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Massive Nitrogen Loss Over the Western Indian Continental Shelf During Seasonal Anoxia: Evidence From Isotope Pairing Technique

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The western Indian continental shelf houses the world's largest naturally formed coastal low-oxygen zone that develops seasonally during the summer monsoon. We investigated multiple reductive nitrogen transformation pathways and quantified their rates in this system through anaerobic incubations with additions of ^{15}N-labeled substrates during the anoxic period for three consecutive years (2008–2010). Addition of ^{15}N labeled ammonium ($^{15}NH_4{}^+$) resulted in low to moderate anaerobic ammonia oxidation (Anammox) rates in about half of our incubations from the oxygen depleted waters. In contrast, incubations with labeled nitrite ($^{15}NO_2{}^-$) led to large production of $^{30}N_2$ over $^{29}N_2$ in all incubation experiments, indicating denitrification to be the dominant N-loss pathway. Rates of dissimilatory nitrate/nitrite reduction to ammonium (DNRA) were found to be highly variable and were lower by an order of magnitude than the denitrification rates. Extrapolation of average rates over the sampling periods and volume of anoxic waters showed large nitrogen removal (3.70–11.1 Tg year^{-1}) which is about three times as high as the previously reported estimate (1.3–3.8 Tg year^{-1}). Despite the small area it occupies, this shallow seasonal anoxic zone may account for as much as 20–60% the of the total annual fixed nitrogen loss in the perennial oxygen minimum zone of the Arabian Sea.

Keywords: seasonal hypoxia/anoxia, denitrification, anammox, DNRA, nitrogen loss

INTRODUCTION

As in other parts of the North Indian Ocean, hydrography and biogeochemistry over the western Indian continental shelf (WICS) are also dominated by seasonal reversals of surface currents associated with the monsoons (Schott and McCreary, 2001; Naqvi et al., 2006a). During the winter or the Northeast Monsoon (NEM) the West India Coastal Current (WICC) anomalously flows poleward, causing downwelling and oligotrophic, well-oxygenated conditions off the Indian west coast. By contrast, the region behaves as a mini eastern boundary upwelling system during the summer or the Southwest Monsoon (SWM) when the WICC flows toward the equator. The equatorward flow induces upwelling, which is weak except off the southwest coast of India and the west coast of Sri Lanka where winds are more favorable. As a result, there is only modest enhancement of primary productivity and consequent oxygen demand in near-bottom waters (Naqvi et al., 2010a). However, despite moderate oxygen demand there are several factors that contribute to development of intense anoxia in near bottom waters: (1) The upwelled water, derived from the upper part of the mesopelagic oxygen minimum zone of the Arabian Sea is oxygen-depleted to begin with, although not anoxic; (2) Due to slow upwelling the upwelled water remains over the shelf for a long time; and (3) The cold, saline upwelled water is capped by a thin (<10 m) warm, fresher lens, unusual for any upwelling system, formed due to intense precipitation in the coastal zone during the SWM, resulting in very strong stratification very close to the surface, thereby greatly restricting oxygen diffusion from the surface. The exact time of formation of this coastal OMZ varies strongly. It seems to gradually evolve from the south to the north. Upwelling along the WICS has been reported to start in the south (8°N, off Cochin) probably during spring (in March) (Stramma et al., 1996; Schott and McCreary, 2001), gradually extending northward. In the north (19°N, off Mumbai) the effect of upwelling is felt at least until early December (Naik, 2003; Shankar et al., 2005). This time span far exceeds the duration of the SWM. However, strong water column stratification starts in June due to intense rainfall in coastal areas, which facilitates the formation of the low oxygen zone. The oxygen deficiency intensifies with time resulting in the prevalence of reducing conditions – conversion the oxidized nitrogen to molecular nitrogen (N_2) followed by reduction of sulfate ($SO_4{}^{2-}$) to hydrogen sulfide (H_2S) – by July–August. In the north (e.g., off Mumbai) such conditions develop later in the season and last up to October–November. Thus, overall, reducing conditions over the WICS prevail for at least 3 months (90 days) (Naik, 2003; Naqvi et al., 2006b). These processes operate over a large geographical area, making the WICS the single largest low-oxygen system of its kind in the world, occupying an area of $\sim$200,000 km^2 ($O_2 < 20$ μM) – an order of magnitude bigger than the area of the famous dead zone of the Gulf of Mexico (area with $O_2 < 62.5$ μM: 22,000 km^2 – Rabalais et al., 2010). The shallow anoxic zone in the eastern Arabian Sea is believed to provide significant feedback to the global climate change through unusually high emissions of N_2O (nitrous oxide) and DMS (dimethyl sulfide) (Naqvi et al., 2000; Shenoy et al., 2012).

As evident from the chemical data – a decrease in nitrate ($NO_3{}^-$) and a concomitant accumulation of nitrite ($NO_2{}^-$) – reductive nitrogen removal from this system typically begins to occur during the later phase of the SWM in July/August and continues till October/early November (Naqvi et al., 2009). The reducing zones can be categorized into "suboxic" (functionally identified by the presence of secondary $NO_2{}^-$, although it must be pointed out that recent work by Thamdrup et al. (2012) has shown that the secondary $NO_2{}^-$-bearing waters are also functionally anoxic) and anoxic (sulfidic); these zones are geographically separated, often located over the mid- and inner parts of the shelf (Naqvi et al., 2009). Direct measurements of N-loss rates and the processes involved are very scarce from WICS. Heterotrophic denitrification ($NO_3{}^- \rightarrow NO_2{}^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), in which bacteria use $NO_3{}^-$ as an electron acceptor for organic matter oxidation (Froelich et al., 1979), was long believed to be the only process removing fixed nitrogen from the ocean, until anammox was recognized to be another major N-loss pathway (Dalsgaard et al., 2003; Kuypers et al., 2003). In previous studies undertaken over the WICS, rates reaching up to 837 nmol L^{-1} d^{-1} were determined based on changes in $NO_3{}^- + NO_2{}^-$ concentration over time at fixed sampling locations (Naik, 2003). Similar rate was also calculated assuming that the initial $NO_3{}^-$ content of 20 μM in the upwelled water was fully utilized over a month (Naqvi et al., 2006b). Overall annual N-loss in the range of 1.2–3.6 Tg was arrived at by scaling up these rates to the entire shelf for the duration of the seasonal anoxia. However, these estimates should be considered as minimum estimates as replenishment of the nitrate pool had not been taken into account. Devol et al. (2006) estimated N-loss at a few coastal stations by incubating samples spiked with $^{15}NO_3{}^-$ on board ship and measuring the growth of ^{15}N-labeled N_2. The average rate was found to be to 33.2 nmol N L^{-1} d^{-1}. The highest rate measured by these authors was 207 nmol N L^{-1} d^{-1}, only about a quarter of the rate inferred from the overall changes in the nutrient concentrations. However, these measurements suffered from several uncertainties. For example, they did not take into account the possible N-loss through anammox. Moreover, the signal of labeled N_2 produced from $^{15}NO_3{}^-$ may be significantly diluted due to mixing of $^{15}NO_2{}^-$, originated during the first step of denitrification pathway, with high ambient substrate pool of $^{14}NO_2{}^-$. This may lead to underestimation (Holtappels et al., 2011). Also, none of the studies undertaken so far have considered the dissimilatory nitrate/nitrite reduction to ammonium (DNRA) that could have major implications for the estimation of the N-loss by labeling experiments (Jensen et al., 2011; Song et al., 2016) as well as for the estimates based on temporal changes in nitrate concentration (i.e., while DNRA removes $NO_3{}^-$ or $NO_2{}^-$, it is not an N-loss process). Moreover, dissolved inorganic nitrogen (DIN) loading in this region is believed to have significantly increased in recent years due to enhanced synthetic fertilizer consumption in South Asia ($\sim 17 \times 10^{12}$ g N a^{-1}), exhibiting an increase by factor of 50 since 1960s (Naqvi et al., 2009). Seitzinger et al. (2005) estimated that the South Asian rivers could account for as much as 4.2 Tg year^{-1} of the global riverine DIN input of 20.8 tgN year^{-1} to the ocean in 1990. However, the concentrations of $NO_3{}^-$ reported from some of the rivers draining into the Arabian Sea are quite moderate (e.g., 8–9 μM from the Zuari estuary of Goa – Bardhan et al., 2015). In addition, Naqvi et al. (2010b) estimated annual deposition of $\sim$0.16 Tg of nitrogen from the atmosphere over the WICS which most probably is also increasing due to fossil fuel burning and industrialization (Duce et al., 2008). Consequently, the N-loss estimated from upwelled $NO_3{}^-$ may be considerably lower than actual.

