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LETTER

Dinitrogen fixation rates in the Bay of Bengal during summer monsoon

Himanshu Saxena1,2,3, Deepika Sahoo1,2, Mohammad Atif Khan1, Sanjeev Kumar1, A K Sudheer1 and Arvind Singh1,3

1 Physical Research Laboratory, Ahmedabad, 380009, India
2 Indian Institute of Technology, Gandhinagar, 382355, India
3 Authors to whom any correspondence should be addressed.

E-mail: arvinds@prl.res.in and himanshu@prl.res.in

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Abstract

Biological dinitrogen (N2) fixation exerts an important control on oceanic primary production by providing bioavailable form of nitrogen (such as ammonium) to photosynthetic microorganisms. N2 fixation is dominant in nutrient poor and warm surface waters. The Bay of Bengal is one such region where no measurements of phototrophic N2 fixation rates exist. The surface water of the Bay of Bengal is generally nitrate-poor and warm due to prevailing stratification and thus, could favour N2 fixation. We commenced the first N2 fixation study in the photic zone of the Bay of Bengal using 15N2 gas tracer incubation experiment during summer monsoon 2018. We collected seawater samples from four depths (covering the mixed layer depth of up to 75 m) at eight stations. N2 fixation rates varied from 4 to 75 μmol N m−2 d−1. The contribution of N2 fixation to primary production was negligible (<1%). However, the upper bound of observed N2 fixation rates is higher than the rates measured in other oceanic regimes, such as the Eastern Tropical South Pacific, the Tropical Northwest Atlantic, and the Equatorial and Southern Indian Ocean.

1. Introduction

1.1. Biological dinitrogen fixation

Nitrogen is a staple element for every living organism in addition to carbon, since all the major cellular components such as genetic materials (e.g., DNA and RNA), proteins, and energy carrier molecules (e.g., ATP) are stemmed from these elements. In the oceans, most of the nitrogen is in the form of dissolved dinitrogen (N2) gas, which is present in an inexhaustible amount. Still it is the bioavailable form of nitrogen (hereafter N, such as NH4+, NO3−, and NO2−) along with other micronutrients that limits the oceanic primary productivity (Broeker 1974, Smith 1984). Therefore, N plays a pivotal role in controlling the oceanic primary production. The scarcity of N limits the phytoplankton growth in most of the world oceans (~80%) located between 30°N and 30°S (Moore et al 2013). On the contrary, low N in waters creates a suitable niche for N2 fixing microorganisms (diazotrophs) which reduce the most abundant but relatively inert N2 to NH4+ (Karl et al 1997, Knapp 2012). Therefore, in N limited marine environments, biological N2 fixation provides a significant source of N for primary production and thus, helps in sustaining ocean fertility (Tyrrell 1999).

Generally, cyanobacteria are the major diazotrophs in oceans and play a central role by providing N to the oceans (Karl et al 2002, Zehr 2011). Oceanic N2 fixation is catalyzed by a few major types of cyanobacteria: filamentous non-heterocystous cyanobacteria, filamentous heterocystous cyanobacteria, unicellular cyanobacteria, and unicellular cyanobacterial symbionts (Luo et al 2012, Thompson and Zehr 2013). One of the cyanobacteria, i.e., Trichodesmium, has been traditionally considered the most dominant diazotrophic genus, but it is now clear that marine diazotrophs are more diverse. Non-cyanobacterial diazotrophs which are not constrained by light may also conduct N2 fixation below the twilight zone (Zehr et al 1998, Riemann et al 2010, 2020).
Luo et al. 2012). They can be detected in a wide range of environments such as nutrient-rich, cold, temperate zones, and the dark and deep oceans (Benavides et al. 2018). But the N\textsubscript{2} fixation rates by non-cyanobacterial diazotrophs are usually near the detection limit or low when compared to cyanobacterial N\textsubscript{2} fixation rates (Moisander et al. 2017).

