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Anammox bacterial abundance, diversity and distribution across surface sediments underlying oxic, hypoxic and suboxic zones of Northern Indian Ocean

The oxygen-depleted environments (ODEs) are reported sites of oceanic fixed nitrogen loss mediated by major microbial pathways like denitrification and anaerobic ammonium oxidation (anammox). The aim of the present study was to understand the diversity, abundance, and distribution of anammox bacteria across surface sediments of the northern Indian Ocean using PCR-based molecular tools. A total of 22 sediment samples were collected from three transects (off Goa, off Mangalore and off Kochi) in Arabian Sea and off Paradip transect in Bay of Bengal, underlying oxic, hypoxic or suboxic water column having a depth of 20 to 2000m. Multiple molecular proxies such as 16S rRNA, hydrazine synthase (HzsA) and hydrazine oxidoreductase (Hzo) gene partial fragment were targeted for characterization of anammox community. Gene abundance was quantified using standard curve method, while for diversity studies a combination of tools such as nested PCR, DGGE, pyrosequencing and metagenomic library construction was used to identify all possible anammox communities. Anammox gene diversity was high in the suboxic zone and dissolved oxygen and salinity could contribute ~89 to 90 % variability. The anammox specific 16S rRNA gene diversity study was able to identify only genus *Scalindua*, though belonging to diverse clades. On the other hand, functional diversity studies targeting hydrazine gene and phylogenetic diversity studies using universal primer suggest the possibility of occurrence of novel anammox community with high diversity and abundance. The gene copy number ranged between $1.5E+04$ and $1.9E+05$ per gram sediment and did not show significant correlation with dissolved oxygen in the overlying water-column. Anammox abundance was high in the coastal stations in comparison to the offshore stations. Within suboxic zones, a noticeable increase was observed in off Kochi transect which is outside the secondary nitrite maxima zone in the eastern Arabian Sea. Our study reports the widespread prevalence of anammox bacterial community across the sampling sites, but not in very high abundance, suggesting a less significant role for anammox in the northern Indian Ocean. However, the positive correlation of anammox gene abundance with temperature poses a serious concern due to a reported expansion of coastal hypoxia induced by global warming.

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