The main aim of the present study is to identify and quantify the multiple potential reductive nitrogen processes operating in the water column over the WICS during the period of anoxia by employing various combinations of ^{15}N-labeled substrates. This is the first report evaluating relative contributions of denitrification and anammox to N-loss and also the significance of DNRA from this region. As the experiments were conducted for three consecutive years the results also provide an idea of the inter-annual variability.

MATERIALS AND METHODS

Sampling Locations and Physicochemical Profiling of Water Column

Samples were collected from several stations located along coast-perpendicular transects during three cruises carried out from 2008 to 2010, on the Coastal Research Vessel *Sagar Sukti* (SaSu#183 and SaSu#205) and using a fishing trawler (CaTS#78). The period of observations was restricted to the time – from late August to late September – when severe oxygen deficiency develops over the WICS. In 2008, only the transect off Goa (15.52^{o}N/73.76^{o}E to 15.423^{o}N/73.283^{o}E) was sampled, whereas in 2009 and 2010 observations along two additional southern transects, off Karwar (14.478^{o}N/74.25^{o}E to 14.325^{o}N/73.768^{o}E) and Mangalore (13.14^{o}N/74.64^{o}E to 12.87^{o}N/74.108^{o}E), were also made (**Figure 1** and **Supplementary Tables S1, S2**). Physicochemical characteristics of the water column (temperature, salinity, dissolved oxygen, inorganic nutrients, chlorophyll *a* and turbidity) were determined at each sampling site. N-loss rates were measured over the inner shelf (depth < 30 m) and mid shelf (depths 30–50 m) where strong reducing conditions prevailed.

A Sea-Bird Electronics CTD system (SBE 911) was used for obtaining continuous profiles of temperature and salinity. Niskin samplers (5 L) fitted with reversing thermometers and mounted on synthetic ropes were used for sampling of seawater from selected depths based on the CTD/O_2 profiles. Dissolved O_2 was measured in each sample following the Winkler titrimetric method (Grasshoff et al., 1983, precision < 1.34 μM). Sub-samples for nutrients (unfiltered) were frozen on *Sagar Sukti* cruises and stored in an ice box on the CaTS field trip for analyses in the shore laboratory using a SKALAR autoanalyzer (precisions ± 0.06, ± 0.01, ± 0.01, ± 0.01 μM for NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}, respectively). In most cases, NO_2^- concentrations were also measured onboard immediately after sampling following the procedure of Bendschneider and Robinson (1952). The lower detection limit of the NO_2^- measurement was 0.05 μM. Deficiency in DIN was estimated

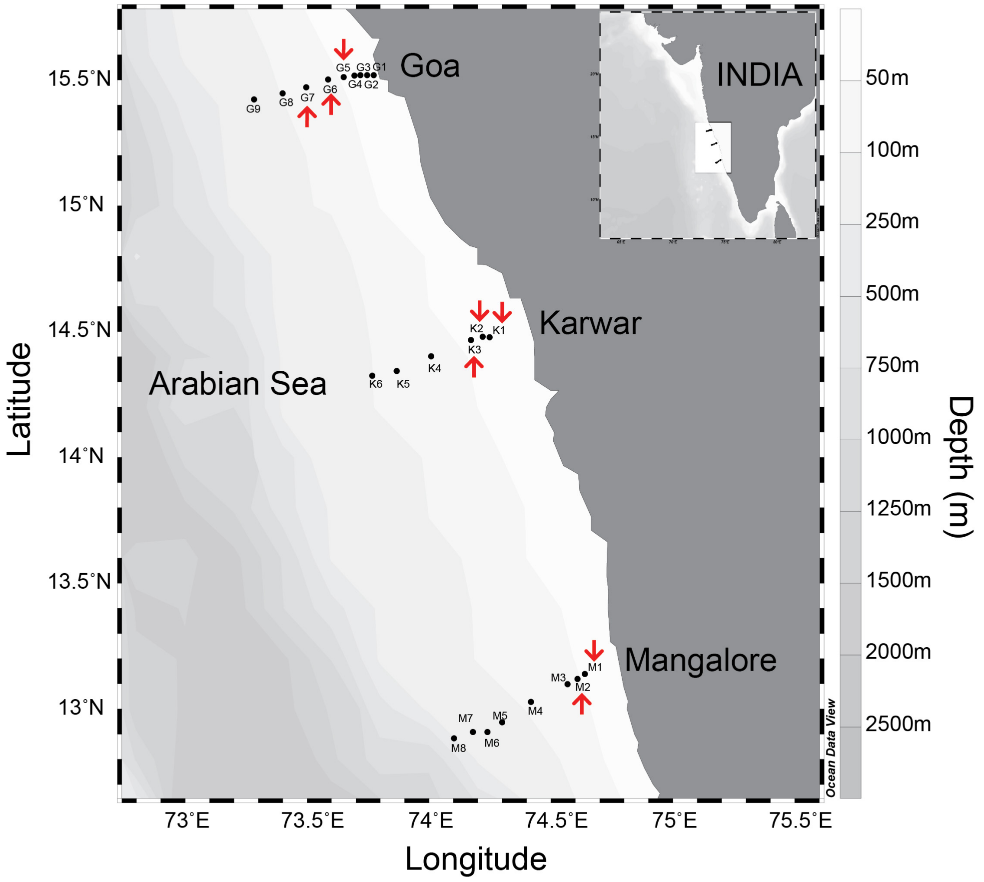

FIGURE 1 | Study area in the eastern Arabian Sea along western continental shelf of India. Biogeochemical monitoring locations on each coast perpendicular transects are indicated by closed circle points. The stations marked with "red arrows" are where ^{15}N-incubations experiments were performed.

from $N^*(\mu M) = [(NO_3^- + NO_2^- + NH_4^+) - 16 \times PO_4^{3-} + 2.9]$ following Gruber and Sarmiento (1997). Chlorophyll *a* samples were collected in 1-liter amber colored HDPE bottles and were filtered immediately through glass-fiber filters (GF/F, 47 mm diameter, 0.7 μm pore size, Whatman) under low vacuum. The filter papers were soaked in 90% acetone and stored at −20°C in the dark to extract the pigments. Quantitative estimation of chlorophyll *a* was done after 24 h spectrofluorometrically at 655 nm wavelength (Strickland and Parsons, 1972). Precision of chlorophyll *a* measurement was ± 0.001 mg m^{-3}.