Marine diazotrophs have profound effects on the net community production, marine N cycle, and organic matter export to the deep ocean (Karl et al. 1997, 2002, Gruber 2008). Above all, there occurs an inextricable link between the world oceans’ N\textsubscript{2} fixation and climate variability. Diazotrophs are entailed in the global feedback mechanisms of the climate system by affecting the concentration of CO\textsubscript{2} in the atmosphere which, in turn, by altering the dust associated Fe supply and the stratification of the upper ocean, would influence the rates of N\textsubscript{2} fixation in the ocean. Thus, it has become necessary to integrate the N\textsubscript{2} fixation rates from the overlooked basins (e.g., the Bay of Bengal) into the global biogeochemical models (Karl et al. 2008).

1.2. The Bay of Bengal: a potential diazotrophic niche

Studies on N\textsubscript{2} fixation in the Indian Ocean are limited compared to the Pacific and the Atlantic Oceans (Sohm et al. 2011, Luo et al. 2014, Benavides et al. 2016). The Arabian Sea and the Bay of Bengal (hereafter the Bay) are the twin basins in the Indian Ocean located at similar latitudes, to the west and the east of the Indian subcontinent, respectively. Both lie in the tropics and receive similar solar irradiation. In the Arabian Sea, a few studies have reported higher N\textsubscript{2} fixation rates compared to that in the world oceans (Gandhi et al. 2011, Kumar et al. 2017, Singh et al. 2019) despite the fact that this region receives N nutrients almost throughout the year via upwelling during summer and convective mixing during winter (Ryther et al. 1966, Madhupratap et al. 1996).

On the other hand, the diazotrophy in the Bay is poorly understood with no database for photic zone N\textsubscript{2} fixation rates. Being oligotrophic, the Bay might also favour comparable or even higher rates of N\textsubscript{2} fixation than the Arabian Sea. The new production (primary production facilitated by new nutrients (Eppley and Peterson 1979)) is similar in the Bay and the Arabian Sea (Kumar et al. 2004b, Singh and Ramesh, 2011 2015). In the Bay, stratification prevents the vertical influx of nutrients in the open ocean. Most of the nutrients coming through rivers are used within estuarine and coastal regions (Prasanna Kumar et al. 2002, Singh and Ramesh 2011), and thus riverine nutrients have negligible contribution on open ocean biological productivity (Duce et al. 2008, Singh and Ramesh 2011). Atmospheric deposition contribution to the nutrients supply is equally low (less than 3%) in the Bay and the Arabian Sea (Singh et al. 2012). Thus, high new production in the Bay suggests the higher probability of N\textsubscript{2} fixation in this basin than the Arabian Sea. Nitrogen isotopic composition of sedimentary organic matter (low $\delta^{15}$N values) also alludes to the presence of diazotrophy in the Bay (Gaye et al. 2005). Although these hypothesized controls on marine N\textsubscript{2} fixation in the Bay are all plausible, none of them have been validated with direct field measurements. Therefore, the main objectives of the present study were to understand the role of stratification and oligotrophic conditions on N\textsubscript{2} fixation rates in the Bay during summer monsoon and to estimate the contribution of N\textsubscript{2} fixation to the primary production in the Sunlit layers of the Bay. We further hypothesized that N\textsubscript{2} fixation might play a crucial role for the primary production in the Bay.

2. Materials and methods

2.1. Sampling and nutrient measurements

Seawater samples were collected at four depths from the top 75 m of the water column at eight locations (SN1 to SN8) during research expedition on-board ORV Sagar Nidhi (SN132) in summer monsoon (12 July – 2 August 2018) in the Bay. The top two depths for sampling were fixed at 10 m and 25 m; the next two depths were mostly 50 m and 75 m as out of these two depths, one depth corresponded to depth of deep chlorophyll maxima (DCM). Samples were collected from Niskin bottles mounted on a CTD (Conductivity-Temperature-Depth) rosette sampler (table 1). Temperature, salinity and density vertical profiles of water column were obtained using the CTD, along with the dissolved oxygen profile using O\textsubscript{2} probe mounted on a CTD rosette sampler. Indian National Centre for Ocean Information Services Real-Time Automatic Weather Stations (I-RAWS) provided wind speed data (Harikumar et al. 2013). Mixed layer depth (MLD) was determined based on the variation in temperature (0.2 °C difference from sea surface temperature (SST)) (de Boyer Montégut et al. 2004). MLD was oxic (data not shown).

