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High-throughput screening of sediment bacterial communities from Oxygen Minimum Zones of the northern Indian Ocean

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ABSTRACT:

The Northern Indian Ocean host two recognized Oxygen Minimum Zones (OMZ): one in the Arabian Sea and the other in the Bay of Bengal region. The next-generation sequencing technique was used to understand the total bacterial diversity from the surface sediment of off Goa within the OMZ of Arabian Sea, and from off Paradip within the OMZ of Bay of Bengal. The dominant phyla identified include Firmicutes (33.06%) and Proteobacteria (32.44%) from the Arabian Sea, and Proteobacteria (52.51%) and Planctomycetes (8.63%) from the Bay of Bengal. Statistical analysis indicates that bacterial diversity from sediments of the Bay of Bengal OMZ is ~48% higher than the Arabian Sea OMZ. Diverse candidate bacterial clades were also detected, whose function is unknown, but many of these were reported from other OMZs as well, suggesting their putative role in sediment biogeochemistry. Bacterial diversity from the present study reveals that the off Paradip site of Bay of Bengal OMZ is highly diverse and unexplored in comparison to the off Goa site of the Arabian Sea OMZ. Functional diversity analysis indicates that the relative percentage distribution of genes involved in methane, nitrogen, sulfur and many unclassified energy metabolisms is almost the same in both sites, reflecting a similar ecological role, irrespective of the differences in phylotypic diversity.

Keywords:

OMZ, sediment bacteria, 454 pyrosequencing, Arabian Sea, Bay of Bengal, functional ecology.

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1. INTRODUCTION

The Northern Indian Ocean comprises of two major ocean basins: the Arabian Sea (AS) in the west and the Bay of Bengal (BB) in the east. Even though both these basins are roughly placed in the same latitude, they differ in many aspects. This includes differences in average salinity, primary productivity, denitrification rates, the intensity of mesoscale eddies, contrasting transport of dissolved oxygen, and organic matter. (McCreary Jr et al., 2013). Both these basins experience intense oxygen depletion below the mixed layer of the water column, where the dissolved oxygen (DO) falls below 20μM (Rao et al., 1994). The sequence of electron acceptor utilization in such an environment, generally follow the thermodynamic energy yield (Froelich et al., 1979). The OMZs are also termed as NMZ (Nitrite maxima >5μM/ Nitrate deficient >10μM) or CMZ (Carbon maxima: DIC, dissolved inorganic carbon >2255-2350 μM kg^{-1}) in the area's where dissolved oxygen depletion is more pronounced (Paulmier et al., 2011). The dissolved Manganese (d-Mn) maxima regions localized within these OMZ likely have an oxygen scavenging effect which intensifies OMZ further (Lewis and Luther III, 2000).

Though the OMZs are inhospitable to aerobically respiring organisms, it acts as a comfortable niche for microorganisms that can use alternative pathways of respiration (Diaz and Rosenberg, 2008). It is vital to understand the dominant microbial taxa's and also their functional ecology to throw light on the biogeochemistry of these oxygen-depleted zones (Rajpathak et al., 2018). Surface sediment underlying OMZs entraps all recent microbial signatures of the water column above (Gerdes et al., 2000), hence will be interesting to explore and compare such benthic OMZ ecosystems, especially those located in shallow zones. The abundant bacterial communities in the eastern Arabian Sea sediments underlying OMZ were attributed to be phylum Proteobacteria and Planctomycetes (Divya et al., 2011). Bacteroidetes, Acidobacteria, Actinobacteria, and Firmicutes dominance are also expected, which form an integral part of soil/sediment habitat (Lv et al., 2014).

With the advent of molecular techniques over the last decade, a large volume of a database has been generated based on 16S rRNA genes that help to elucidate the bacterial community structure (Hodkinson and Grice, 2015). Phylogenetic profiling, using next-generation sequencing (NGS) techniques like 454 (longer reads) and Illumina (shorter reads) offer high-resolution data from complex environments (Claesson et al., 2010). By utilizing algorithms such as PICRUSt, it is also possible to predict functions from 16S rRNA microbiome data (Langille et al., 2013). The available data on the microbiome of Northern Indian Ocean OMZ using such high-throughput sequencing technique has chiefly restricted to pelagic OMZs (Fernandes et al., 2019; Rajpathak et al., 2018). A

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similar high-resolution study from benthic OMZ is limited to some functionally significant groups rather than total bacterial community (Fernandes et al., 2018). Fine details of sediment bacterial communities beneath the OMZs of northern Indian Ocean are lacking and needs special attention.

The objective of our work was to compare the surface sediment bacterial diversity within two major OMZs in the northern Indian Ocean utilizing NGS technique. We used long-read 454 pyro-sequencing technology, targeting v1-v3 hypervariable region of 16S ribosomal RNA (rRNA) gene to examine bacterial community composition in perennial OMZ locations in the Arabian Sea, (off Goa: GS1A) and the Bay of Bengal (off Paradip: PS1B). Functional gene profiling was done utilizing PICRUSt algorithm to understand the relative distribution of ecologically significant genes. Our data shows intense oxygen depletion at both sampled locations, i.e., near-bottom water dissolved oxygen was 2±0.4 μM at the time of sampling and had comparable salinity as well as water column depth. Diversity studies are essential in understanding ecosystem processes and defining the role of microbes relevant to the study area.