Isotope Pairing Experiments

Depending on the depth and thickness of the perceived reducing layer, incubation experiments were restricted to 1–3 depths. In 2008, incubations were conducted at two stations at two depths each off Goa (**Figure 2**, O_2 panel marked in diamond symbol). In 2009, three stations each along Goa and Karwar and 1 station off Mangalore were sampled. In 2010, three stations were sampled off Goa, two off Karwar and one off Mangalore (**Figure 2**, **Table 1**, and **Supplementary Figures S2, S3**). Water samples for incubation experiments were taken immediately after the retrieval of samplers in 250-ml glass serum bottles. Water approximating three times of the volume of the bottle was allowed to overflow before the sample was sealed using butyl rubber septum, taking care not to trap any air bubble, and crimped with aluminum cap. Samples were purged with helium for 20 min to reduce the background N_2 concentration as well as to remove oxygen that might have been inadvertently introduced during sampling. After purging with helium for 15 min, various substrates ($^{15}NH_4^+$, $^{15}NO_2^-$ and $^{15}NO_2^- + {}^{14}NH_4^+$, Sigma-Aldrich stable isotope labeled products) were added to serum bottles and purging was continued for another 5 min to ensure removal of oxygen added along with the substrates and also proper mixing. The final concentration of each substrate was 8 μM. Samples were then quickly transferred into 12-ml Labco Exetainers carefully avoiding any atmospheric contamination and incubated in dark at near *in situ* temperature for 36–48 h. Microbial activities were terminated at approximately equal time intervals by adding 100 μl saturated $HgCl_2$ solution after introducing 2 ml of helium headspace (Holtappels et al., 2011). Exetainers were stored upside

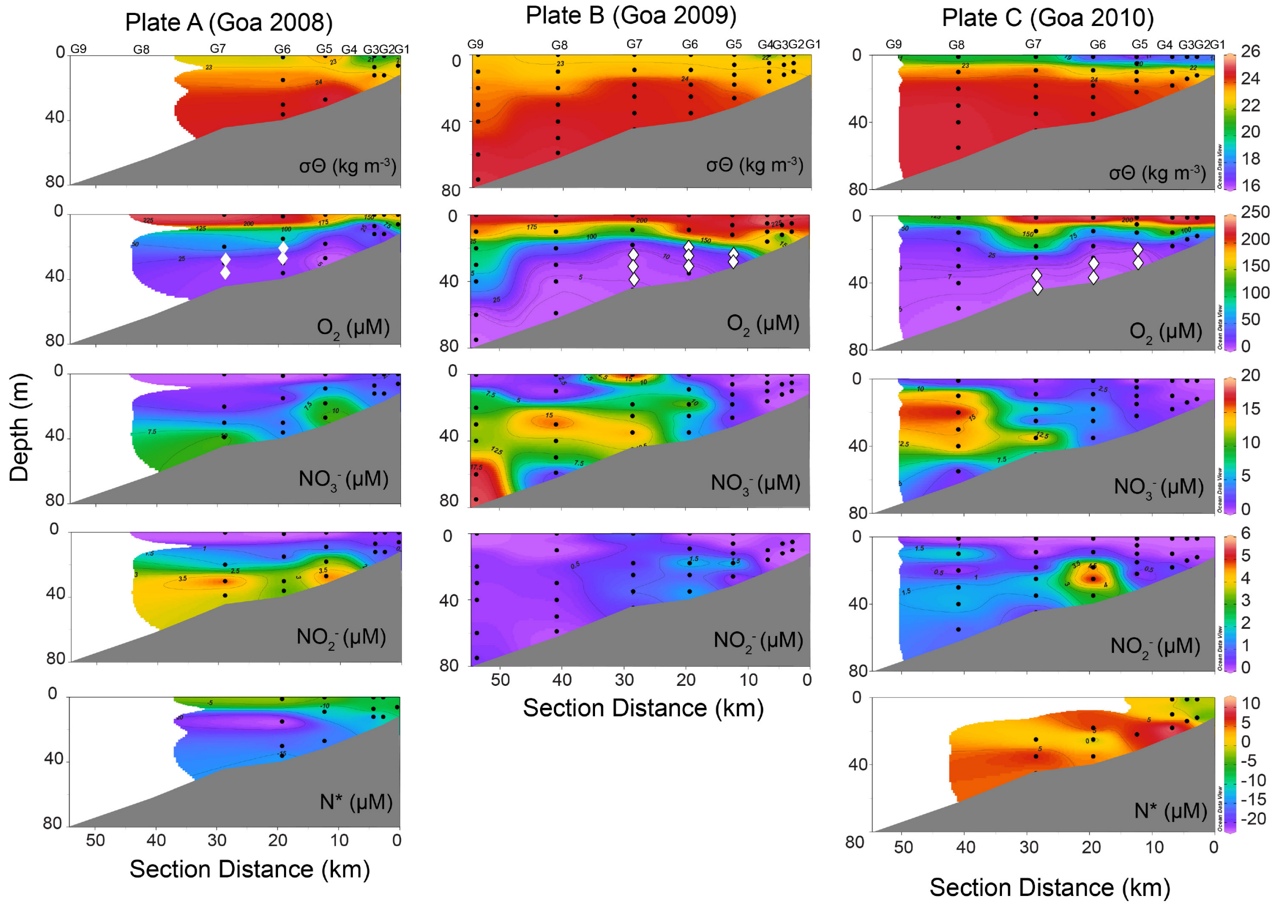

FIGURE 2 | Distribution of physicochemical properties including potential density (σ_θ), dissolved oxygen (O_2), nitrate (NO_3^-), nitrite (NO_2^-), and nitrogen deficit (N*) in the water column along coast perpendicular transect off Goa based on observations carried out during three consecutive years Plate A. 2008, Plate B. 2009, and Plate C. 2010. Depths marked as “diamond-◇” on O_2 panels in all 3 years represents the depths where ^{15}N-incubation experiments were conducted.

TABLE 1 | Calculated rates (mean and stdev values) of different nitrogen transformation process (anammox rates obtained from both 15NH4+ and 15NO2− incubations) along with ancillary chemical data.