For nutrient measurements, 50 ml seawater from each depth was collected. Samples were immediately stored at −20 °C on-board. Nutrients were measured using an auto-analyzer (Skalar Auto-analyzer) at the onshore laboratory. Detection limits for NO\textsubscript{3} + NO\textsubscript{2}, NO\textsubscript{2}, PO\textsubscript{4}\textsuperscript{3−} and SiO\textsubscript{4}\textsuperscript{4−} were 0.16 μmol l\(^{-1}\), 0.06 μmol l\(^{-1}\), 0.02 μmol l\(^{-1}\) and 0.31 μmol l\(^{-1}\) respectively. In addition, samples for dissolved inorganic carbon were also taken and colorimetrically analyzed (data not shown) for C uptake rate calculation.
Table 1. Latitude, longitude, sea surface temperature (SST), sea surface salinity (SSS), N:P, N:Si, mixed layer depth (MLD), depth integrated N₂ fixation rates, and primary production of all the stations.

| Station | Latitude (°N) | Longitude (°E) | SST (°C) | SSS | N:P | N:Si | MLD (m) | Integrated N₂ fixation rate (μmol N m⁻² d⁻¹) | Primary production (mmol C m⁻² d⁻¹) |
|---------|---------------|---------------|----------|-----|-----|------|--------|------------------------------------------|---------------------------------|
| SN1     | 07° 29' 99"   | 88° 14.23'    | 28.8     | 34.19 | 1.2–6.0 | 0.1–0.9 | 64 | 11 ± 5 | 30 ± 8 |
| SN2     | 08° 47' 66"   | 88° 13.44'    | 28.1     | 34.39 | 4.3–9.4 | 1.1–1.4 | 49 | 6 ± 6  | 87 ± 11 |
| SN3     | 12° 29' 60"   | 88° 06.52'    | 28.8     | 33.11 | 0.8–12.4 | 0.1–1.4 | 52 | 27 ± 16 | 39 ± 4 |
| SN4     | 14° 13' 64"   | 88° 03.63'    | 28.5     | 32.78 | 1.5–12.4 | 0.1–1.7 | 48 | 20 ± 4 | 24 ± 2 |
| SN5     | 16° 30' 49"   | 88° 00.42'    | 28.2     | 33.48 | 1.0–9.2 | 0.1–1.4 | 43 | 4 ± 4  | 69 ± 5 |
| SN6     | 16° 28' 19"   | 87° 05.02'    | 28.7     | 33.44 | 0.0–6.4 | 0.0–0.6 | 77 | 12 ± 8 | 58 ± 13 |
| SN7     | 15° 42' 30"   | 85° 28.94'    | 28.7     | 33.62 | 1.7–11.1 | 0.1–1.5 | 41 | 75 ± 98 | 81 ± 12 |
| SN8     | 15° 42' 30"   | 85° 05.32'    | 29.1     | 33.04 | 1.8–4.6 | 0.1–0.6 | 66 | 41 ± 6 | 45 ± 4 |

