First evidence of denitrification vis-à-vis monsoon in the Arabian Sea since Late Miocene

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In the Arabian Sea, South Asian monsoon (SAM)-induced high surface water productivity coupled with poor ventilation of intermediate water results in strong denitrification within the oxygen minimum zone (OMZ). Despite the significance of denitrification in the Arabian Sea, we have no long-term record of its evolution spanning the past several million years. Here, we present the first record of denitrification evolution since Late Miocene (~10.2 Ma) in the Eastern Arabian Sea, where the SAM generates moderate surface water productivity, based on the samples retrieved during the International Ocean Discovery Program (IODP) Expedition 355. We find that (i) the SAM was persistently weaker from ~10.2 to 3.1 Ma; it did not intensify at ~8 Ma in contrast to a few previous studies, (ii) on tectonic timescale, both the SAM and the East Asian Monsoon (EAM) varied synchronously, (iii) the first evidence of denitrification and productivity/SAM intensification was at ~3.2–2.8 Ma that coincided with Mid-Pliocene Warm Period (MPWP), and (iv) the modern strength of the OMZ where denitrification is a permanent feature was attained at ~1.0 Ma.

Oxygen minimum zones (OMZs) - the regions of dissolved oxygen deficient ($O_2 < 20 \mu M$) water located in the tropical oceans - have been proposed to expand in the present scenario of global warming1,2. OMZs play a significant role in producing N$_2$O - a powerful greenhouse gas through the process of denitrification (a process by which nitrate and nitrite are reduced to nitrogen gas) when the dissolved O$_2$ levels fall below 1 $\mu$M3. A perennial OMZ develops between 150 and 1000 m water depth in the Arabian Sea due to various natural factors such as high surface water productivity and reduced ventilation of intermediate water4. The anoxic zones of these OMZs occupy only ~0.8% of the world ocean but are responsible for the highest production of N$_2$ through denitrification (~35% of the global production) out of which the Arabian Sea contributes the largest proportion (~17% of global N$_2$ production)5. The balance between nitrogen fixation and its removal through N$_2$ production is a key to carbon assimilation by primary production and CO$_2$ regulation in the atmosphere6,7. In the Arabian Sea, most of the studies have examined denitrification variability over the past 100 kyr or younger; the longest record available goes back to 1 Ma in the Western Arabian Sea7. Hence, there is a lack of information regarding the long-term evolution of denitrification spanning the past several million years, especially from the Eastern Arabian Sea. Here, we examine samples from Site U1456 in the Eastern Arabian Sea retrieved during the IODP Expedition 355 (Fig. 1).

To reveal the long-term OMZ variability and its coupling with surface water productivity, we analyzed multiple isotopic and geochemical proxies viz. nitrogen and carbon isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$), total organic carbon and total nitrogen (TOC and TN) concentrations, and carbon to nitrogen (C/N) weight ratio of sedimentary organic matter (SOM).

Study Area
Site U1456 is located at 16°37.28′N, 68°50.33′E in the Eastern Arabian Sea (EAS) (Fig. 1), ~475 km away from the Indian coast, and ~820 km from the modern mouth of the Indus River, and within the Laxmi Basin which is flanked by the Laxmi Ridge to the west and the Indian continental shelf to the east. The Laxmi Basin is characterized by a 200–250 km wide depression that runs in a northwest–southeast direction parallel to the west coast of India8. The site is situated at a water depth of 3640 m, which lies well above the modern lysocline (~3800 m)

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Three distinct water masses identified by Rochford in the Arabian Sea are Arabian Sea High Salinity Water (~50 m to 75 m) (ASHSW), Persian Gulf Water (~25 m to 70 m) (PGW), and Red Sea Water (~600 m to 900 m) (RSW). ASHSW shows greater seasonal variability than PGW and RSW and is considered as the main source of oxygen in the Western Arabian Sea (WAS). Thus, the subsurface denitrification intensity in the WAS is controlled by the surface productivity as well as the supply of oxygen from the water masses. However, in the EAS, the subsurface denitrification is expected to be controlled mainly by the extent of surface productivity. An Argo float-based study in the Arabian Sea revealed the presence of high salinity water with inter-seasonal to inter-annual variability. The vertical mixing of PGW and RSW between ~250 m to ~800 m result in the formation of the Arabian Sea Intermediate Water. The deep water masses of the Indian Ocean comprise Antarctic Bottom Water (AABW), Circumpolar Deep Water (CDW), and Indian Deep Water (IDW). IDW forms in the Indian Ocean itself by the process of diffusion and upwelling and is characterised by low oxygen content and relatively enriched nutrients because of its aging. The present-day bottom water in the Arabian Sea flows northward and upwells into the layer of North Indian Deep Water (~1500–3500 m).