Year	Transects	Site	Depth (m)	O_2 (µM)	NO_3^- (µM)	NO_2^- (µM)	NH_4^+ (µM)	Denitrification (µmol N_2L^{-1} d^{-1})	Anammox (µmol N_2 L^{-1} d^{-1}) based on $^{15}NH_4^+$ incubation	Anammox (µmol N_2 L^{-1} d^{-1}) based on $^{15}NO_2^-$ incubation	DNRA (µmol$N_2L^{-1}d^{-1}$)
2008	Goa	G6	30	11.1	3.20	2.7	1.58	1.90 ± 0.16	0.02 ± 0.004	0.52 ± 0.1	n/m
			35	13.9	4.08	2.6	0.06	0.91 ± 0.08	0.01 ± 0.003	0.68 ± 0.001	n/m
		G7	35	10.4	4.05	4.4	0.89	1.93 ± 0.24	0.01 ± 0.001	0.89 ± 0.1	n/m
			40	BDL	11.9	3.03	0.24	1.21 ± 0.95	nd	n/m	n/m
2009	Goa	G5	18	BDL	2.19	2.1	n/m	4.37 ± 0.20	nd	n/m	nd
			25	BDL	0.98	0.07	n/m	8.62 ± 1.10	nd	n/m	nd
		G6	18	32.0	12.6	1.9	n/m	0.45 ± 0.01	nd	n/m	nd
			25	10.5	4.9	0.8	n/m	1.15 ± 0.48	detected	0.91 ± 0.45	nd
			35	2.88	4.67	1.11	n/m	2.25 ± 0.37	nd	n/m	0.32 ± 0.07
		G7	25	4.4	12.6	0.8	n/m	0.53 ± 0.13	detected	0.20 ± 0.10	Nd
			35	BDL	15.9	1.1	n/m	1.31 ± 0.27	detected	0.50 ± 0.21	0.03 ± 0.01
			45	4.5	12.4	0.6	n/m	0.90 ± 0.04	detected	0.01 ± 0.05	nd
	Karwar	K1	15	BDL	1.6	3.08	n/m	9.29 ± 4.52	detected	nd	nd
			20	BDL	2.7	2.83	n/m	7.75 ± 2.25	nd	n/m	nd
		K2	10	24.7	n/m	6.4	n/m	9.34 ± 1.32	nd	n/m	0.14 ± 0.02
			20	7.3	n/m	6.8	n/m	0.66 ± 0.10	nd	n/m	nd
			30	3.9	n/m	6.8	n/m	0.25 ± 0.03	detected	0.07 ± 0.02	0.19 ± 0.009
		K3	18	39.9	18.2	0.5	n/m	0.15 ± 0.03	nd	n/m	nd
			25	5.7	13.6	3.5	n/m	0.33 ± 0.06	nd	n/m	nd
			35	2.9	9.5	2.7	n/m	0.19 ± 0.03	nd	n/m	nd
			44	3.7	11.4	4.9	n/m	0.21 ± 0.01	nd	n/m	0.02 ± 0.006
	Mangalore	M1	24	BDL	n/m	2.5	n/m	10.03 ± 1.7	nd		0.1 ± 0.02
2010	Goa	G5	15	17.9	1.1	0.5	8.3	3.54 ± 2.3	0.02 ± 0.001	0.08 ± 0.29	nd
			25	4.7	1.9	0.1	9.1	0.43 ± 0.08	0.43 ± 0.02	0.79 ± 0.12	0.15 ± 0.006
		G6	30	3.8	4.4	5.5	3.7	0.61 ± 0.02	0.003 ± 0.001	0.35 ± 0.15	nd
			35	3.8	2.6	2.7	4.0	0.24 ± 0.1	0.01 ± 0.003	0.33 ± 0.21	nd
		G7	40	3.9	4.6	0.4	2.1	2.83 ± 0.66	0.24 ± 0.008	1.88 ± 0.43	nd
	Karwar	K2	22	3.8	0.9	1.1	3.3	3.05 ± 1.16	0.014 ± 0.002	1.19 ± 0.68	nd
			26	2.48	5.7	0.1	4.3	0.12 ± 0.03	0.011 ± 0.001	0.12 ± 0.03	nd
		K3	35	6.7	15.7	1.2	3.1	0.03 ± 0.006	0.03 ± 0.01	0.05 ± 0.02	nd
			50	2.0	2.7	0.1	5.2	0.03 ± 0.004	0.01 ± 0.001	0.04 ± 0.02	nd
	Mangalore	M2	20	n/m	11.8	5.9	1.8	3.66 ± 2.11	0.03 ± 0.001	nd	0.04 ± 0.002
			35	17.6	15.8	8.4	2.2	2.77 ± 0.50	0.05 ± 0.001	nd	nd

n/m = not measured, nd = not detected; BDL = below detection limit.

down until analysis to prevent gas loss from the headspace through the septa.

Analysis and Calculation of Denitrification, Anammox, and DNRA Rates

The stable isotopic composition of N_2, measured as $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ in the headspace, was determined within a few weeks of collection using a continuous flow mass spectrometer coupled with a custom build gas chromatograph (Fisons VG Optima, Manchester, United Kingdom). 100 µl of headspace sample was slowly and carefully drawn from the vial and injected into the GC-IRMS system using gas-tight syringe. Deionized water was used to compensate for the slight under-pressure in the headspace arising from outward He diffusion during storage as well as to make up for the volume of the gas withdrawn. Air was injected as the standard before and after each batch of eight samples. Concentrations of $^{29}N_2$ and $^{30}N_2$ produced during incubation were calculated as described by Holtappels et al. (2011). After analysis of the labeled N_2, $^{15}NO_2^-$ amended samples were used for DNRA rate measurements. The isotopic composition of NH_4^+ in the samples was determined after chemically converting it to N_2 with sodium hypobromite (NaOBr) solution using the same mass spectrometer (Warembourg, 1993; Preisler et al., 2007).

The rates were computed as the slope of linear regression of ^{15}N-production over time. Concentrations of N_2 produced via denitrification and anammox pathways were calculated following Thamdrup and Dalsgaard (2002). N_2 production via denitrification was computed from $^{15}NO_2^-$ incubation as:

Denitrification = $^{30}N_2 \times [F_{NO2}]^{-2}$

Where, F_{NO2} refers to the ^{15}N-mole fraction in the NO_2^- pool.

Anammox was quantified from the $^{15}NH_4^+$ incubation considering the mole fractions of ^{15}N in the substrate pool (F_{NH4}) and excess $^{29}N_2$:

Anammox=$^{29}N_2 \times [F_{NH4}]^{-1}$

In some cases (at stations G6 and G7 off Goa, and station K2 off Karwar in 2009) where NH_4^+ concentrations were not available, anammox rates were calculated from $^{15}NO_2^-$ incubation as:

Anammox=$^{29}N_2 \times [F_{NO2}]^{-2} - [\text{Denitrification} \times 2 \times (1 - F_{NO2})]$

Dissimilatory nitrate/nitrite reduction to ammonium rates were calculated from the slope of linear regression of ^{15}N-labeled NH_4^+ production with time in the NO_2^- spiked samples (Jensen et al., 2011).

RESULTS

Physicochemical Characteristics

The physicochemical conditions along various transects visited in the 3 years were very similar in that water column was strongly stratified in all cases (as exemplified by transect off Goa in **Figure 2**). In 2008 and 2009, surface water had potential density (σ_θ) of ~20–22.5 kg m^{-3} that increased to 23.5–24.5 kg m^{-3} in the cooler and more saline subsurface upwelled water below the thermocline (**Figure 2**, Plates A and B). Due to a more intense rainfall in 2010, surface σ_θ values were much lower (**Figure 2**, Plate C). In all cases, surface waters were mostly oxygen saturated or slightly supersaturated (>200 μM) but O_2 concentrations declined sharply (<10 μM, often reaching non-detectable limit) below the thin surface layer. Presence of sulfide was noticed by its strong odor along all transects in 2009. Surface water was mostly nutrient depleted. Over the deeper outer shelf (depth > 50 m) NO_3^- levels were higher (>15 μM) below the surface, but the concentrations decreased steadily toward the coast. Lower NO_3^- levels in oxygen-depleted water over the inner- and mid-shelf regions often coincided with elevated NO_2^- (up to 6 μM) indicating vigorous anaerobic microbial NO_3^- reduction (**Figure 2**). Dissolved NH_4^+, whenever measured, showed some accumulation in NO_3^- reducing water, either being produced through organic matter mineralization and/or DNRA or originating from the underlying sediment. Except for the year 2010, off Goa, strongly negative N* values in subsurface waters over the shelf indicated high fixed N-loss under suboxic/anoxic conditions (**Figure 2**, Plates A and C). The two southern transects (off Karwar and Mangalore) also showed similar physicochemical characteristics (**Supplementary Figures S2, S3**).

Nitrogen Reduction Pathways Over the WICS

Denitrification was detected in all samples, as indicated by significant ($r^2 \geq 0.7$) production of $^{30}N_2$ in $^{15}NO_2^-$-spiked incubations. In fact, most of the N-loss over the shelf appears to be through this process (**Table 1** and **Supplementary Figure S4**, 1 to 33). Production of $^{30}N_2$ exceeded that of $^{29}N_2$ in a majority of experiments, maintaining the theoretical production ratio of 2 $(1-F_{NO2})/F_{NO2}$ through random isotope pairing as proposed by Nielsen (1992). In 2008, denitrification rates along the Goa transect ranged from 0.91 ± 0.08 to 1.93 ± 0.24 μmol N_2 L^{-1} d^{-1}. Higher denitrification rates were observed in 2009 at the inner shelf stations. At station G5, the denitrification rate averaged 6.49 ± 0.65 μmol N_2 L^{-1} d^{-1}; even higher rates were measured along the two southern transects, 9.34 ± 1.32 μmol N_2 L^{-1} d^{-1} off Karwar and 10.03 ± 1.7 μmol N_2 L^{-1} d^{-1} off Mangalore (**Table 1**). In 2010, denitrification rates were considerably lower over the inner shelf as compared to those in 2009. However, at the mid shelf stations inter-annual variations were less pronounced. Using all the 3 year's data, the average denitrification rate was calculated as 2.45 ± 0.63 μmol N_2 L^{-1} d^{-1}.