2.2. Incubation experiments

For N₂ fixation rate and C uptake measurements, duplicate samples were taken from each of the four depths in pre-cleaned ~2.35 l polycarbonate bottles (Nalgene, USA). All bottles were filled without headspace (bubble free). 2 ml of 0.2 mol l⁻¹ NaH¹³CO₃ (99 atom% ¹³C enriched, Cambridge Isotope Laboratories, USA) was added to each bottle (final ¹³C enrichment of 9% in each bottle) for C uptake measurements. Further, 2 ml of ¹⁵N₂ gas (98 atom% ¹⁵N, final ¹⁵N enrichment of 9.3% in each bottle) was injected through the septum caps to the same bottles using a chromatographic gas-tight syringe (Hamilton) for N₂ fixation measurements (Montoya et al. 1996). The bottles were gently shaken for 5 min and covered immediately with calibrated light filters to mimic the light levels similar to the corresponding depths from where the samples were taken. Bottles were incubated for 4 h (from 10:00 to 14:00 h) except at SN8 where bottles were incubated for 24 hours (due to unavailability of CTD during light period) in an on-board incubator in which seawater from surface ocean was circulated to maintain the temperature. After incubation, samples were filtered by gentle vacuum filtration (<200 mm Hg) in dark, sequentially through pre-combusted (400 °C for 4 h) 0.7 µm pore sized Whatman GF/F filters (47 mm diameter) and rinsed with filtered seawater. The filters were dried overnight in an oven at 50 °C and stored in acid cleaned Petri dish for further mass spectrometric analysis. In addition to the samples for incubation, samples (~4.7 l of seawater) for measuring the C and N isotopic composition of natural particulate organic carbon (POC) and nitrogen (PON), from each of the four depths were also filtered and stored in the same manner.

2.3. Chemical and isotopic analysis

For C isotopic analysis, filters were decarbonated to remove inorganic carbon using HCl (36%) fumes for 4 h and dried overnight in an oven at 50 °C following (Lorrain et al. 2003). POC, PON, atom% ¹⁵N and atom% ¹³C in the samples were measured using an Isotope Ratio Mass Spectrometer (Thermo Delta V Plus) attached to an Elemental Analyser (FLASH 2000) at PRL, Ahmedabad. Volumetric rates of N₂ fixation were calculated following (Montoya et al. 1996):

\[ \text{N}_2 \text{ fixation} = \left( \frac{1}{t} \right) \left( \frac{A_{\text{PN}} - A_{\text{PN}0}}{A_{\text{N}} - A_{\text{PN}0}} \right) \left[ \text{PON} \right] \]

where \( t \) = incubation time, \( A_{\text{PN}0} = \text{¹⁵N atom% in PON at the end of incubation}, A_{\text{PN}0} = \text{¹⁵N atom% in PON at the start of incubation}, A_{\text{N}} = \text{¹⁵N enrichment in the dissolved form after the tracer addition at the start of incubation}. \) In the above equation, all the terms are measured except \( A_{\text{N}} \), which is defined as:

\[ A_{\text{N}} = \left( \frac{\text{¹⁵N tracer} \times \text{tracer conc.} + \text{¹⁵N natural} \times \text{natural conc.}}{\text{tracer conc.} + \text{natural conc.}} \right) \]

The natural concentration of dissolved N₂ was calculated using the formula given in Weiss (1970). C uptake rates were calculated likewise to N₂ fixation rates following (Slawyk et al. 1977). Column C uptake and N₂ fixation rates were calculated by the trapezoidal integration of volumetric rates with depth. The error in atom% ¹⁵N and atom% ¹³C measurement, expressed as standard deviation, was less than 1% (excluding SN7 and SN8, where it was 3%) and 2%, respectively. IAEA-N-2 ((NH₄)₂SO₄) for nitrogen, and IAEA-CH-3 (Cellulose) for carbon were used as standards for isotopic measurements.

The N demand for sustaining the observed C uptake rates was calculated using the Redfield ratio (C:N=106:16) (Redfield 1958), by dividing the observed C uptake rates (in molar units) by 6.625. The N₂ fixation rates were divided by N demand to calculate the percentage contribution of N₂ fixation to primary production.
3. Results

3.1. Hydrography or environmental conditions

SST was higher than 28 °C at all the stations (figure 1, table 1). The lowest SST (∼28.1 °C) was observed at SN2 and the highest at SN8 (∼29.1 °C). Salinity varied between 32.78 and 34.39, with the lowest value at SN4 and the highest value at SN2. Surprisingly, no north-south gradient in SST and SSS was observed in the Bay. Wind speed ranged between 3.6 to 24.5 m s$^{-1}$ (average, 11.5 m s$^{-1}$) during study period and MLD ranged between 41 and 77 m, with the lowest observed value at SN7 and the highest at SN6.