Results and Discussion

The drilled section at Site U1456 is divided into four lithologic units based on a variety of sediment properties (Fig. 2a); Unit I (~121 m thick and Pleistocene nannofossil ooze interbedded with very thin turbidites), Unit II (~240 m thick and late Pliocene to early Pleistocene sand and silt), Unit III (~370 m thick and late Miocene to late Pliocene clay/claystone, sand/sandstone, nannofossil chalk, and nannofossil-rich claystone), and Unit IV (~380 m thick and older than late Miocene claystone, calcarenite, calcilutite, and conglomerate/breccia). These lithologies are characterized by different mineralogical and geochemical properties.

Since the drilled core is very long (1109.4 m) and the site is quite deep (3640 m), the isotopic ratios of the SOM should be evaluated for the diagenetic alterations related to the lithology. Diagenesis of the organic matter begins within the photic zone of the water column, which continues during sinking. It further maintains unceasingly within the bioturbated mixed layer of sediment (a few cm to ~10 cm depth) and only a few percent (1 to 0.01%) of organic matter is finally buried/preserved in the sediment. Although microbial activity has been found even up to several hundred meters deep into the sedimentary sequence, diagenesis reduces significantly with increasing depth. Popp et al. suggested that despite the loss of organic matter due to remineralization, the δ13C of SOM remains almost unchanged with increasing depth. Similarly, a very small δ15N offset was found between core top sediments and sinking particles in the equatorial Pacific region; the loss of organic matter due to diagenesis in the upper section of the core top shows no corresponding δ15N change. Core top studies from the Western Arabian Sea reported no correlation between TN and δ15N, which indicates that diagenesis does not affect δ15N variation. We also obtain no relationship between TN and δ15N (r² = 0.19; Supplementary Fig. 1). Thus, diagenesis appears to cause no significant alteration in δ13C and δ15N values of SOM at Site U1456.

The C/N ratio of marine organic matter generally ranges from 8 to 10. Terrestrial organic matter predominantly consists of compounds like cellulose and lignin with much low nitrogen content. The C/N ratios of land-derived organic matter, therefore, are much high in the range between 20 and 100. The mean δ13C values...
of the marine organic matter, C4, and C3 plants are about −21‰, −13‰, and −27‰, respectively21. The C/N ratio together with δ13C of SOM has been widely used to determine the origin of organic matter20. At Site U1456, the δ15N values vary from −18‰ to −25‰ and most of the C/N ratios range from 6 to 10, indicating that SOM is mostly of marine origin (Fig. 2e,f and Supplementary Fig. 2).

Based on surface sediment analysis of more than 100 locations in the Central and Eastern Arabian Sea (most of them are located in the Eastern Arabian Sea), the δ15N values of SOM have been found to vary from 6‰ to 11‰22. In most of the oxygenated basins, the δ15N values do not exceed 6‰ while those from the oxygen deficient basins are highly enriched with mostly higher than 6‰ 7,22,23,24. Thus, the periods with δ15N values higher than 6‰ may signify denitrification associated with strong OMZ. At Site U1456, the δ15N values of SOM vary between 2.4‰ to 8.2‰ (Fig. 2b). The maximum TOC and TN values are 2.42% and 0.17%, respectively (Fig. 2c,d). The Mid-to Late Pliocene (~3.2 to 2.7 Ma) is characterized by high δ15N values (>6‰) along with high TOC and TN values, indicating denitrification/strong OMZ (Fig. 2). Another period of denitrification/OMZ intensification (δ15N > 6‰) takes place from ~1.0 Ma to the core top (0.03 Ma) (Fig. 2b). During these periods of intense denitrification, the surface water productivity indicators viz. TOC and TN contents also represent an increasing trend (Fig. 2c,d). Intense wind-induced productivity and particle flux occur in the Arabian Sea during the monsoon seasons25. Modern climatological chlorophyll a data show that the surface water productivity in the Eastern Arabian Sea is driven by both the summer and the winter monsoons26. Thus, surface water productivity variability in the Eastern Arabian Sea is a manifestation of the SAM variability, which can be linked to denitrification/OMZ intensification.