2. MATERIALS & METHODS

2.1. Sample collection and site characteristics

Sediment samples were collected from off Goa region of AS-OMZ, GS1A ($15^0$13'N, $72^0$56'E; February 2013) and off Paradip region of BB-OMZ, PS1B ($19^0$57'N, $86^0$46'E; August 2014), located in the northern Indian Ocean and corresponding water column depth was ~200m. Box corer was used to retrieve the undisturbed sediment sample. The sediment core was carefully sub-sampled using acrylic core liner (25 mm ID, ~30 cm length), from the center of the core in order to avoid mixing of the sample. The 0-5 cm subsections of samples were transferred into a sterile screw-cap container. Samples were handled aseptically and preserved at -20°C until further analysis. The Temperature/Salinity profiling of the water column above the sediment was carried out using a Sea-Bird Electronics CTD (conductivity-temperature-depth) - rosette sampling system, Model SBE9, fitted with Niskin/ Go-Flo bottles. Dissolved oxygen (DO) sensor (RINKO from ALEC, Japan) was attached to the same.

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111 **2.2. Sediment characterization**

112 The sediments were freeze-dried, homogenized, and ground in an agate mortar prior analysis. Total carbon
113 (TC) and nitrogen (TN) were analyzed in CN analyzer (FISONS NA1500) using the method described (Bhushan et
114 al., 2001). Total organic carbon (TOC) content was estimated using a colorimetric based wet oxidation method,
115 which is reported to be highly reproducible (Azam and Sajjad, 2005). Inorganic carbon (TIC) was determined as the
116 difference between TC and TOC (Bernard et al., 1995). Organic matter (OM) was calculated by multiplying TOC
117 with Van Bemmelen's factor 1.724, based on the assumption that humidified organic matter of soil contains 58%
118 carbon, but it could vary from 40-60% (Nelson and Sommers, 1982). For $CaCO_3$ calculation, TIC was multiplied
119 with 8.33 to get the relative percentage (Bernard et al., 1995).
120
121 **2.3. Genomic DNA extraction and 454 Pyrosequencing**
122 Total genomic DNA was extracted from 400-500 mg of the sediment samples in triplicates, using the Fast
123 DNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA). The purified DNA was quantified using Nanodrop
124 2000 spectrophotometer (ThermoScientific, USA) and visualized on an Agarose gel (0.8%) to determine the quality
125 of the extracted DNA. The gel was viewed using AlphaImager Gel documentation system after staining with
126 Ethidium Bromide (EtBr). The extracted DNA was pooled and amplified using barcoded fusion primers targeting
127 the v1–v3 region of the 16S rRNA gene using the universal primer 9F (AGAGTTTGATCMTGGCTCAG) and 541R
128 (ATTACCGCGGCTGCTGG). The mixed amplicons were subjected to emulsion PCR and then deposited on
129 picotiter plates (Agilent). Amplification condition are as follows: initial denaturation at 95°C for 5 min,
130 followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30
131 sec, with a final elongation at 72°C for 5 min. The detailed procedure of pyrosequencing is described elsewhere
132 (Suh et al., 2014). Sequencing was performed by Chunlab Inc. (Seoul, Korea) using the 454 GS FLX Titanium
133 Sequencing system (Roche Branford, CT, USA) per the manufacturer's instructions.
134
135 **2.4. Sequence data processing**
136 Amplicon pyrosequencing data were processed using the QIIME software package, ver. 1.7. (Caporaso et
137 al., 2010). Chimaera's and primer mismatch was removed from 454 PCR amplicons by Amplicon Noise software,
138 ver.1.27 (Quince et al., 2011), using the FLX Titanium sequence data platform implemented in QIIME. Sequences

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were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the program CD-HIT (Edgar, 2010). Average read length of PCR amplicons was 378±45 bp. The resulting reads were taxonomically classified based on similarity scores in both the BLASTN searches (E-value $>10^{-5}$) through EzBioCloud 16S database (2014.07.01) and pairwise alignments using the Greengenes database (release 13.5) based on RDP classifier method (ver.14) (Im et al., 2012). The cut-off values used for taxonomic assignments were as follows (x = similarity): species ($x \geq 97\%$), genus ($97\% > x \geq 94\%$), family ($94\% > x \geq 90\%$), order ($90\% > x \geq 85\%$), class ($85\% > x \geq 80\%$) and phylum ($80 > x \geq 75\%$). If the similarity was lower than the specific cut-off value, the sequence was assigned as 'unclassified,' un (Chun et al., 2007). The richness and diversity indices were calculated using the Mothur platform (Schloss et al., 2009). CLCommunity™ v.3.46 was used for data visualization.

2.5. Functional prediction of metagenome

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) algorithm was used to predict the functional composition of a microbial community metagenome from its 16S rRNA profile (Langille et al., 2013). It predicts gene families that are already known and included in the orthology reference used in Kyoto Encyclopaedia of Genes and Genomes (KEGG). The final output of this workflow was quantified in terms of predicted gene abundances per sample per OTUs. Only genes whose function which are ecologically significant were considered for a graphical representation (Fig. 5).

3. RESULTS

3.1. Sample characteristics

In the present study, both sampled sites showed intense oxygen depletion, i.e., near bottom DO was ~2µM, hence in the underlying sediments predicted DO level would be much lower. The OM was highest in GS1A and accordingly TOC and TN. The TOC/TN ratio was slightly high for PS1B sample. TIC was significantly different between the two sites, i.e., in GS1A, 8.11%, while PS1B was as low as 0.29%. Near bottom water, characteristics were comparable, including salinity and DO, whereas temperature was 3°C apart, possibly due to the 40m difference in depth. Complete details are presented in Table 1.