Figure 1. Depth profiles of temperature (T), salinity (S), and density ($\sigma_t$) at the eight sampling locations based on the CTD data.
3.3. N2 depths with an average of 4.5 relative to the N provided by N2. For the primary production, in general, sunlight, water, CO2, and nutrients are needed.

4. Discussion

3.2. Nutrients

The concentration of all the nutrients was above the detection limit, except NOx (i.e., NO3− + NO2−) in the upper 25 m at SN3. In general, NOx concentration was lower at surface and increased with depth (figure 2). Similar to NOx, PO43− also increased with depth. N:P was lower than the Redfield ratio and varied from ~0 to 12.4. N:Si varied from ~0 to 1.7 and was higher than the Redfield ratio (N:Si = 8.57) except at SN1, SN3 and SN4 (table 1). P* calculated as [PO43−] − [NO3−]/16, was positive throughout the study period, which indicated PO43− concentration is in excess of NOx (Deutsch et al 2007). The δ15N showed increasing trend with depth. The δ15N of natural PON samples varied between 1 to 6‰ at surface with an average of 3.2‰ and 1 to 8‰ at lower depths with an average of 4.5‰.

3.3. N2 fixation and C uptake rates

Volumetric N2 fixation rates varied from ~0 (below detection limit) to 0.56 nM N hr−1 with the highest rate at SN7 (figure 3). Except at surface of SN7, the rates were less than 0.08 nM N hr−1. The N2 fixation rates were higher near surface and decreased gradually with depth. Surface N2 fixation rates contributed 39 to 86% of the whole water column N2 fixation activity (surface N2 fixation contribution was measured by taking the percentage of surface rates with the sum of water column N2 fixation rates). C uptake rates varied from 1.4 to 282.2 nM C hr−1 (figure 3) with the highest rate at SN7. The depth integrated N2 fixation and C uptake rates ranged between 4 and 75 μmol N m−2 d−1, and 24 to 87 mmol C m−2 d−1, respectively, with the highest depth integrated N2 fixation rate at station SN7 (table 1).

N demand estimated based on the Redfield ratio for sustaining the observed primary production was almost two orders of magnitude greater than the N provided by observed N2 fixation (figure 4). Contribution of N2 fixation to primary production was almost negligible (<0.61%) at all the stations. Since, the demand was high relative to the N provided by N2 fixation, the observed primary production must be sustained by the already present adequate amount of NOx nutrients in the seawater.

4. Discussion

For the primary production, in general, sunlight, water, CO2, and nutrients are needed (Capone et al 2008). In tropical oceans, sunlight is usually not a limiting factor, and water and CO2 in oceans are abundant enough to not limit the primary production. But the availability of macronutrients (such as N and PO43−) and micronutrients (such as Fe) limit the primary productivity in the Sunlit layer of the oceans (Capone et al 2008). The Bay receives nutrients through river run off, atmospheric deposition, eddy, upwelling, and cyclones (which are frequent in the Bay; (Kumar et al 2004b)). Primary producers can actively assimilate anthropogenic N also to meet the N demand for their cellular activity. Most of the N associated with agricultural and non-agricultural activities in the northern India drains into the Ganges River system, which ultimately meets the Bay, potentially bringing large amount of anthropogenic N. However, it is reported that ~96% of this N does not reach to the open Bay and is removed in the estuarine and the coastal regions (Kumar et al 2004b, Singh and Ramesh 2011, Krishna et al 2016), leaving ~0.45 mmol N m−2 d−1 as the fluvial input to the open ocean in the Bay (Kumar et al 2004b). In the Bay, it has been reported that atmospheric N input is about 0.46 Tg N year−1 (Singh et al 2012).