The origin and evolution of the SAM are still a topic of debate. According to the previous hypothesis based on a study from the Western Arabian Sea (Ocean Drilling Program (ODP) Site 722), the initiation/intensification of the SAM occurred at around 8.5 Ma and continued until 6 Ma27 (Fig. 3g). Another study from the same ODP Site 722 shows that the onset of the SAM took place at ~12.9 Ma and a major intensification occurred at ~7 Ma28. In contrast, a decrease in G. bulloides abundance was found at 8.5 Ma (Fig. 3f) from the ODP Site 722 implying reducing SAM29. A recent study from the inner seas of the Maldives (IODP sites U1465-71) postulates a proto-monsoon from 25–12.9 Ma and an abrupt increase in the monsoon at ~12.9 Ma30 (Fig. 3d). The δ13C values of paleosols from the Siwalik Group sediments in the northern Pakistan spanning the past 18 Myr showed a marked shift from C-3 to C-4 dominated plants at ~7.4 Ma, which may be associated with SAM inception and again the flood plains were mostly occupied by C-4 grassland in Plio-Pleistocene31 indicating monsoon
intensification (Fig. 3h). Recent records of Himalayan weathering represented by the chemical index of alteration (CIA) and K/Al ratios (Fig. 3c) demonstrated that SAM attained the maximum strength at 15 Ma, remained high until 10.5 Ma, gradually weakened until ~3.5 Ma, and again increased from the Late Pliocene to Pleistocene. The Sr isotope and clay mineral data also suggested weaker SAM after 8 Ma. Our record from Site U1456 spans ~10.2 to 0.03 Ma, but includes several hiatuses dated to ~8.2–9.2 Ma, ~3.7–5.4 Ma, and ~1.6–2.2 Ma. Nevertheless, we interpret that surface water productivity in the Eastern Arabian Sea was low from 10.15 Ma to 3.2 Ma as evident from uniformly low values of TOC and TN (3a and 2b). Additionally, during this period, the δ15N did not reach the threshold value (~6‰) indicative of denitrification (Fig. 3a). This implies that neither the surface water productivity (TOC, TN) nor the OMZ intensity supports any major intensification in SAM strength from ~10 to ~3.2 Ma.