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3.2. Diversity analysis

A total of 17,784 reads were pyro-sequenced, from which, 10,069 high-quality reads with a mean length of approx. 470-480 bp., were obtained, after read pre-processing combining two stations. The OTU richness estimators such as Chao1, ACE, Jack-knife, and OTU diversity estimators like Simpson and Shannon index indicate sediments underlying oxygen-depleted water columns in the northern Indian ocean is rich in bacterial diversity. The PS1B site of BB-OMZ is more diverse than GS1A site of AS-OMZ (Table 2). The rarefaction curve calculated with Mothur at 97% similarity also showed a similar pattern (Fig. 1). The Shannon index values are 4.37 and 6.97 for GS1A and PS1B, respectively. Simpson diversity index ranged between 0 and 1, where a value of 1 represents infinite diversity, while 0 represents no diversity. The obtained values were 0.934 (GS1A) and 0.998 (PS1B), which are close to 1, thus confirming that the microbial population in the sampled area is highly diverse. Alpha diversity indicator Chao 1 and ACE compute asymptotic species richness for abundance-based data, while Jackknife gives incidence-based data, the latter being a cross-validation technique to estimate the bias of species richness estimators used (Colwell and Coddington, 1994). The predicted bias-corrected estimator like Jackknife suggests that the OTU richness is 3 to 4 fold higher than that is observed. The estimated OTUs were highest in PS1B. The Goods nonparametric coverage estimator indicates that the present, long read 454 pyrosequencing technique was successful in recovering ~70 and 90 % of bacterial phylotypes from PS1B and GS1A respectively.

3.3. Taxonomic composition

Slightly different results were obtained for BLASTN searches, and pairwise alignments, however dominant communities and their relative percentage remained the same. A total of 30 phyla were identified using BLASTN search, of which 24 phyla were common to both zones based on Greengenes database v.13.5. While BLASTN search using EzBioCloud 16S database resulted in a total of 48 phyla, of which 29 were specific to both site. This difference mainly occurred because the unclassified bacterial phylum was differentiated further into 9 unnamed phyla of which 4 phyla identified are novel and also to 10 classified phyla of which 8 were candidate phyla. The comparison from phylum to species obtained through EzBioCloud is presented in Fig. 2, which shows the number of taxa that are unique to each site and common in both areas. Analyzing through Greengenes database helped to classify as specific clades, which are commonly found in the literature (Fig. 3), whereas EzBioCloud was useful for species-level taxonomic assignment.

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The dominant bacterial phyla are Firmicutes (33.06%), Proteobacteria (32.44%), Bacteroidetes (17.19%), and Chloroflexi (4.93%) in GS1A and Proteobacteria (52.51%), Planctomycetes (8.63%), Actinobacteria (6.64%), Firmicutes (5.99%) Acidobacteria (4.51%) and Chloroflexi (4.1%) in PS1B, contributing >80% of total bacterial community (Fig. 4). The other dominant phyla (≥1%) recovered from site GS1A are Actinobacteria (2.12%) and Planctomycetes (1.08%) and from PS1B site are Bacteroidetes (2.42%), Cyanobacteria (1.67%), Gemmatimonadetes (1.19%) and Nitrospirae (1.19%). The relative abundance of identified candidate phyla ranged between 1.84 to 1.85% based on the Greengenes database, while Ezbiocloud analysis suggests its contribution is 2.88% and 2.23% respectively in GS1A and PS1B. This difference in the results arises from the fact that Ezbiocloud could assign more OTUs as candidatus and other low diverse unclassified phyla contributed ≤2%. For more details, please refer Supplementary information A1 and A2. Taxonomic composition of shared OTUs from phylum to species level decrease from 61.07% to 16.73%; whereas the unique OTUs of PS1B increase from 21.28% to 58.21%; and for GS1A it is 17.02% to 25.06% (Fig. 2), suggesting PS1B is highly diverse in comparison to GS1A, but dominance wise an inverse relationship was obtained. These differences could mainly be attributed to differences in sediment characteristics in the sampling sites (Table 1).

Analysis of 105 bacterial classes recovered from the current data set showed that there is still >50% similarities between the communities in the two sites. This might indicate that these shared classes might play ecologically significant roles in the OMZ sediment-water interface. The dominant classes in GS1A include Bacilli (32.97%), Gammaproteobacteria (18.49%) and Flavobacteria (17.14%) and in PS1B include gamma, alpha delta-Proteobacteria (23.88%, 19.49%, 10.91% respectively) followed by Planctomycetales (6.76%) making up a total of 60-70% of the total bacterial community in northern Indian Ocean OMZ. The other Proteobacteria sub-classes like beta (0.12%) and epsilon (0.24%) were recovered only from the PS1B site, but their overall contribution is not very significant. Dominant bacterial orders at 10% cut-off, recovered exclusively from AS-OMZ include Bacillales (32.97%), majorly Planococcaceae (26.09%) family members followed by Flavobacteriales (17.14%) and Oceanospirillales (13.16%), while from PS1B Steroidobacter_o (12%) and Rhizobiales (11.07%) were the most dominant bacterial order.

Bacteria which make up ≥1% were considered to be abundant (Fig.3). In GS1A most abundant groups were Planococcaceae_uc (24.09%) which were identical to *Paenisporosarcina quisquiliarum* like sp., followed by Arctic sea ice bacterium_ARK9985_un (16.94%) similar to *Salegentibacter mishustinae*. Both these communities were not

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present in PS1B site. Similarly, other dominant groups like Alcanivoracaceae_uc (3.84%) similar to *Alcanivorax venustensis*, *Photobacterium*_uc (2.69%) similar to *Photobacterium indicum* and *Bacillus agaradhaerens*_uc (2.62%) were unique to GS1A site. This emphasizes the spatial variability in bacterial diversity between the two OMZ sites within the northern Indian Ocean. Gammaproteobacteria_uc (11.34%) were highly abundant in GS1A as well as in PS1B (18.79%). Deltaproteobacteria; NB1-j_uc was equally distributed in both sites (1.63%). Similarly, Bacilli_uc (3.92/1.65%) and Rhodobacter_uc (2.54/1.04) were also recovered in significant proportions from both sites. High dominance of Alphaproteobacteria_uc was recovered (13.45%) from PS1B site, while in GS1A its contribution was only a tenth (1.21%) of that recovered from PS1B. All remaining groups recovered were ≤5%, which include clades like LO133, koll13_ JTB31, RB25, BPC102, SOGA31, and Hyd89-23. The relative distribution of all dominant clades in both sites is represented in Fig. 3.