Upwelling in the Bay is confined within ~40 km of coastal region along the south western boundary during summer (Shetye et al 1991, Prasanna Kumar et al 2002). Copious riverine freshwater discharge (1.6 x 1012 m3 yr−1; Subramanian 1993) and heavy rainfall (2 m annually over Bay; (Bigg 1994, Prasad 1997)) during summer monsoon cause a considerable decrease in sea surface salinity. This leads to intensive stratification of sea surface. Wind force...
over the Bay is insufficient of disrupting surface stratification (Prasanna Kumar et al 2002, Shenoi et al 2002, Patra et al 2007). During our observations in the peak monsoon time, wind speed went up to 24.5 m s$^{-1}$ but MLD was never deeper than 77 m. Thus, strongly stratified layers might have inhibited vertical mixing and the supply of nutrients from sub-surface water to surface (Gopalkrishna and Sastry 1985, Prasanna Kumar et al 2002). Therefore, based on the above findings, we hypothesised that to sustain the primary productivity in the central Bay, N$_2$ fixation might play a crucial role.

Figure 3. Vertical profiles of volumetric rates of N$_2$ fixation and C uptake at each station. Error bars represent the standard deviation of duplicates.
Diazotrophs need warm and N depleted water to survive (Capone et al. 2008, Gandhi et al. 2011). Surface water in the Bay is N depleted. Along with the need of N depleted environment, on the contrary, diazotrophs need a PO₄⁻³ and Fe rich environment (Sanudo-Wilhelmy et al. 2001, Mills et al. 2004, Singh et al. 2017). Positive P′ at all the stations indicate the PO₄⁻³ concentration in excess of NO₃⁻. N:P ratio is also less than the Redfield ratio (table 1). Positive P′ (or N:P < 12) indicate that the PO₄⁻³ is not limiting the N₂ fixation rates. Thus, N₂ fixation might be regulated by Fe availability. The dry-deposition fluxes of Fe to the Bay are significantly higher compared to those over the Arabian Sea (Bikkina and Sarin 2013) and surface dissolved Fe ranged from 0.2 to 0.5 nM in the northern Bay (5 °N to 18 °N) (Grand et al. 2015, Chinni et al. 2019). These reported dissolved Fe concentrations indicate that diazotrophs in the Bay are not Fe limited. This in conjunction with warm sea surface water (SST varied between 28 to 29 °C), might create favourable conditions for the growth of diazotrophs. Hence, surface NO₃ depleted and warm water in the Bay might facilitate the sustenance of diazotrophs and their N₂ fixation activity.

The δ¹⁵N of PON depends on the isotopic composition of inorganic nitrogenous sources (6–8‰ of NH₄⁺, 3–7‰ of NO₃⁻, and 0‰ of atmospheric N₂) (Miyake and Wada 1967, Kumar et al. 2004a). Thus, δ¹⁵N of PON is likely to be close to 0‰ in the presence of diazotrophs (Altabet and Mccarthy 1985). The δ¹⁵N of natural PON in our study supports the N₂ fixation activity (figure 2). The N₂ fixation rates showed a maximum at surface than subsurface. This overall decreasing trend of N₂ fixation rates with depth suggests the role of NO₃ nutrients as detrimental to N₂ fixation activity. Therefore, under oligotrophic conditions, N₂ fixation might play an important role in compensating the N demand of primary production. But the N demand observed for C uptake rates in the oligotrophic Bay during our study is ~300 times higher than the N provided by N₂ fixation and thus, the contribution of N₂ fixation to primary production is <1% (figure 4).