Anammox activity was confirmed by results of $^{15}NH_4^+$ incubations that led to significant $^{29}N_2$ production in over half (60%) of the all experiments (**Table 1** and **Supplementary Figure S5**, 1 to 20). Anammox rates were calculated using $^{29}N_2$ production rates from both $^{15}NH_4^+$ and $^{15}NO_2^-$ incubations; these are presented in **Table 1**. The rates were significantly different from each other (Independent samples *t*-test, $p < 0.05$) with consistently higher rates from $^{15}NO_2^-$ incubations. This is expected due to the differences in ^{15}N enrichment in the substrate pool (Bulow et al., 2010 and references there in). For consistency, in the present study we considered anammox rates derived only from $^{15}NH_4^+$ incubation which is recommended to be more direct and precise. In 2008, anammox rates ranged between 0 and 0.02 ± 0.004 μmol N_2 L^{-1} d^{-1}. This process was mostly undetectable in 2009. In 2010, overall anammox rates were higher but highly variable from below detection to 0.43 μmol N_2 L^{-1} d^{-1}. The average anammox rate derived from all our $^{15}NH_4^+$ incubations was ~0.06 ± 0.004 μmol N_2 L^{-1} d^{-1}. These numbers are two orders of magnitude lower than the above-mentioned denitrification rate.

Dissimilatory nitrate/nitrite reduction to ammonium activities were seen only sporadically. In both 2009 and 2010, DNRA activity was found at one depth along the Goa transect, and anammox was not detected in 2008. Off Karwar DNRA was found at 3 depths in 2009, but it was absent in 2010. A completely opposite trend was observed off Mangalore. The average DNRA rate (109.25 ± 22.1 nmol N_2 L^{-1} d^{-1}) was higher than that observed over the Omani shelf (highest ~40 nmol N_2 L^{-1} d^{-1}).

Spatial and Inter Annual Variability of Nitrogen Reduction Processes

The spatio-temporal trends in denitrification and anammox rates were tested using non-parametric Mann Whitney *t*-test (two-tailed). Due to large intra-annual variability, no inter-annual variability was evident in both processes (**Supplementary Figures S10a,b**). However, denitrification rates along the Goa coast were significantly lower than that at the Karwar coast at $p \leq 0.1$ (p: 0.10), and Mangalore coast at $p < 0.05$ (P: 0.04). Anammox rates were different only between Karwar and Mangalore at a significance level of $p \leq 0.1$ (p: 0.07). Denitrification was found to be more active at the oxic-suboxic/anoxic interface compared to deeper water (**Figures 3A,B**). In 2009, low rates of denitrification were measured at the base of the oxycline at stations G6 (**Figure 3B**) and K3 where O_2 concentrations were > 30 μM (**Table 1**). These O_2 levels probably inhibited denitrification at these stations. In fact, with just a few exceptions, low levels of denitrification (only 10–25% of the mean of all measured rates) were consistently found in waters containing measurable O_2. Anammox activity

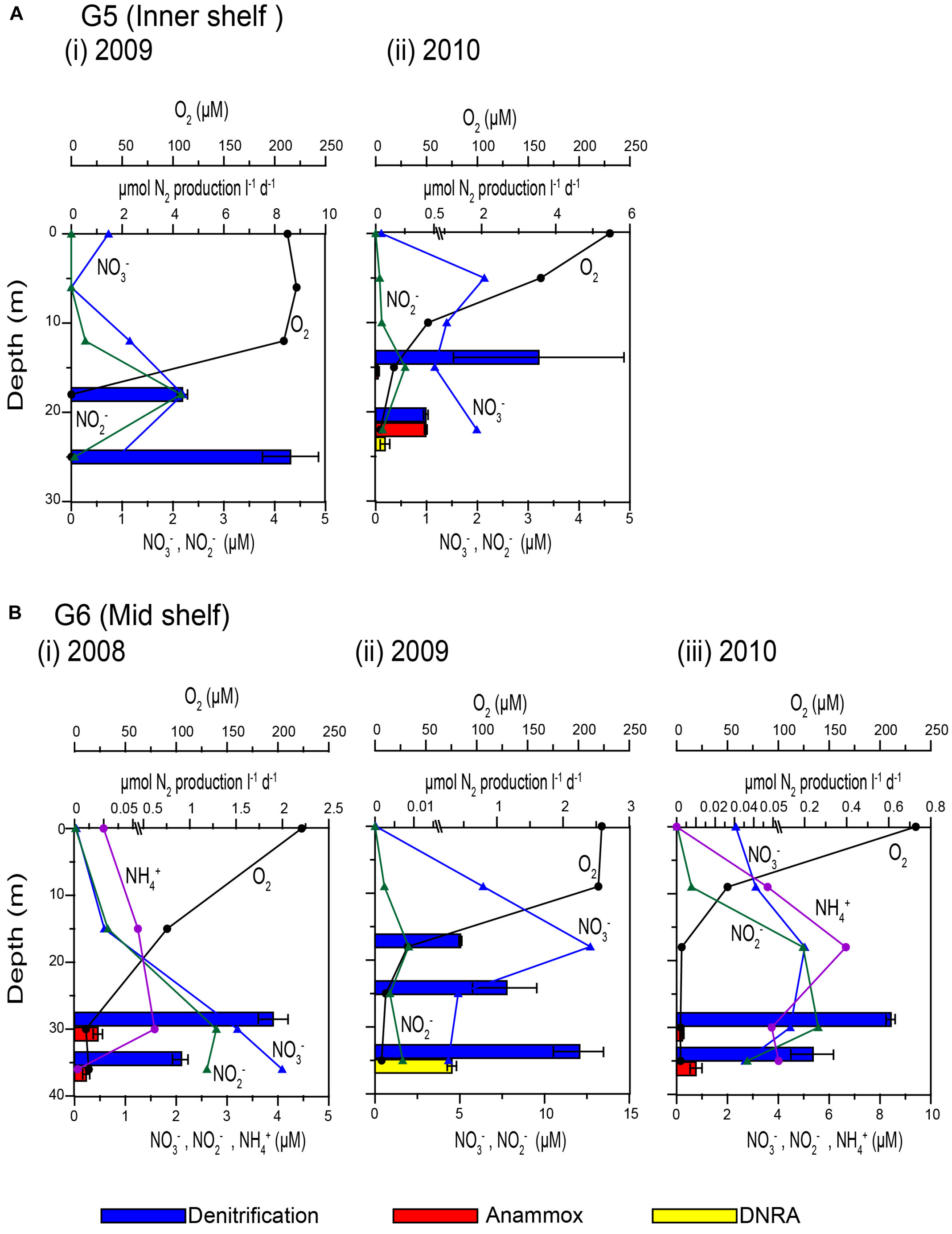

FIGURE 3 | Depth wise variations in concentrations of dissolved oxygen, nitrate, nitrite and ammonium (connected vertical profiles) and N_2 production rates measured by isotope pairing experiments (horizontal bars): **(A)** G5 inner shelf station off Goa (i) 2009 and (ii) 2010 and **(B)** G6 mid shelf station off Goa during (i) 2008, (ii) 2009, and (iii) 2010.

was low and stochastic to infer any trend in its depth distribution (**Table 1** and **Figures 3A,B**). In 2010, anammox rates in bottom waters at stations G5 and G7 off Goa, were not only higher than those at the oxycline but also the highest recorded in this study (**Table 1** and **Figures 3A,B**). However, at most of the depths where the anammox activity was detected O_2 levels were low but often measurable (<5 μM).

