results suggested that relatively higher N_2 fixation rates occurred in the CE region associated with high concentrations of phosphate than in the ACE region. The photic zone integrated N_2 fixation rates displayed significant positive relation with photic zone integrated chl a and photic zone mean phosphate concentrations, thus highlighting that phosphate limitation for N_2

fixation in the BoB. Phosphate enrichment experiments further revealed that increase in N₂ fixation rates by 1.2 to 8 times from that of *in situ* phosphate concentrations with higher enhancement in the ACE than CE confirming phosphate limitation in the Bay. Removal of dissolved inorganic phosphate within the estuaries before it is released to the coast, less deposition from the atmosphere and weak inputs through vertical mixing due to strong stratification may be potential reasons for phosphate limitation for N₂ fixation in the BoB. The N₂ fixation contributed <0.5% of the new production in the BoB. However, episodic events, such as cyclones and depressions, may enhance phosphate through mixing and therefore enhanced N₂ fixation in the BoB. High time-resolution measurements are required to evaluate impact of such events on N₂ fixation rates in the BoB.

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Table 1: Mixed layer mean temperature, salinity, chl *a*, nutrients and N₂ fixation rates in the mixed layer and integrated to the photic zone in the ACE, CE and NE regions

| Variable | ACE | CE | NE |
|--|-----------|-----------|-----------|
| Temperature (°C) | 30.3±0.1 | 29.3±0.2 | 29.5±0.3 |
| Salinity | 33.2±0.1 | 33.9±0.1 | 32.7±0.8 |
| chl <i>a</i> (µg/L) | 0.03±0.01 | 0.05±0.02 | 0.12±0.17 |
| Nitrate (µM) | 0.02±0.05 | 1.2±0.7 | 0.7±1 |
| Phosphate (µM) | 0.04±0.05 | 0.10±0.04 | 0.06±0.05 |
| Silicate (µM) | 1.9±0.3 | 2.0±0.8 | 2.2±0.8 |
| N:P | 0.5±0.3 | 26.2±6 | 20.1±3 |
| Mixed layer mean N ₂ -fixation (µmole m ⁻³ d ⁻¹) | 0.17±0.1 | 0.37±0.2 | 0.22±0.1 |
| Photic zone integrated N ₂ fixation ((µmole m ⁻² d ⁻¹) | 6.5±1 | 10.5±2 | 8.8±1 |

Table 2: N₂ fixation rates in the euphotic zone of different regions in the world Ocean

| Region | N ₂ fixation ($\mu\text{mol m}^{-2} \text{d}^{-1}$) | Reference |
|------------------------------------|---|--|
| Equatorial Pacific | 18-358 | Bonnet et al., 2009 |
| North Pacific | 24-134 | Karl et al., 1997; Church et al., 2009 Gundersen et al., 1976; Montoya et al., 2004 |
| South Pacific | 50-137 | Lee et al., 2002; Deutsch et. el., 2001; Shiozaki et al., 2014 |
| North Atlantic | 48-254 | Michaels, et al., 1996; Gruber and Sarmiento, 1997; Moore et al., 2009 |
| South Atlantic | 20-41 | Goering et al., 1966; Moore et al., 2009 |
| Tropical Atlantic (10°N) | 3.7-267 | Voss et al., 2004 |
| Mediterranean Sea | 0-76 | Ibello et al., 2010 |
| Equatorial & Southern Indian Ocean | 10 \pm 5 | Shiozaki et al., 2014 |
| Arabian Sea (spring) | 40-129 | Capone et al., 1998; |
| Arabian Sea (spring) | 100-3400 | Gandhi et al., 2011 |
| Arabian Sea (winter) | 0.8-1739 | Ahmed et al., 2017 |
| Bay of Bengal (spring) | 35-729 | Srinivas and Sarin, 2013 |
| Bay of Bengal | 80 | Naqvi et al., 2011 |
| Bay of Bengal (winter) | 0 | Loscher et al., 2020 |
| Bay of Bengal (summer) | 2-23 | Present study |