Diazotrophic communities in the photic zone of the Bay include both the cyanobacterial and non-cyanobacterial groups. During pre‐summer monsoon, Wu et al. (2019) observed Trichodesmium spp. (more abundant) and unicellular diazotrophic cyanobacteria along with proteobacteria (mainly alpha-, beta-, and gamma-proteobacteria) with higher abundance in the equatorial region. During winter monsoon, Löscher et al. (2020) observed Synechococcus and Trichodesmium with no evidence of unicellular diazotrophic cyanobacteria. Diatoms-associated diazotrophs have also been reported during summer monsoon for same sampling stations as ours (Bhaskar et al. 2007). Thus, diazotrophic cyanobacteria were observed to be the dominant group in the Bay. Diazotrophs require cellular energy to split the N₂ molecule and for this reason, sunlight is an important factor for controlling N₂ fixation by supplying adequate energy to diazotrophs (Karl et al. 2008). Therefore, performing N₂ fixation during the light period has the advantage that this energy-demanding process can then directly be supported by photosynthesis (Fredriksson et al. 1998). It has been reported that the nitrogenase (the specific enzyme used to fix N₂) activity of diazotrophic cyanobacteria is closely related to photosynthesis (Gallon 2001, Karl et al. 2008). The diel cycle of nitrogenase activity in Trichodesmium thiebautii results from anew synthesis of nitrogenase each morning and degradation of nitrogenase in the late afternoon and night (Capone et al. 1990). In addition, there are reports of an N₂ fixing endogenous rhythm in some diazotrophic prokaryotes (such as Synechococcus RF-1 (Capone et al. 1990) and Trichodesmium thiebautii (Grobbelaar et al. 1986)). It has also been reported that N₂ fixation is regulated by light energy supplies (Cai and Gao 2015). For this reason, N₂ fixation occurs predominantly in surface waters (Luo et al. 2014). Despite of a favourable niche for N₂
fixation in the Bay i.e., NO$_3^-$ poor or PO$_4^{3-}$ rich, the rates observed are low in terms of percentage contribution to primary production. This points towards the fact that factors like cloud cover and turbidity might be playing a crucial role in restraining the N$_2$ fixation in the Bay. Being situated in the tropical region, in general, sunlight might not be a limiting factor for the biological production. However, during summer monsoon, increased cloudiness and sediment load due to enhanced riverine flux decreases the light penetration in the Bay. It has been reported that cloud cover and turbidity during summer monsoon are the reasons for low productivity in the Bay than the Arabian Sea (Gomes et al 2000, Prasanna Kumar et al 2010). Therefore, it is likely that cloud cover and turbidity might have contributed towards the low rates of N$_2$ fixation in the Bay.

The N$_2$ fixation rates observed in our study are higher than the recently reported rates in the Bay for January 2014 (Löscher et al 2020) where the rates at DCM were below detection limit though they found the clusters of N$_2$ fixing microbes. Löscher et al (2020) targeted oxygen minimum zones for heterotrophic N$_2$ fixation rates, while most of the N$_2$ fixation occurs in the surface waters. Carbon uptake rates in their study ranged between 286–1855 nmol C l$^{-1}$ d$^{-1}$ at the depth of DCM whereas in our study, it varied from 1.4 to 282.2 nM C h$^{-1}$ (or 17 to 3386 nM C d$^{-1}$) in the Sunlit layer of the Bay. This suggests the Bay to be more productive during summer than the winter monsoon. Another study by Madhupratap et al (2003) in summer monsoon 2001 (Jul–Aug), observed NaH$^{14}$CO$_3$ based depth integrated C uptake rates from 7.5 to 18.3 mmol C m$^{-2}$ d$^{-1}$ along 88°E and 27.3–43.3 mmol C m$^{-2}$ d$^{-1}$ along the coastal transect. Our rates are between 24–87 mmol C m$^{-2}$ d$^{-1}$ along 88°E and 45-81 mmol C m$^{-2}$ d$^{-1}$ for SN6 to SN8. Although there are methodological differences, a ~ 5 fold increase in primary production is observed during summer monsoon in the Bay within the period of almost two decades. This is in agreement with the report that states global marine primary production have increased over the past several decades (Chavez et al 2011).