Overall denitrification rates did not show large inter annual variations over the 3 years period of the study. Over the inner shelf, average denitrification rate was higher in 2009 (6.49 ± 0.65 μmol N_2 L^{-1} d^{-1}) compared to 2010 (1.98 ± 1.1 μmol N_2 L^{-1} d^{-1}, respectively). In comparison, average anammox average rate was the highest in 2010 (0.07 ± 0.004 μmol N_2 L^{-1} d^{-1}), seven times more than the rates measured in 2008 (0.01 ± 0.002 μmol N_2 L^{-1} d^{-1}). In 2009, near-bottom waters at most inner shelf stations were found to be sulfidic (**Supplementary Figure S1**). At these stations denitrification rates were exceedingly high while anammox activity was not detected (**Figure 3A**).

N-Loss Rates Over the WICS

Despite the intra-annual variability observed at individual stations/sections, our data show quite similar average denitrification rates for 3 years. The mean values of our 3-year N-loss rates through denitrification and anammox were 2.45 ± 0.6 and 0.06 ± 0.004 μmol N_2 L^{-1} d^{-1}, respectively. For up-scaling these values over the entire shelf we followed the assumption previously made by Naqvi et al. (2000) and Naik (2003). Naqvi et al. (2000) estimated the area of the hypoxic zone over the WCSI to be 180,000 km^2. However, O_2 levels over this entire area are not low enough to support reductive N-loss, and as a lower limit about one third of the entire shelf, 60,000 km^2, was assumed to functionally anoxic. The total volume of reducing water was estimated to range between 1.2 and 3.6×10^{12} m^3 taking the thickness of the reducing layer to be 20 m. Applying our 3 years average N-loss rates to this volume and assuming the reducing condition to last for 90 days, the overall N-loss would range from 3.70 ± 0.091 to 11.1 ± 2.72 Tg $year^{-1}$ via denitrification, and from 0.09 ± 0.01 to 0.27 ± 0.03 Tg $year^{-1}$ via anammox (**Table 2**).

DISCUSSION

Oxygen Deficiency and Associated Biogeochemical Properties

Consistent with earlier observations (Naqvi et al., 2009, 2010a), suboxic (Winkler $O_2 < 10$ μM; NO_3^- and $NO_2^- > 0$ μM) and anoxic ($O_2 = 0$ μM; $H_2S > 0$ μM) conditions were found to prevail over the inner-shelf and mid shelf regions in the present study as well. This oxygen deficient environment that evidently supported reductive nitrogen transformations (Hannig et al., 2007; Lavik et al., 2009; Naqvi et al., 2010a) was chosen for ^{15}N labeling experiments (**Figure 2**, depths marked by diamond in O_2 panels). Oxygenated surface water had extremely low NO_3^- concentrations (< 1 μM at most of the stations) with very few exceptions. Average surface chlorophyll *a* concentration was ∼2 mg m^{-3}, with the highest concentration (7.01 mg m^{-3}) occurring at a station M8. Earlier observations made during the SWM also revealed high surface chlorophyll levels (e.g., 2.63 mg m^{-3} at station G5 – Maya et al., 2011). Bhavya et al. (2017) reported average carbon uptake rate of 0.23 mg C l^{-1} d^{-1} water over the coast of Mangalore; this was significantly higher than production during the pre-monsoon but slightly lower than that during the post-monsoon period. In the coastal Arabian Sea primary production has been reported to be the largest contributor to the carbon stock (Barber et al., 2001). Therefore, the low NO_3^- levels in surface waters observed in the present study are probably due to biological uptake which in turn makes organic matter available to support microbial respiration in sub surface layers, increasing the O_2 demand (Devol et al., 2006). Hypoxic water below the surface over the outer shelf was NO_3^- rich (∼15 μM), but NO_3^- was gradually depleted as the water upwelled and moved up the shelf. Accumulation of NO_2^- in the suboxic water over the inner- and mid-shelf points to active denitrification that apparently varied in strength spatially. When the NO_3^- was fully utilized, sulfate reduction commenced leading to buildup of H_2S and NH_4^+. The highest NH_4^+ concentrations (up to 9.1 μM) in low-oxygen waters (**Table 1**) are consistent with earlier measurements by Naqvi et al. (2009). Concentration of H_2S was not measured in the present study; however, its frequent presence over the inner shelf was indicated by strong odor. Soon after our observations off Goa in 2009, station located on Goa transect was visited for time series sampling and a build-up of sulfide in subsurface water was noticed (**Supplementary Figure S1**, reaching concentration as high as ∼12 μM (Shenoy et al., 2012). Overall, our results show the presence of intensely O_2-depleted waters underlying a shallow and sharp pycnocline suitable for suboxic/anoxic microbial processes like denitrification, anammox and DNRA.

Regulation of Nitrogen Reduction Pathways Over the WICS

In the present study, denitrification was found to be the dominant fixed nitrogen removal process. The average denitrification rate (2.45 ± 0.6 μmol N_2 L^{-1} d^{-1}) was more than double of the previously reported value derived from the observed $NO_3^- + NO_2^-$ loss over time (Naik, 2003) and almost two orders of magnitude higher than the estimates derived from incubation of samples spiked with $^{15}NO_3^-$ (Devol et al., 2006). Contrastingly, anammox process, measured and detected for the first time over the WICS, was found to occur to a much lesser extent (∼0.06 ± 0.004 μmol N_2 L^{-1} d^{-1}). In $^{15}NH_4^+$ incubations, production of $^{30}N_2$ was not detected thereby ruling out significant involvement of micro-aerobic ammonium oxidation as reported from the Black Sea (Lam et al., 2007). Nevertheless, our anammox rate over the Indian shelf in the eastern Arabian Sea is comparable with the rate measured off Oman in the western Arabian Sea where this process was found to be the dominant nitrogen loss pathway (Jensen et al., 2011). This indicates that anammox is not limited by the substrate availability as both NO_3^- and NH_4^+ were mostly available in near-bottom waters sampled by us. The growth rate of

TABLE 2 | Total nitrogen loss (mean and standard deviation values) from eastern Arabian Sea from present study and comparison with earlier data extrapolated to the OMZ area and thickness adopted from Naqvi et al. (2000).

Source	Area of Coastal OMZ (Km^2)	Thickness OMZ (m)	Average Denitrification (μmol N_2 L^{-1} d^{-1})	Average Anammox (μmol N_2 L^{-1} d^{-1})	Removal of Fixed Nitrogen (Tg N $year^{-1}$)	
					Denitrification	Anammox
Present study	60–180	20	2.45 ± 0.6	0.06 ± 0.004	3.70 ± 0.91–11.11 ± 2.72	0.09 ± 0.01–0.27 ± 0.03
Devol et al., 2006	60–180	20	0.033	n/m	0.05–0.15	n/m
Naik, 2003	60–180	20	0.83 ± 0.6	n/m	1.3–3.8	n/m

n/m = not measured.

anammox bacteria are reported to be very slow, with doubling times of 10–30 days in bio-reactors under controlled conditions (Strous et al., 1998). Lower doubling times of 5.5–7.5 days were obtained in specialized membrane bioreactor (van der Star et al., 2008); however, the growth of anammox organisms is believed to be even slower in the OMZs (Dalsgaard et al., 2012). In the eastern Arabian Sea non-sulphidic (suboxic) anaerobic conditions generally persist only for a few weeks and within this relatively short time anammox bacteria may not be able to build the necessary biomass to utilize the relatively abundant ammonia. In this study DNRA was independently found to occur in some samples in the absence of detectable anammox activity. Along the shelves off Peru and Oman, occurrence of DNRA and its close coupling with anammox has been reported to account for the production of $^{30}N_2$ in experiments conducted with $^{15}NO_2^-$ (Kartal et al., 2007; Jensen et al., 2011). However, our incubations with $^{15}NO_2^- + {}^{14}NH_4^+$ did not show any effect on $^{30}N_2$ production compared to incubations with $^{15}NO_2^-$ alone. If DNRA-anammox were coupled, then addition of $^{14}NH_4^+$ would have diluted the $^{15}NH_4^+$ pool thereby resulting in a lower $^{30}N_2$ yield (Kartal et al., 2007; Song et al., 2016). Furthermore, only ~24% of the samples yielded positive results in our direct measurement of $^{15}NH_4^+$ production from $^{15}NO_2^-$ in 2009 and 2010, and showed no correlation with the anammox rates. The average DNRA rate (109.6 ± 22.1 nmol N_2 L^{-1} d^{-1}) was higher than that observed over the Omani shelf (highest ~40 nmol N_2 L^{-1} d^{-1}). This indicates that while DNRA could make some contribution to the observed NH_4^+ accumulation over the Indian shelf, the sedimentary flux of NH_4^+ is perhaps more important as Pratihary et al. (2014) reported very high benthic NH_4^+ efflux (3.74 mmol m^{-2} d^{-1}) based on whole core incubations in this region.