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Legend to Figures

Figure 1. The study region with station locations and identified station numbers. The color shading shows the Sea Surface Height Anomaly (SSHA) variations depicting the surface circulation and meso-scale cyclonic eddies (CE) and anticyclonic eddy (ACE) in the Bay of Bengal. Vectors denote the direction and speed of the geostrophic currents at the surface. The magnitude of current vector is shown in the left-top corner. The track of each eddy is shown as a line and the date of origin and date of sampling. The locations of discharge by different rivers are shown. The N₂ fixation experiments were conducted at all stations except stations 11,13,16 and 18.

Figure 2: Vertical distributions of (a) temperature (°C), (b) Salinity, (c) Chlorophyll-a (mg m⁻³) and d) wind speed along the cruise track in the BoB. Color code scale is shown for each parameter on the right side of each figure. Station numbers and the stations encompassing the CE, and ACE are also shown. The depth of mixed layer (yellow line) and euphotic depth (white line) are shown .

Figure 3: Vertical distributions of (a) DIN (μM), (b) phosphate (μM), and (c) Silicate (μM) in the color scale along the cruise track in the BoB. Color code scale is shown is shown for each variable on the right side of each figure. Station numbers and stations encompassing the CE and ACE regions are shown.

Figure 4: Vertical distributions of (a) N₂ fixation rates (μmol m⁻³ d⁻¹), and (b) isotopic composition of nitrogen in particulate nitrogen (δ¹⁵N_{PON}) along the cruise track in the BoB. Color code scale is shown for each variable on the right side of each figure. Station numbers and the stations encompassing the CE and ACE are also shown.

Figure 5: Relationships between photic zone integrated N₂ fixation rate and a) mixed layer mean phosphate and b) photic zone integrated Chl-a. One point (station 9) which is away from the regression line in Fig. 5b was not taken into consideration while constructing regression line.

Figure 6: a) The box plot showing the variations in mixed layer mean N:P ratio with distance from station 1 to 17 and b) N₂ fixation at the surface with natural phosphate (blue bar) and addition of 0.5 μM of phosphate (red bars). N:P ratios were shown as ND wherever phosphate concentrations are below or close to detection limits. The station numbers are plotted above the bar and stations considered under CE and ACE were marked. The horizontal bar in the box shows median value.

Figure 1:

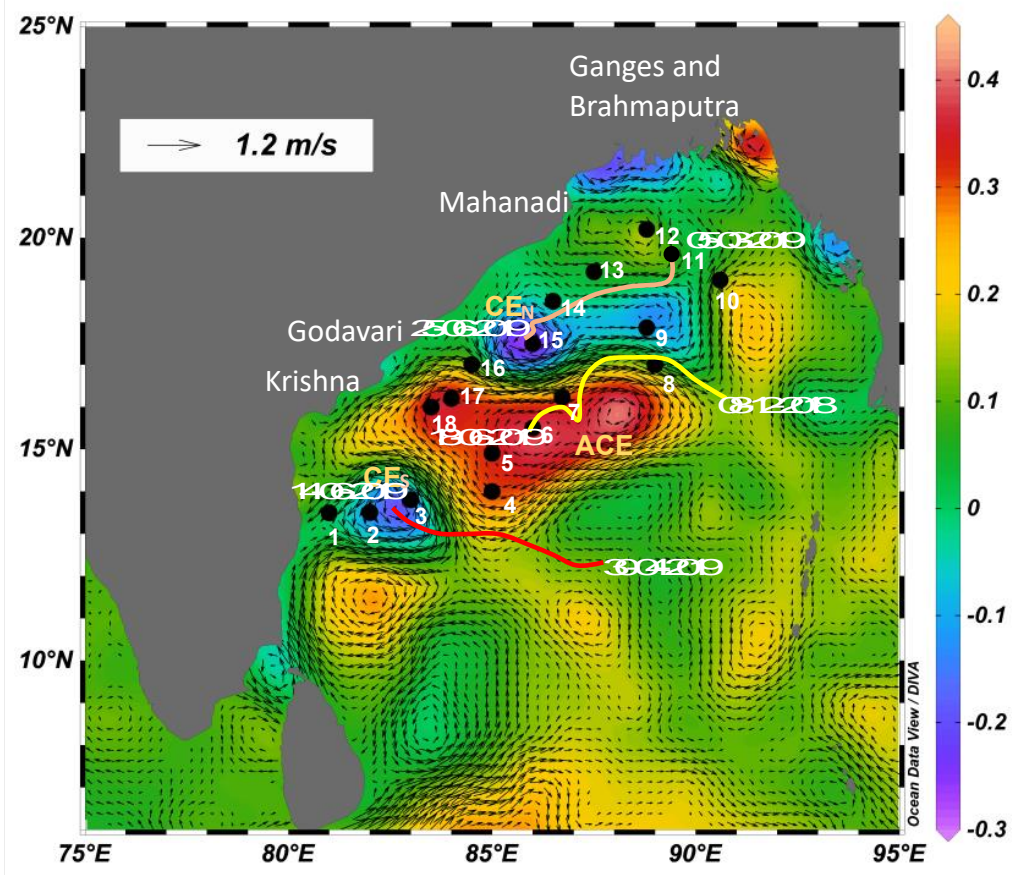


Figure 2:

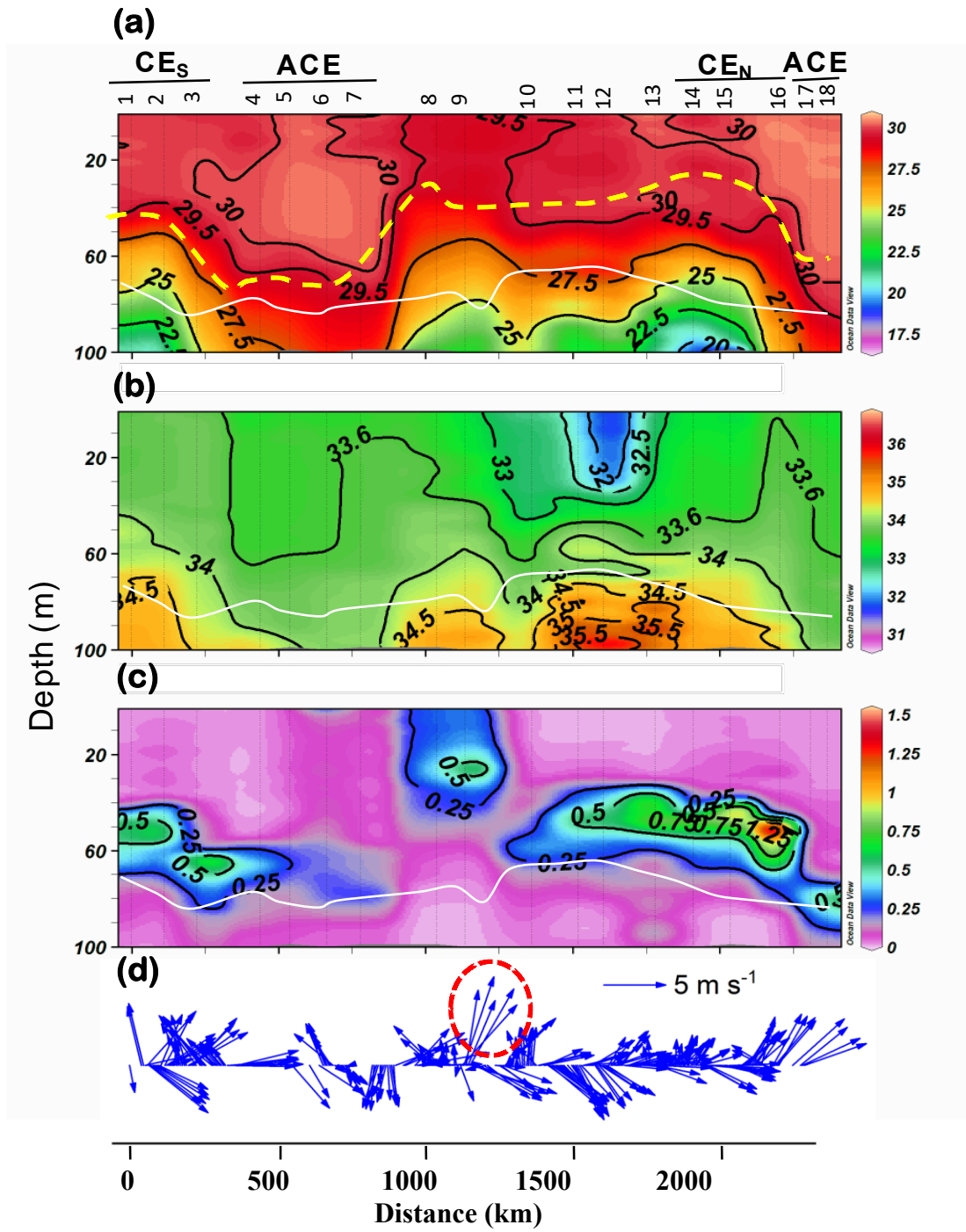


Figure 3:

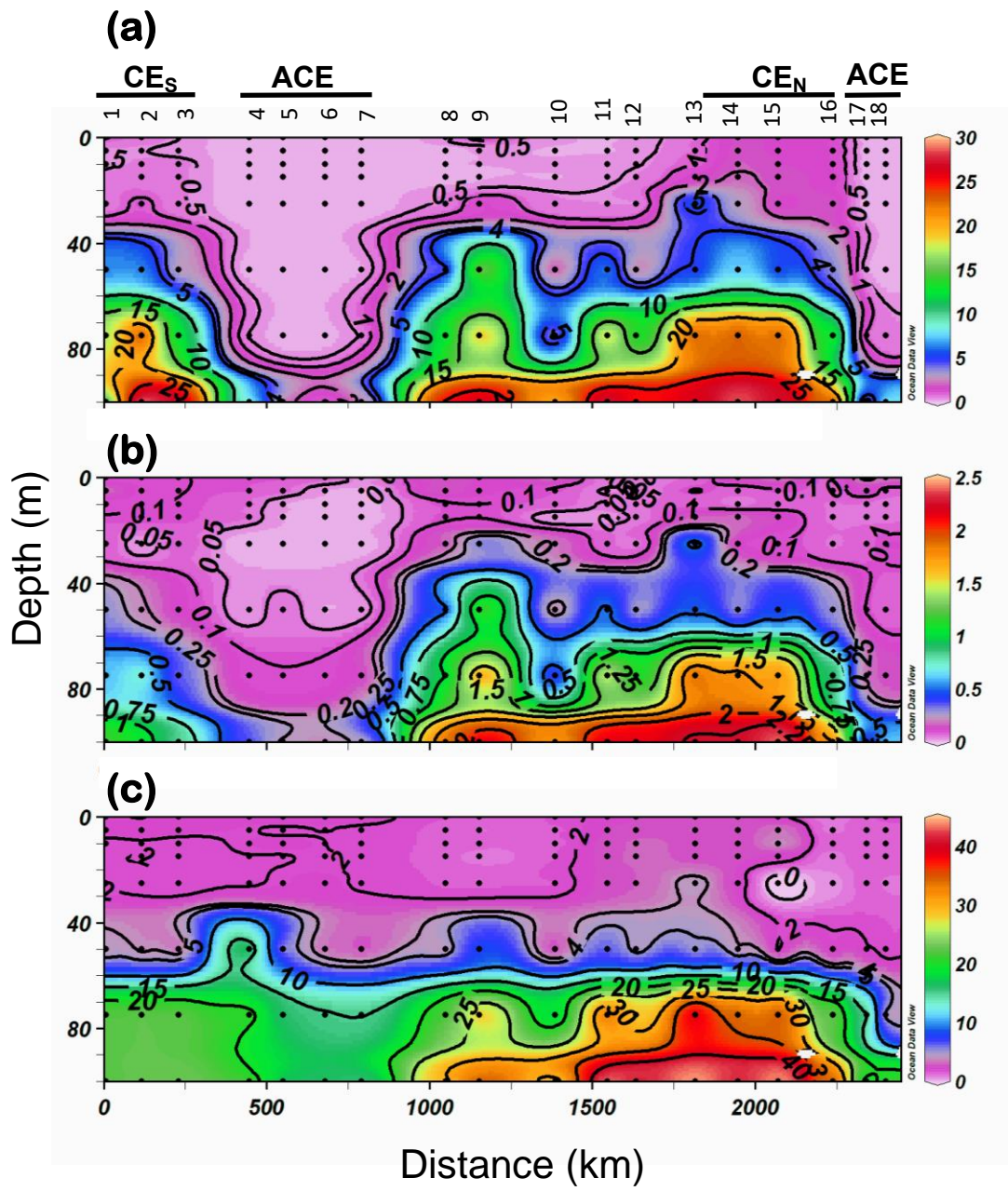
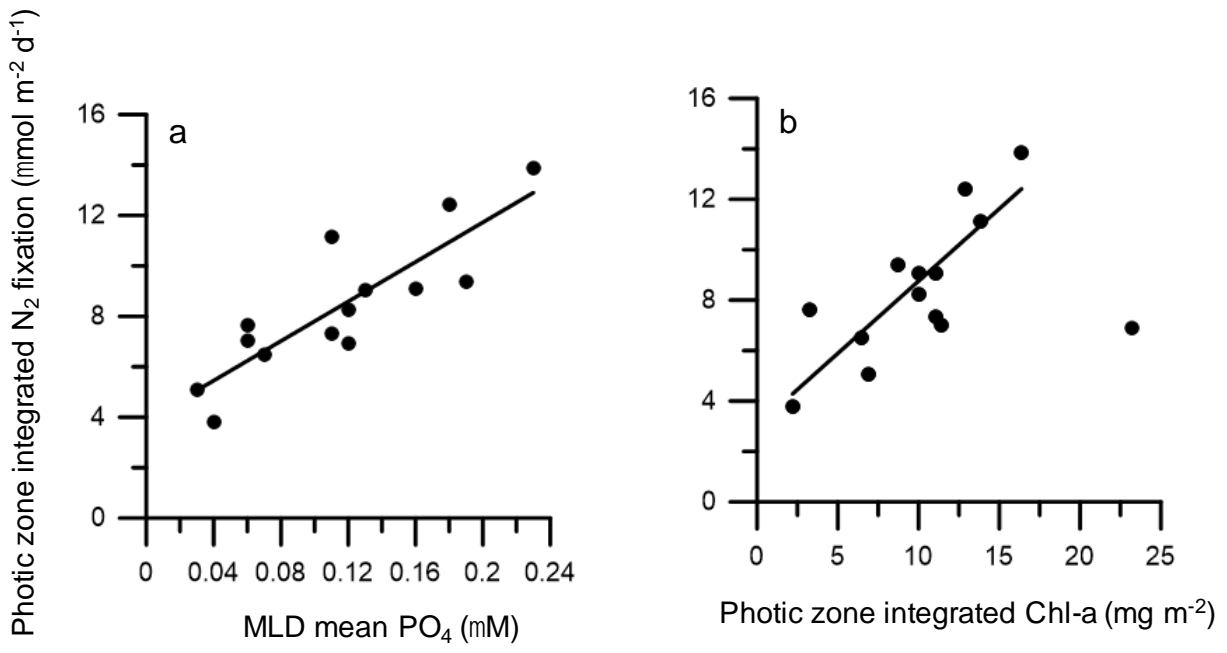