3.4. Predicted functional ecology

PICRUST analysis has identified a high proportion of genes involved in methane metabolism, followed by nitrogen and sulfur metabolisms (Fig. 5). For a large proportion of the genome, functions could not be assigned clearly, as many novel phyla were recovered in the present study. As Firmicutes was abundant in GS1A, sporulation genes were 4 fold higher. Carbon fixation by non-photosynthetic bacteria outcompete photosynthetic bacteria as sample were from ~ 200m water column depth, where light penetration is negligible, and their proportion was higher in GS1A site of AS-OMZ. Genes involved in metabolizing inorganic ions like iron, manganese, and similar redox-sensitive elements were equally abundant as the sulfur metabolic genes. The PICRUST analysis also suggests the existence of many unclassified metabolic pathways, which might correspond too many secondary metabolite pathways, xenobiotic compound degradation pathways, etc. Putative bacterial representatives groups involved in biogeochemical cycling were identified, and their functional role is discussed in detail in the discussion section, and these results are also supported by predictive functional profiling.

4. DISCUSSION

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4.1. Northern Indian Ocean OMZ characteristics

The BB-OMZ (10μM) is reported to be less intense than AS-OMZ (2μM) (Paulmier, 2009). In the present study, both sampled sites showed intense oxygen depletion, i.e., DO 2±0.4 μM (Table 1). Intensified OMZ has been reported previously as well in the BB, especially in the shallow zones. During the summer monsoon, due to the influence of riverine nutrient loading, the DO concentration had gone below the detection limit (Sarma et al., 2013). The AS-OMZ between the water depths of ~100/150 – 1000/1200m is the thickest, compared to Pacific Ocean OMZs, and is identified as the primary site of fixed nitrogen loss (Naqvi et al., 2006). However, in the BB-OMZ, nitrogen loss is not very significant as its limited by substrate availability (Bristow et al., 2017). Also, BB is reported to be less productive compared to AS, (Prasanna Kumar et al., 2002); hence, the organic carbon and nitrogen load in sediments are lower. Such a difference is reflected in our study, as well. Despite comparable DO level, the TOC and TN content was 0.64 and 0.56 fold lower in BB-OMZ to AS-OMZ but were still higher than non-OMZ surface sediments which are as low as 0.2 and 0.02 wt. %, respectively (Pattan et al., 2013). The possible reason for higher TN values in sediment underlying OMZ could be due to early nitrogen burial (Robinson et al., 2012), or due to of active re-mineralization processes that are reported to be very common in such ecosystems (Bohlen et al., 2011). The OMZs enhance preservation of organic matter and thus reported values of TOC ranges from ~1-2 to 6-7% (Cowie et al., 2014) and TOC/TN ratios within 7.3 – 12.3 (van der Weijden et al., 1999). The TIC difference could be attributed to the difference in $CaCO_3$ content caused by increased carbon sequestration (Sarma et al., 2007) and also because of abundant shelled meiobenthic fauna (Ramaswamy and Gaye, 2006) in AS compared to BB region of northern Indian Ocean.

4.2. OMZ sediment bacterial diversity and richness

Pyrosequencing based 16S rRNA gene surveys are increasingly utilized to study bacterial community structure distribution (Youssef et al., 2009). Although this has been replaced by massively parallel sequencing technique like Illumina, the pyrosequencing based approach is more advantageous, for taxonomic assignments, because of the long read length. The bacterial community composition from different marine realms reveals that microbial taxonomic richness is highest in OMZ (Walsh et al., 2015). In marine sediments, Shannon index (H), an indicator of diversity, can be as high as 6.76, which is still lower compared to freshwater and intertidal sediments (Wang et al., 2012). An earlier study carried out in deep benthic eastern AS-OMZ utilizing traditional sequencing

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277 method has reported a similar Shannon diversity index of 4.4 (Divya et al., 2011). Reports are scarce from BB-
278 OMZ benthic OMZ; however, in pelagic OMZ comparable values are recorded at 200m. i.e., 6.221 for Shannon and
279 0.95 for Simpson index (Fernandes et al., 2019). This regional differences between AS and BB OMZ are not
280 uncommon, e.g., in south and east China Sea sediment samples, H index is 2.52 and 7.96 respectively (Zhu et al.,
281 2013;Dang et al., 2008), while in our study H index was 4.37 and 6.97 for OMZs of AS and BB. The predicted
282 richness estimators suggest that sediment OMZ bacterial communities are even richer than coastal microbial mats
283 (Bolhuis and Stal, 2011). In the pelagic BB-OMZ Chao 1 predictor has estimated 4697 OTUs (Fernandes et al.,
284 2019) while in the benthic OMZ based on our study it was estimated as high as 7617, almost 0.6 fold higher, thus
285 indicating that the sediment below BB-OMZ is more diverse than the sediment below AS-OMZ and pelagic OMZ of
286 BB. Fig. 2 depicts that the number of unique OTUs were more in BB-OMZ, and according to BLAST similarity
287 search, many clades seems to be novel and assigned taxa's are mostly environmental clusters. In the present era of
288 next-generation sequencing, immense data on the microbiome have accumulated and hence utilizing tools of
289 diversity indices are required to compare microbial community structures.
290
291 **4.3. Bacterial community structure in OMZ**
292 In the eastern AS-OMZ surface sediment, nearly 14 phyla were identified using Sanger sequencing technique,
293 majority of which were Proteobacteria (52%), Planctomycetes (12.7%) and Chloroflexi (8.8%) (Divya et al., 2011).
294 Similarly, in another study carried out utilizing NGS technique confirms Proteobacteria to be the dominant phylum
295 making up 70-75% in all six sites within benthic OMZ of AS followed by Bacteroidetes. Chloroflexi and Firmicutes
296 were also recovered in considerable number, (Fernandes et al., 2018). The dominance of Proteobacteria is well
297 documented in the marine ecosystem (Wang et al., 2012). The sediments collected from off Paradip port, which is
298 roughly 27 nautical miles from PS1B, close to 40 phyla's were reported utilizing the high-throughput technique. The
299 relative contribution of the phylum Proteobacteria was only 17%, which was lesser than Bacteroidetes (23%) and
300 Firmicutes (19%) (Pramanik et al., 2016), and these results are comparable to GS1A, which is geographical separated.
301 However, Proteobacteria was the most divergent phylum in the studied AS-OMZ as well with 215 OTUs. The notable
302 differences in the relative dominance of various taxa suggest that more than DO, factors such as the availability of
303 nutrients or organic carbon alter the benthic bacterial community structure (Fierer and Jackson, 2006). However, in
304 pelagic OMZ, DO play a significant role in structuring bacterial community (Stewart et al., 2012).