The ambient O_2 concentration did not show any clear control over denitrification ($r = 0.22$, $p > 0.05$) and anammox ($r = 0.17$, $p > 0.05$) in present study. However, it must be noted that the Winkler O_2 measurements at near-zero levels may result in systematic overestimation. There are several reports on the sensitivity of denitrification to ambient O_2 concentrations ranging from 0.2 to 5 µM in non-sulphidic O_2-depleted waters (Dalsgaard et al., 2014, 2012; Bristow et al., 2017). For the first step of denitrification – nitrate reduction to nitrite – a somewhat high O_2 tolerance (>25 µM) was noticed from the OMZ waters off Namibia and Peru (Kalvelage et al., 2011) than what is inferred from the field data (Thamdrup et al., 2012). Earlier observations in the Baltic Sea (Rönner and Sörensson, 1985) showed that O_2 concentrations of ~9–11 µM could inhibit denitrification; this is supported by the results of Jensen et al. (2009) from the Mariager Fjord. Like denitrification, anammox is also sensitive to the ambient O_2 concentration. In the Black Sea, anammox bacteria were found to be active at low levels of O_2 (4–8 µM) but showed complete inhibition at 13–14 µM (Jensen et al., 2008). Elsewhere in the oceans tolerance of anammox to O_2 has been found to vary from nanomolar levels in the OMZs to as high as ~15 µM in shallow waters (Kalvelage et al., 2011; Dalsgaard et al., 2012). As stated earlier, the Winkler O_2 data used in some of these studies are not very reliable at vanishingly low levels. However, if real, this variability may be explained either by adaptation of anammox organisms to variable O_2 conditions in coastal waters or by the presence of aggregated particles providing anaerobic micro-niches (Kalvelage et al., 2011; Klawonn et al., 2015). Time series observations by Shenoy et al. (2012) carried out for 3 days at station G5 showed highly fluctuating O_2 concentrations on very short time scales (hours), indicating that the anammox and possibly also denitrifying bacteria might be better adapted to these varying O_2 concentrations. Thus, due to multiple uncertainties it is not possible to categorically address the issue of O_2 control on rates measured in the present study.

In many of the incubation experiments carried out by us, an initial lag phase of 10–12 h occurred before linear increase of ^{15}N labeled N_2 production. Around 70% of experiments with $^{15}NO_2^-$ amendments in which denitrification were detected and ~30% experiments with $^{15}NH_4^+$ which confirmed anammox activity showed such a lag phase. The most likely explanation of this time lag, also observed by us in similar incubations of anoxic reservoir samples (Naqvi et al., 2018), is that the microbes that perform denitrification and anammox were present in the water but were activated only when O_2 level fell below the threshold (functionally anoxic). The possible causes of the presence of O_2 in our samples subjected to incubation are discussed below. The data from the lag phase were not considered for calculation of rates and only the linear portion of the curve was used for this purpose. In some cases, a large (exponential) increase in labeled N_2 production, departing from the linear trend, was also seen toward the end of the experiments. These data points, ostensibly arising from the so-called "bottle effect," were not used for the rate calculation either. For these reasons, the rates computed by us should be considered as conservative. It is worth mentioning that average denitrification rates estimated from all experiments without lag phases (1.78 ± 0.19 μmol N_2 L^{-1} d^{-1}) were marginally lower than those from experiments with lag phases (2.74 ± 0.8 μmol N_2 L^{-1} d^{-1}). Even our lower mean values are more than twice the earlier estimates from the WICS (0.83 μmol N_2 L^{-1} d^{-1} – Naik, 2003). Contrastingly, in case of anammox, rates calculated from $^{15}NH_4^+$ experiments with lag phase in $^{29}N_2$ production were relatively far lower (0.01 ± 0.003 μmol N_2 L^{-1} d^{-1}) than those from experiments showing linear production from the beginning (0.08 ± 0.004 μmol N_2 L^{-1} d^{-1}). This suggests that anammox rates could not have been overestimated.

In isotope pairing technique (IPT) a time lag of ∼40 h in labeled N_2 production has been observed previously in $^{15}NO_3^-$ incubations (Dalsgaard et al., 2003). This can be attributed to the time taken for the conversion of NO_3^- to NO_2^- pool before N_2 production starts. In modified IPT involving $^{15}NO_2^-$ as the substrate, time lag could arise from the inhibitory effect of traces of O_2 on denitrification/anammox in the incubated samples. The length of time lag has been found to be directly proportional to the O_2 concentration (Baumann et al., 1997). Results of O_2 amendment experiments by Jensen et al. (2008) revealed that the presence of ∼13.5 μM of O_2 could produce 30 h lag before labeled N_2 production commenced. There are several possibilities of O_2 contamination occurring during experiments involving the IPT. Dalsgaard et al. (2014) pointed out that O_2 could leach out from the PVC samplers to anoxic water samples. However, purging with helium for 15 min in 250-ml samples collected in serum bottles should take care of such contamination; this has been practiced as standard operating protocol in many previous studies, both in water from oceanic OMZs (Dalsgaard et al., 2003; Kuypers et al., 2005; Jensen et al., 2008; Holtappels et al., 2011) and anoxic freshwaters (Naqvi et al., 2018). However, there still remains a possibility that some O_2 contamination could occur while transferring samples from serum bottles to Labco exetainers. De Brabandere et al. (2014) also demonstrated that Labco exetainers often used for incubations, as was also done in our study, could suffer from significant O_2 diffusion through their butyl septa. Considering O_2 consumption rates of 0.13 μmol l^{-1} h^{-1} in $^{15}NO_2^-$ incubation (Jensen et al., 2008), ∼1.3 μM O_2 might have produced a time lag of 10–12 h in our experiments. The initial time lag was seen in samples having measurable O_2 levels (often > 10 μM). We strongly believe that He purging for 20 min was sufficient to completely remove O_2 initially and that the production of lag phase is most likely due to subsequent contamination. Interestingly, no time lag was observed in the case of samples that contained traces of sulfide, as in our 2009 experiments. We hypothesize that traces of O_2 introduced through contamination could have been quickly utilized for the oxidation of sulfide, but as discussed below we do not rule out the possibility of the occurrence of chemolithotrophic denitrification in the presence of sulfide (Lavik et al., 2009).

Over the Peruvian and Namibian shelves, re-suspension of sediments and presence of copious marine snow are expected to be most conducive for creating particle associated micro-anaerobic niches within which substantial N-loss could occur (Kuypers et al., 2005; Woebken et al., 2007; Kalvelage et al., 2011, 2013). Co-variation of turbidity and anammox rates was observed in these areas. The water column over the western Indian shelf is more turbid (1–4 NTU, measured in 2009), than off Peru and Namibia (0.05–0.1 NTU; Kuypers et al., 2005). In the present study, water turbidity (**Figure 4A**) showed statistically significant correlation with denitrification ($r = 0.47$, $p < 0.05$). This suggests N removal through denitrification may also occur in particle-associated micro-anaerobic niches over the WICS.