The upper bound of observed N$_2$ fixation rates in our study is higher than many of the other oceanic regimes (Benavides and Voss 2015, Singh et al 2019), such as Eastern Tropical South Pacific (Knapp et al 2016), Tropical Northwest Atlantic (Goering et al 1966). Depth integrated rates of N$_2$ fixation in the Arabian Sea and Equatorial Southern Indian Ocean during winter, ranged from about 24.6 to 34.7 and 6.27 to 16.6 μmol N m$^{-2}$ d$^{-1}$ respectively (Shiozaki et al 2014). This suggests the Bay is more diazotrophically active during summer than the Arabian Sea and Equatorial Southern Indian Ocean during winter. We compared the N$_2$ fixation rates with primary production of this study and found the contribution of N$_2$ fixation to primary production of about 1.1 to 2.8% for the Arabian Sea and 0.1 to 1.6% for Equatorial Southern Indian Ocean which is not much higher than the Bay. Chen et al (2008) reported N$_2$ fixation rates of 1 to 13 μmol N m$^{-2}$ d$^{-1}$ during different seasons in the South China Sea and the contribution of N$_2$ fixation to primary production calculated by us from their data is about 0.03 to 0.16%. Church et al (2009) reported N$_2$ fixation rates between 20 and 307 μmol N m$^{-2}$ d$^{-1}$ over a three year period in the North Pacific Ocean and the contribution of N$_2$ fixation to primary production in this region calculated by us is about 1 to 4%. Thus, in our study the percentage contribution of N$_2$ fixation to primary production in the Bay is comparable to other oceanic regimes. We require sustained observations of N$_2$ fixation in the Bay as our study is limited to one season (Hermes et al 2019). There is a chance of high N$_2$ fixation during spring and autumn—when high rates of N$_2$ fixation observed in the Arabian Sea (Gandhi et al 2011, Singh et al 2019).

At elevated temperature due to recent ocean warming, the higher rates of respiration and the relative low oxygen concentration in warm seawater are advantageous to keep the nitrogen-fixing cells anoxic for a better nitrogenase activity. Trichodesmium erythraeum bloom in high pCO$_2$ waters of the Bay has been reported (Shetye et al 2013). Therefore, in future, as climate becomes warmer due to rise in CO$_2$, increase in N$_2$ fixation could be observed due to increase in temperature (Boyd and Doney 2002) or high CO$_2$ (Hutchins et al 2007, Levinat et al 2007, Fu et al 2008). N$_2$ fixation may stimulate primary productivity in the N-limited oligotrophic Bay and thus, provide a negative feedback on rising atmospheric CO$_2$ levels. Our study may help in understanding the future advancement of N$_2$ fixation in the Bay as a consequence of global warming.

5. Conclusions

In this study, we have presented the first N$_2$ fixation study within the Sunlit layer of the Bay of Bengal. The oligotrophic Bay encounters warm water, excess PO$_4^{3-}$ and Fe which are prerequisites for diazotrophs to flourish and thus, we hypothesized high N$_2$ fixation in the Bay. But our findings were contrary to our hypothesis. The observed N$_2$ fixation rates are low in the Bay in terms of percentage contribution (almost negligible) to primary production. Due to summer monsoon, turbidity due to copious riverine discharge and cloud cover over Bay might have contributed towards the low rates of N$_2$ fixation. Nevertheless, the upper bound of such seemingly low rates is higher than many world oceanic regimes. This underscores the global importance of the Bay in the global marine nitrogen cycle. However, our results give an imprint of summer monsoon only. Therefore, a more
detailed study is needed to estimate the N\textsubscript{2} fixation rates in the Bay, to understand the effect of different seasons on the rates of N\textsubscript{2} fixation, and at large to establish the role of N\textsubscript{2} fixation for the primary production in the Bay.

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ORCID iDs

Himanshu Saxena 🐓 https://orcid.org/0000-0001-5141-5802
Arvind Singh 🐓 https://orcid.org/0000-0002-3060-891X

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