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The candidate phyla GN02, OD1, TM6, TM7, and WS3, were prevalent in ESP pelagic OMZ as well (Ulloa et al., 2013; Ganesh et al., 2014), implying that they have an essential role in OMZ nutrient cycling. Candidate phyla GN02, OP3, OP8, were unique to benthic OMZs of northern Indian Ocean. A total of 13 candidate phyla were obtained, the prevalence of such "microbial dark matter" bacterial communities with such vast diversity in the OMZ suggests, that they have a critical role in ocean biogeochemistry, however, the roles they play are not understood yet. The single-cell genomics approach is required to understand the coding potential of these "bacterial dark matter" in OMZ (Rinke et al., 2013).

The clade Woeseiaceae/JTB255 is recognized as the most abundant clade in marine sediment, having a cosmopolitan distribution. Moreover analyzed metagenomes of JTB255 are known to encode truncated denitrification pathway to nitrous oxide (Mußmann et al., 2017). Since denitrification mediated nitrogen loss is reported to be dominant in Arabian Sea OMZ, we expected to get more hits in our microbiome (Ward et al., 2009). However few representative sequences of JTB31 and JTB38 has identified which might be having a similar role to play. We assume that the present 454 pyrosequencing approach, targeting the v1-v3 variable region may have failed to detect sub taxonomic level information, concerning some groups. The other dominant bacterial representatives reported from pelagic OMZ of Pacific Ocean, such as SUP05, ARCTIC96BD-19, and Arcobacteriacea was absent in our metagenomic data sets (Glaubitz et al., 2013; Ulloa et al., 2013). However, the sulfate-reducing bacterial communities and candidate phyla reported in Pelagic OMZ of Pacific were retrieved in our study as well. Deep in the underlying sediments of AS-OMZ diverse sulfur reducing bacterial and archaeal OTUs were recovered (Fernandes et al., 2018), some of which were common to the studied surface sediments as well. Our finding suggests that northern Indian Ocean OMZ which is well studied for different nitrogen transformation pathways should also be treated as a laboratory to understand more on sulfur cycling at least in the areas where ultra-low DO is noticed. Similarly, the possible biogeochemical roles played by candidate phyla must be investigated with priority.

4.4. Dominant bacterial communities and their functional ecology

In the PICRUSt analysis, the proportion of gene families relevant in biogeochemical cycling were highest for methane metabolism, followed by nitrogen and sulfur. In northern Indian Ocean pelagic OMZs, nitrogen cycling is reported to be very active (Naqvi et al., 2006), while in the studied benthic OMZ, gene proportion of methanotrophs

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and methanogens outcompetes in respect to gene proportion of nitrogen cycle members. This could be basically due to the difference in the level of oxygen depletion within the benthic and pelagic ecosystem, as in the studied site the near bottom recorded ultralow DO level. Recent studies have linked methane oxidation to nitrite denitrification through the unique oxygen-producing intra-aerobic methanotrophic pathway by candidatus NC10 bacterial phylum (Padilla et al., 2016). This was supported by studies carried out in freshwater reservoir, where methane stimulated massive nitrogen loss (Naqvi et al., 2018). As denitrification is reported to be dominated over anammox in northern Indian Ocean OMZ (Ward et al., 2009) where organic carbon is fuelling heterotrophic denitrifying communities, the coupling of methane oxidation and denitrification might be advantageous.

Global annual denitrification rate in sediment would be approximately 200 Tg N, and majority contributed from sediments underlying OMZ, where its reported 2 to 4 times higher (Devol, 2015). Members of Steroidobacter order, which is known to perform denitrification coupled with methane oxidation (Liu et al., 2014), make up 12% of the BB-OMZ metagenome. Flavobacteriales members are also known to perform denitrification (Horn et al., 2005), and their abundance in GS1A site was 17.14%. In PS1B, Planctomycetes (9.48%) were identified to be the second dominant phylum. Hits corresponding to anammox order Brocadiales of phylum Planctomycetes were very low in the sediments analyzed, making up only 0.03 and 0.3% in GS1A and PS1B site and made up only $1/10^{th}$ of Planctomycetes community. The low dominance of anammox group suggests their contribution to nitrogen cycle was negligible even within benthic OMZs of the northern Indian Ocean, as observed in pelagic OMZs of AS (Ward et al., 2009). As Planctomycetales is known to encode a large number of sulfatase genes, which makes them as a specialist for the initial breakdown of sulfated hetero-polysaccharides (Wegner et al., 2013), the allocated role could be carbon capture deep in the sediments.