Figure 3. Comparative records of the South Asian Monsoon and East Asian Monsoon since Mid-Miocene. (a) δ15N and total organic carbon (TOC) from IODP site U1456, (b) Magnetic susceptibility record of Chinese loess plateau and Hm/Gt (40 point moving average) from the South China Sea ODP site 1143, (c) Chemical Index of Weathering (CIA) from the Indus river fan, (d) Mn/Ca record from the Maldives inner Sea, (e) Magnetic susceptibility record of the southern Bay of Bengal ODP site 758, (f) G. bulloides abundance from ODP site 722, (g) G. bulloides abundance from ODP site 722, and (h) δ13C of calcretes from the Potwar Plateau. The green arrows represent the strengthening of monsoon and the purple indicate the weakening of monsoon. The yellow band marks the arid period when many of the studies including the present study show the weakened monsoon while the green bands indicate the periods of strengthened monsoon.
During the study period, for the first time, the OMZ intensified to the level that denitrification takes place was at ~3.2–2.8 Ma (Fig. 2b). During this period, the surface water productivity (Fig. 2c,d) was also enhanced, indicating stronger SAM, which coincides with MPWP40. Earlier studies, based on magnetic susceptibility (Chinese Loess Plateau, Fig. 3b; southern Bay of Bengal, Fig. 3e) and hematite to goethite ratio (Hm/Gt, South China Sea, Fig. 3b), also reported the enhanced SAM and EAM during ~3.6–2.6 Ma37,38. A new magnetostratigraphy study from Chinese Loess Plateau spanning from ~8.2 Ma to 2.6 Ma documented long-term East Asian Summer Monsoon (EASM) intensification. Both proxy, as well as numerical climate model assessment, show that the Antarctic glaciation was an important driver for the long-term trend of late Miocene-Pliocene EASM intensification39. To examine the responsible mechanisms, a modeling experiment, using the NCAR climate model CCM3, with idealized Himalayan-Tibetan Plateau elevations explains the observed increase of the EAM as a result that the Himalayan-Tibetan Plateau attained modern extension along its eastern and northern margins34. It was speculated that it might not have affected the SAM circulation pattern34. The present study, based on the multi-proxy records, suggests that the SAM was also enhanced in parallel with the EAM and therefore the intensification can be ascribed to the same mechanism. A recent review41 investigated the role of the Tibet Plateau in affecting SAM, and found that it simply acts as a physical barrier for northerly cool, dry winds. Its role as an elevated heat source is of secondary importance in affecting the SAM. EAM dynamics is also affected by the Tibet Plateau, which is located in the path of subtropical jet streams41. The increase in both the EAM and SAM during 3.6–2.6 Ma could have resulted in the increased weathering and organic carbon burial, as evident by higher TOC (Fig. 2c), leading to atmospheric CO2 drawdown that would have possibly contributed to Northern Hemisphere Glaciation (NHG) at 2.7 Ma37. Thereafter, from 2.8 Ma to ~1.0 Ma, δ13N values as well as the surface water productivity declined in parallel, indicating relatively weaker SAM. Previous studies also reported the weakened EAM and SAM after ~2.6 Ma37,38, confirming our results, which coincides with the onset of NHG. Finally, the OMZ reached its modern strength, i.e., denitrification became a permanent feature, at about ~1.0 Ma closely following the enhanced surface water productivity. It implies that SAM intensified from ~1.0 Ma as reported in earlier studies viz. the enhanced sedimentation rate in the Indus Fan42, the increased chemical weathering from the Bengal Fan33 and the South China Sea41, the rise of magnetic susceptibility (Fig. 3b) and mean sediment flux from the Indian Ocean38.

### Methods

The samples used in the present study were obtained onboard the JOIDES Resolution, 5–15 cm long whole-round core sections at the interval of every core or every alternate core were squeezed using titanium steel squeezing device to obtain the interstitial water. The remaining sediments are named ‘squeez cake’. The samples were dried to remove the moisture at 45 °C before processing. Around 10 to 20 g of sediment aliquots were taken for further analysis. Dried samples were finely ground for homogenization. Homogeneous samples were divided into two batches for geochemical and isotopic analyses - (i) 2 N HCl treatment for total organic carbon (TOC) and δ13C measurement and (ii) untreated for determination of total nitrogen (TN) content and δ15N values. 20 ml of 2 N HCl solution was added to 5–10 g of fine sediment powder. The mixture was shaken mechanically and allowed to stand for ~12 hours. The sample was then washed with ultrapure demineralized water and approximately 25 mg of treated sample was used for TOC and δ13C analysis. For TN and δ15N measurement, approximately 40 mg of bulk ground sediment was used. The δ15N and δ13C values were determined using isotope ratio mass spectrometer coupled with an element analyzer at Marine Stable Isotope Lab, National Centre for Antarctic and Ocean Research, Goa, India and Department of Oceanography, Pusan National University, Busan, Korea. The standard used was ammonium sulfate (IAEA-N-1) and cellulose (IAEA-CH-3). The analytical precision for δ15N and δ13C is ±0.12‰ and ±0.06‰, respectively. Similarly, TN and TOC were determined using sulfanilamide as the standard. The analytical precision for TN and TOC is ±0.63% and ±0.84%, respectively.

### Data Availability

The data used in this study are included in the supplementary information files.

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Author Contributions

M.T. and B.K.K. designed the research and collected the samples onboard Joides Resolution aided by IODP Expedition 355 Scientists; S.T. and J.L. did the sample processing and analysis; S.T. and M.T. supported by B.K.K and J.L. wrote the manuscript, which was edited by all the IODP Expedition 355 Scientists.

Additional Information

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