Figure 5:



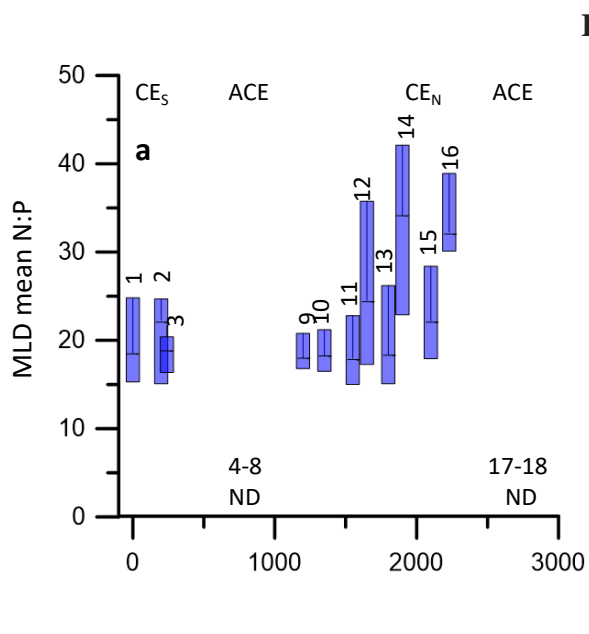
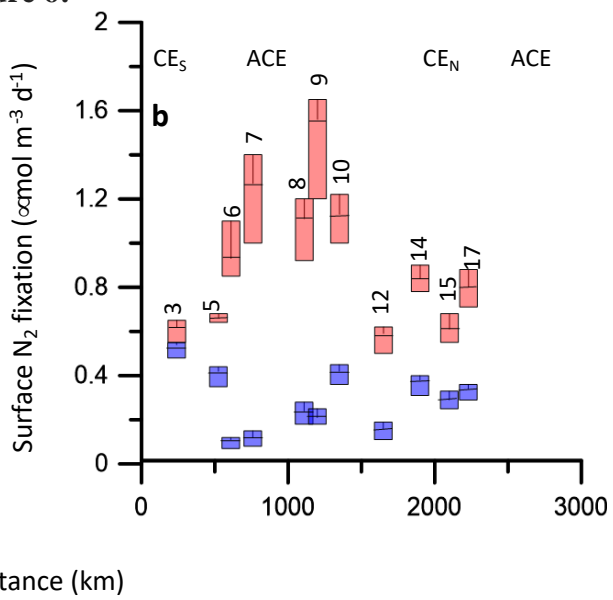


Figure 6:



Supplementary Material

We have conducted an experiment by de-gassing water to prepare N₂ gas spike. ~100 ml of water taken and degassed in vacuum line with rotary pump and the other set was degassed by purging with high purity (99.99995%) helium for 30 minutes and 1 ml of 99.5% N₂ gas spike was dissolved. Surface sea water samples were collected from the shelf region of Visakhapatnam at two locations (given below) where *Trichodesmium* spp. was detected into 1 L Nalgene bottles of 4 nos and two bottles each spike with vacuum degassed and He sparged spikes. The results are given below:

| Location | Spike method | N ₂ fixation rate (μmol m ⁻³ d ⁻¹) |
|----------------|------------------|--|
| 17.49N: 83.58E | Vacuum degassing | 2.54 |
| 17.53N: 83.52E | Vacuum degassing | 2.42 |
| 17.49N: 83.58E | He Purging | 2.60 |
| 17.53N: 83.52E | He Purging | 2.49 |

The mean N₂ fixation rates by two methods are statistically insignificant.