Other notable nitrogen cycle representatives recovered are Oceanospirillales, Chromatiales, Nitrospirales, Syntrophobacteriales, Thiotrichales, and NB1-j which is known to encode nitrogen metabolic genes such as norB, nosZ, nifD, and nrfA (de Voogd et al., 2015), and contributed 14.61% in GS1A and 7.47% in PS1B site respectively. Alphaproteobacterial order Rhizobiales which make up 2.69% and 11.08% in GS1A and PS1B site, primarily contain nitrogen-fixing bacteria. This order also includes *Methyloligella* sp., a methylotrophic bacteria. Their occurrence is ascribed to methane production in oligotrophic waters (Damm et al., 2010). Frankiales, whose relative abundance was 0.32% found in PS1B site, is also known to fix nitrogen (Sellstedt and Richau, 2013). ~2.4% recovered Vibrionales were *Photobacterium indicum* which are bioluminescent chemoorganotrophic symbionts. They have widespread

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occurrence in marine sediment and is a well-known methylotroph (Xie and Yokota, 2004; Stucki et al., 1981). Few sequences related to SAR-11 division was also recovered from OMZ of northern Indian Ocean, which is probably involved in the conversion of DMSP (Dimethylsulfoniopropionate) to DMS (Dimethylsulfide), which in turn acts as a shield and is reported to protect radiation effects (Hodkinson and Grice, 2015). DMS concentration as high as 525 nM is reported from upwelling areas of eastern AS (Shenoy and Kumar, 2007), suggesting their occurrence is not uncommon.

Many sulfur cycle members were also recovered in our study. The dominant community-identified was *Sulfitobacter dubius* (4.32%). *Sulfitobacter* sp. of class Alphaproteobacteria are known to perform sulfite oxidation, and all known species were isolated from marine habitats (Sorokin, 1995;Long et al., 2011). *Thermodesulfovibrio* of phylum Nitrospira, which made up ~1% in both sites are categorized as sulfate reducers (Schunck et al., 2013). Sequences corresponding to sulfur reducers like Desulfobacterales (0.82%/1.64%) and Syntrophobacterales (0.76%/1.36%) was previously identified in sediments of Black Sea sulfate-methane transition zone as well as in Arabian Sea OMZ water columns and sediments (Fernandes et al., 2018;Fuchs et al., 2005;Leloup et al., 2007).

In our analysis, a certain proportion of gene families were identified to perform photosynthesis, and major contributors in the analyzed microbiome are Chromatiales (0.56%/3.05%), Rhodospirillales (0.13%/5.67%) and members of phylum Cyanobacteria (0.03%/1.7%). Chromatiales, a group of purple sulfur bacteria can perform anoxygenic photosynthesis (Manske et al., 2005). Similarly, Rhodospirillales, an alphaproteobacterial member is primarily chemoorganotrophs and photoheterotrophs and mainly include purple non-sulfur bacteria (Luo and Moran, 2015), can also perform anoxygenic photosynthesis (Manske et al., 2005). It's interesting to note that around 70 Cyanobacterial sequences were retrieved from PS1B, where water column depth was ~245m, but only two representatives from GS1A, which was located at ~200m depth, suggesting its contributions to total phytoplankton is higher in low productive areas like BB in comparison to AS. Here the observed Chroococcales is assumed to be low-light adapted group (West et al., 2001).

The predominant Bacillales member in GS1A was *Paenisporosarcina quisquiliarum* like species, which makes up one-fourth of the total hits, was utterly absent in PS1B. The draft genome of *Paenisporosarcina* sp. shows that it encodes several genes for spore formation, including sporulation kinase, sporulation initiation phosphotransferase, spore coat protein, and spore germination protein (Im et al., 2012). Their abundance could be a sign of recent sporulation event during the time of sampling. Moreover, as indicated by the TIC value of GS1A, carbon

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remineralization process was very active due to increased availability of organic carbon. In PICRUST analysis as well 4 fold difference in sporulation related genes identified. Other dominant groups recovered, which make up ~1/5th of the population were identified to be the common soil/sediment inhabitants with a prime role is remineralization of carbon (Schimel and Schaeffer, 2015). These are classified under phyla Acidobacteria, Actinobacteria, Bacteroidetes, and Gemmatimonadetes (Janssen, 2006). Similarly, Anaerolinaeles of phylum Chloroflexi, which contributes 2-3% of the total hits, has also identified with a similar role and were specific to areas which shows very low or zero oxygen.

5. CONCLUSION

The present 454 pyrosequencing data shows high variability in bacterial community structure in AS-OMZ and BB-OMZ surface sediment, despite the comparable water column depth and near-bottom DO concentrations, but differing sediment carbon and nitrogen load. Our data shows that the BB-OMZ is more diverse and unexplored in comparison to AS-OMZ, based on diversity indices. Some dominant bacterial phylotypic representatives of GS1A site of AS-OMZ was utterly absent in PS1B site of BB-OMZ. However, 45% of the communities remain common at bacterial order level to both sites. It is also interesting to note that even though the phylogenetic diversity was different, the relative contribution of functional genes was almost the same. Based on PICRUST analysis, specific putative roles in assimilation, mineralization, oxidation, and reduction of sulfur, nitrogen, methane, and iron compounds are suggested and assumed to be participating in biogeochemical cycles. The prevalence of candidate phyla in the present dataset, which was also identified from other recognized OMZ suggests they have an essential role to play in 'Low oxygen metabolism.' Many new environmental clusters are reported here from sediments underlying OMZ of the northern Indian Ocean, whose sequence information could be utilized to target different bacterial communities. The taxonomic assignment utilizing two databases were successful in providing a better understanding of the OMZ community structure.

DATA AVAILABILITY

All pyrosequencing reads were submitted to the NCBI Genebank database under accession number KU821783 - KU831324 and MG860544 - MG860851. The supporting information is available as supplementary information.

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APPENDICES

A1: Greengenes taxonomy files showing the relative dominance of specific bacterial clades recovered from sediments of northern Indian Ocean OMZ.

A2: EzBioCloud taxonomy files comparing the relative dominance of bacterial communities up to species level recovered from GS1A and PS1B sites of northern Indian Ocean OMZ.