The availability of NO_3^- and organic matter are expected to regulate heterotrophic denitrification (Ward et al., 2008; Dalsgaard et al., 2012) as well as anammox (Kalvelage et al., 2013; Callbeck, 2017) in oceanic oxygen-deficient zones. In our study, in some cases, we noticed high denitrification rates to be associated with higher chlorophyll *a* (a proxy of organic matter availability) as well as lower NO_3^- levels (reflecting denitrification/anammox activities). NO_3^- showed statistically significant inverse relation ($r = -0.43$, $p < 0.05$), whereas chlorophyll *a* yielded non-significant correlations with the measured denitrification rates ($r = 0.20$; $p > 0.05$) (**Figure 4B**).

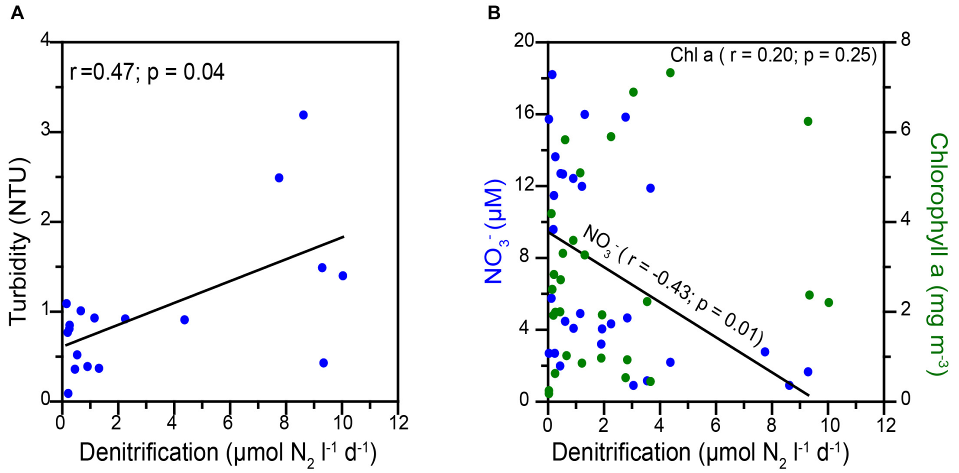

FIGURE 4 | Correlations between **(A)** Denitrification versus turbidity and **(B)** denitrification versus nitrate (blue circles) and chlorophyll *a* (green circle).

This indicates that the N-loss was not limited by organic matter over the Indian shelf.

Sulfide has been reported to inhibit anammox activity (Dalsgaard et al., 2003; Jensen et al., 2008), and this may account for lower anammox rates in our samples in 2009. Recent studies have also shown that sulfide can act as an electron donor for autotrophic denitrification. Various groups of microbes capable of sulfide driven chemolithoautotrophic denitrification have been detected in anoxic waters of the Baltic Sea (Brettar et al., 2006; Hannig et al., 2007), in Saanich Inlet (Zaikova et al., 2010), off Namibia (Lavik et al., 2009) and off Peru (Schunck et al., 2013). These bacteria are known to be fast growing and capable of forming blooms within days to weeks (Lavik et al., 2009). Therefore, it is not unlikely that the high rate of denitrification measured in 2009 arises at least in part from autotrophic denitrification by sulfide-oxidizing microbes. However, it must be pointed out that based on results of incubations alone one cannot distinguish between heterotrophic and autotrophic denitrification processes. Nevertheless, since all our denitrification rates were determined in waters with measurable nitrate/nitrite concentrations and were calculated from the linear increase in N_2 production, it is clear that these processes, whether autotrophic or heterotrophic, is mainly responsible for the apparent N-loss over the Indian shelf. The denitrification rate is of the same order of magnitude but more than double as the NO_3^- loss rate observed in the region (Naik, 2003). On the other hand, the estimated anammox rates are much lower.

N-Removal Versus Uptake Over the Western Indian Continental Shelf During Seasonal Anoxia

The overall nitrogen removal over the WICS is estimated to range between 3.70 ± 0.91 and 11.1 ± 2.72 Tg annually. This corresponds to ∼8–24% of the N-loss estimated for the open ocean OMZ of the Arabian Sea by Bulow et al. (2010) and as high as 24–60% of the model-based value (DeVries et al., 2012). In comparison, anammox contributes only 0.09 ± 0.01–0.27 ± 0.03 Tg to the N-loss per year. Our estimate is also almost three times higher than the previous estimate derived from changes in nitrate concentrations over time (Naik, 2003). The main shortcoming of this estimate that is based on complete removal of an initial NO_3^- concentration of ∼24 μM in the upwelled water over a month at a fixed sampling location (Naik, 2003), is that it ignores additional inputs of NO_3^-. A number of autochthonous and allochthonous sources of fixed nitrogen exist over WICS during the SWM season. As mentioned earlier, atmospheric deposition and land runoff contribute significantly toward the nitrogen inventory (Seitzinger et al., 2005; Naqvi et al., 2010b; Bardhan et al., 2015) which is believed to have increased in recent times. Moreover, high (millimolar) concentrations of NO_3^- are also found in ground water of Goa very close to coast and the submarine groundwater discharge is expected to be a significant but hitherto poorly quantified source of fixed nitrogen to coastal waters.

A considerable amount of fixed nitrogen is removed by the phytoplankton. The chlorophyll *a* (averaging to 2.74 mg m^{-3}) observed in the present study indicates moderately high primary production. Bhavya et al. (2017) reported a carbon uptake rate of 38.8 μmol C l^{-1} d^{-1} from coastal waters of the eastern Arabian Sea during the SWM. Using the Redfield stoichiometry (Redfield et al., 1963) this would correspond to a fixed N demand of 5.8 μmol L^{-1} d^{-1}. However, most of this nitrogen is expected to be regenerated in subsurface waters and thus made available to microbes facilitating the N-loss processes. Once the bottom waters of the WICS turn SO_4^{2-} reducing, after the oxidized N has been fully utilized, the nitrogen regenerated is in the reduced form (NH_4^+). However, it is eventually expected to be oxidized to NO_3^-. Thus, the nitrogen taken up by the autotrophs does not leave the system. The overall N-loss from the system through denitrification and anammox with the former dominating far exceed inputs through upwelling.

CONCLUSION

The present study provides the first results, based on direct measurements using the isotope pairing method of denitrification, anammox and DNRA rates over the world's largest naturally formed coastal low-O_2 zone that develops seasonally over the western Indian continental shelf. The results reveal that denitrification is the predominant process responsible for N-loss at rates that are among the highest reported from any aquatic system. Although anammox was also commonly detected in low O_2 waters its rates were much lower. DNRA was less commonly observed, and at still lower rates. Large spatial and inter annual variations in environmental conditions seem to control the magnitude of different pathways. Denitrification rates were higher over the inner shelf and at the interface of the oxic-suboxic/anoxic layers. Higher rates were observed off Karwar and Mangalore than off Goa. Evolution of sulfate reducing condition and occurrence of dissolved hydrogen sulfide might inhibit anammox activity but stimulate denitrification through chemolithoautotrophy. As in case of the OMZ off Peru, where the measured N-loss rate is the highest over the shelf but the accumulated N* signal is maximal in the offshore OMZ (Kalvelage et al., 2013; Thomsen et al., 2016), strong exchange of water between the coastal and the open ocean OMZ may export N-loss signals (i.e., N* and N_2/Ar ratios, Devol et al., 2006; DeVries et al., 2012) to the open ocean in the Arabian Sea as well.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SN and MK designed the experiments. AS, SN, AP, GS, and HN performed all the experiments. AS and SN analyzed all the samples. AS, SN, and GL processed the data. AS wrote the manuscript with inputs from SN and GL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00678/full#supplementary-material

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