AUTHOR CONTRIBUTION

JLVJ prepared the manuscript and performed the experiments and bioinformatics analysis. CSM conceived the idea and designed the experiment.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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LEGENDS

Table 1: Characteristics of samples collected from northern Indian Ocean OMZ

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Table 2: Summary of pyrosequencing results and statistical analysis of bacterial sequences retrieved from OMZ.

*OTUs (operational taxonomic unit) were calculated using Mothur (3% distance).

§Good's coverage is proportional to non-singleton phylotypes in all sequences.

Fig. 1: Rarefaction curve of bacterial OTUs (operational taxonomic units) associated with sediments underlying oxygen-depleted waters in the Northern Indian Ocean.

Fig. 2: Taxonomic composition of OTUs from phylum to species level retrieved from both the sampling locations based on EzBiocloud16S rRNA database BLAST search.

Fig. 3: Dominant bacterial taxa retrieved at 1% cut-off based on pairwise alignment in Green Gene database.

Fig. 4: Double Pie chart showing bacterial community composition at the phylum and family level from the sampling location.

Fig. 5: Predictive functional profiling of the metagenomes utilizing PICRUSt.

TABLES

Table 1

Sampling Details			**Sediment Characteristics**						**Near-bottom water profile (CTD)**		
Station code	Date	Sampling depth	TOC	TIC	TN	CaCO3	OM	TOC/TN	DO	Temp	Salinity
			%						µM	°C	PSU
GS1A	02-02-2013	200m	2.012	8.11	0.28	67.556	3.469	7.174	2.313	15.584	35.345
PS1B	27-08-2014	244m	1.297	0.289	0.157	2.407	2.236	8.279	1.666	12.326	35.018

Table 2

Sample name	**Optimized reads**	**OTU richness***				**OTU diversity***		**Good's coverage§**
		Observed	Chao1	ACE	Jackknife	Shannon	Simpson	
GS1A	5,944	955	2,506	4,305	3,450	4.37	0.934	0.893
PS1B	4,125	1,889	4,447	7,616	6,242	6.97	0.998	0.695

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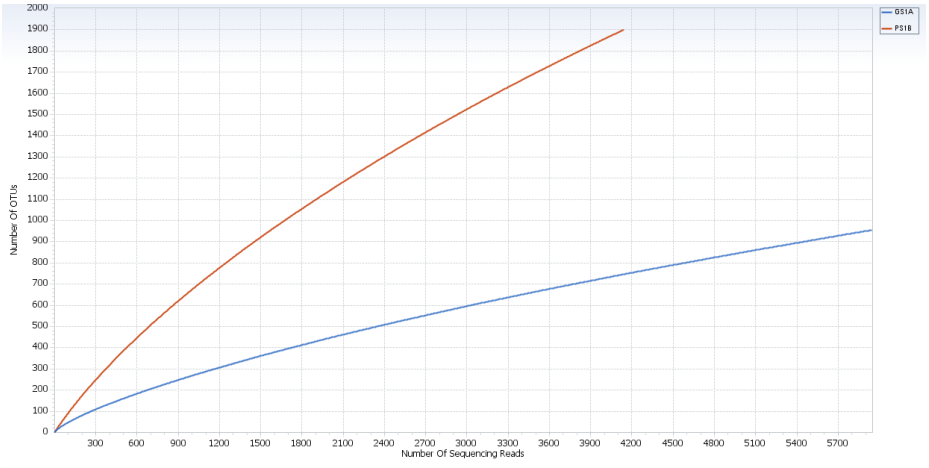
466 **FIGURES**

467



468

469 **Fig. 1**

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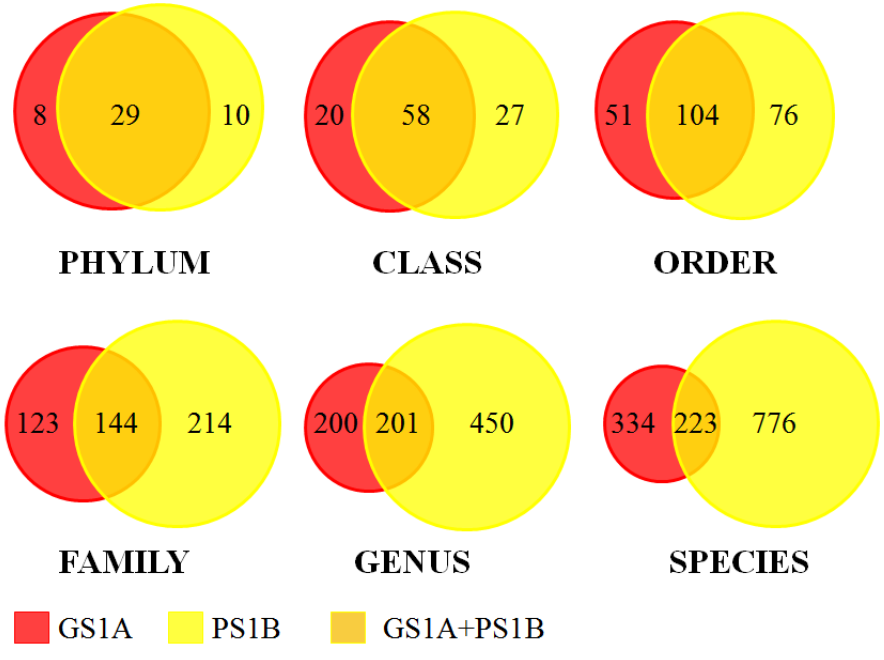

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472 **Fig. 2**

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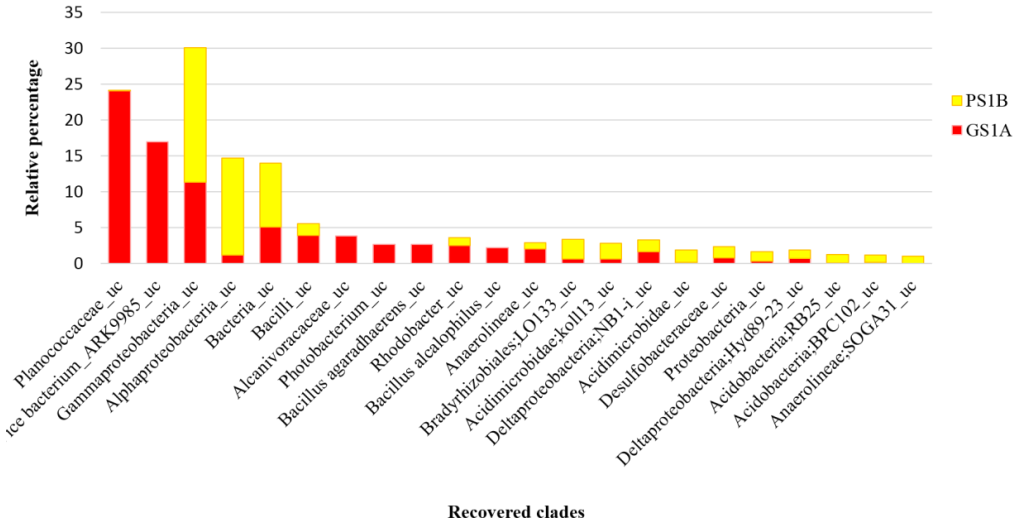

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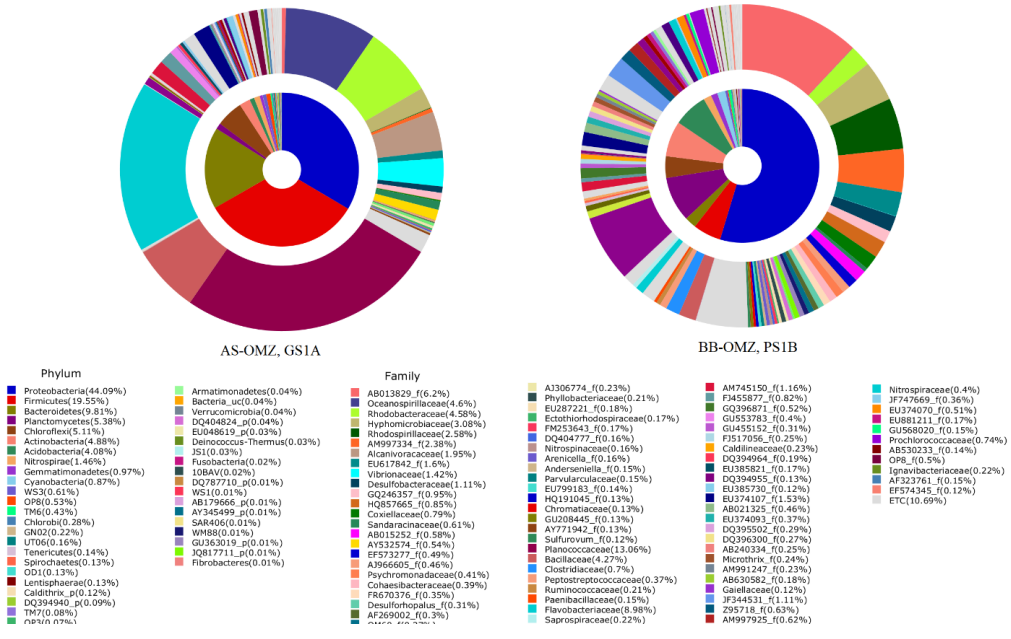
476 **Fig. 3**



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479 **Fig. 4**

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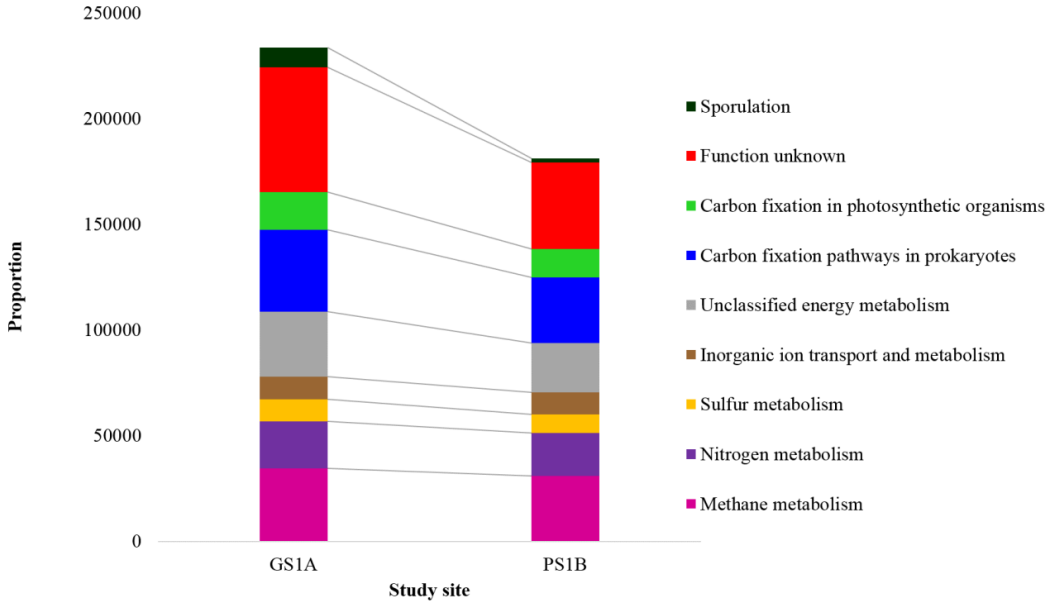

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483 **Fig. 5**

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