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# *Interactive comment on* "High-throughput screening of sediment bacterial communities from Oxygen Minimum Zones of the northern Indian Ocean" *by* Jovitha Lincy and Cathrine S. Manohar

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We are incredibly thankful to referee #1 for the detailed and constructive comments on our manuscript. Significant revisions were made as per comments received.

Two major modifications made in the bioinformatics analysis part which is listed below: 1. 'SILVA Release 132,' database was used for taxonomic assignment (earlier Greengenes database, release 13.5 used for the same) 2. 'Piphillin' algorithm used for predictive functional profiling of 16S rRNA amplicon dataset utilizing KEGG functional database updated October 2018 (previously PICRUSt v.1.1.4 used with KEGG database older version). New literature was added from flexible benthic OMZs. Simi-



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larly, the statements which will not benefit readers were removed and also other ambiguous statements pointed out by referee #1. Conclusion part revised thoroughly.

Please find below our point-to-point response to the review of referee #1. Referee comments are numbered; Our response to each comment is posted in the next paragraph, which is differentiated with a symbol. Also, changes made in the manuscript are highlighted in a double inverted “comma”, and the previous statement in single inverted ‘comma’. There are four sections: General comments, Specific comments, Technical corrections and Additional adjustments.

General comments:

1. The manuscript by Lincy and Manohar presents a study comparing one amplicon sequence dataset from the Arabian Sea sediments to another amplicon dataset from the Bay of Bengal sediments. The authors performed a phylogenetic analysis and a diversity study using some of the traditional indices used for community diversity studies.

→ Yes, we have compared the sediment bacterial community structure between the two major OMZs in the northern Indian Ocean. The generated pyrosequencing data were subjected to phylogenetic and diversity analysis as pointed out. Also, we tried to predict the putative functions of the identified bacteria utilizing algorithms based on KEGG functional database.

2. The manuscript is very hard to read for two reasons, the first of which is the quality of the language (see specific comments), the second of which, and this is the more severe point, it lacks a clear storyline.

→ Yes, we agree that English needs to be improvised and express our sincere gratitude to the referee Dr Carolin Löscher for pointing out all grammatical error. Details of such revisions are included under the heading ‘technical correction’.

→ In the revised version we have incorporated some additional references from flexible

OMZ benthic communities as per referee #1 suggestion, and hope it has provided more clarity and direction.

3. The authors base their analysis on two samples and come up with various claims that could possibly also just be a sequencing artifact given the difference in sequencing quality and depth of the two (!) sampling points. First, they claim a significant difference between the samples which is statistically impossible to claim, second, they claim a variability within the Indian ocean, again based on two samples, third, the diversity indices are not reliable because of the difference in sequencing quality and finally, based on this, the functional diversity model doesn't give any reliable result.

→ Around 7715 low-quality sequences were removed prior to OTU clustering, and only 10,069 sequences were selected further, i.e., in GS1A, 5944 sequences and PS1B, 4125 sequences were subjected for taxonomic assignment and predictive functional profiling. Sequences filtered out are those with ambiguous bases, homopolymers of $\geq$6 bp, and sequence length of $\leq$300bp; reducing sequencing artifact to a greater extent.

→ At four points, the referee expressed doubts which we are trying to clear through the following statement.

ïČŸ Diversity analysis indicates a very high variability between the samples. It happened because, in GS1A site, the relative abundance of Paenisporosarcina sp., Salegentibacter sp., and Amphritea sp. make up $\sim$50% of the microbiome, whereas the first three abundant taxa in PS1B contribute only 16% of the total microbiome. Hence even after subsampling the dataset, high variability is identified between the sites which is impossible to neglect.

ïČŸ Variability between the Arabian Sea and the Bay of Bengal OMZ is a well-proven fact. We are just supporting this fact through our data and no other claims.

ïČŸ We admit that two amplicon pyrosequencing dataset is not enough to make any





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significant claim. However, this opportunistic sampling has provided sample from two core OMZ regions, one in the Arabian Sea and other in the Bay of Bengal with comparable DO (∼2micro-mol), salinity (35ppt) and depth (200m for GS1A, and 244m for PS1B) and results are also exciting and suggest a broad scope for similar studies.

ïČŸ In the original version 'PICRUSt' algorithm was used for metagenome function prediction, in the revised manuscript, we used 'Piphillin' algorithm, as referee suggested to make use of other improved function prediction tools. Piphillin algorithm is more straightforward independent of any proposed phylogenetic tree, leveraging contemporary functional databases and not obliged to any singular data pre-processing protocol, as claimed by developers. Using Piphillin algorithm, more specific information on 'nitrogen' and 'sulfur' metabolic pathway are extracted. However, we admit such algorithms are affected by similarity cutoff and can detect only a subset of the population whose sequence information are updated in the KEGG database.

4. Another point is that the authors constantly compare their sediment data to water column metagenomes, which just doesn't make any sense and is very confusing.

→ The related statements comparing sediment data with water-column are provided for the kind reference. We think all points are relevant. Some statements are rephrased to get more clarity, still if the suggestion is to remove we are ready to do so. One statement regarding 'SAR-11' cluster already removed as it was poorly represented in our dataset and probably must have derived from water-column.

Section.4.2.

ïČŸ 'The present study, as well as the similar available literature from the AS-OMZ surface sediments, has documented similar value, 4.4 for Shannon diversity index (H) (Divya et al., 2011). Reports are scarce from BB-OMZ benthic zones; however, in pelagic OMZ comparable value of 6.6±0.5 are recorded at 200m (Fernandes et al., 2019).'

ïČŸ ‘In the pelagic BB-OMZ Chao 1 predictor has estimated 4697 OTUs (Fernandes et al., 2019) while in the benthic OMZ based on our study the estimated value is 7617, almost 0.6 fold higher, indicating that the sediment below BB-OMZ is more diverse than the pelagic zones.’

Section.4.3.

ïČŸ ‘The candidate phyla GN02, OD1, TM6, TM7, and WS3, were prevalent in ESP (eastern south pacific) pelagic OMZ as well (Ulloa et al., 2013;Ganesh et al., 2014), implying that they have an essential role in OMZ nutrient cycling.’

ïČŸ ‘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), suggesting the pelagic and benthic OMZ bacterial community might be different. However, many of the sulfate-reducing bacterial communities reported in Pelagic OMZ of Pacific were retrieved in our study too, might be because of similar levels of oxygen depletion.’ Section.4.4. ïČŸ ‘In northern Indian Ocean OMZs, nitrogen cycling is reported to be very active (Naqvi et al., 2006). In our analysis gene proportion of methanotrophs and methanogens outcompetes the nitrogen cycle-related genes. One reason could be due to the difference in the level of oxygen depletion within the benthic and pelagic ecosystem. The second reason is as methane cycle is more complex and a significant number of genes are ascribed to various pathway.’

ïČŸ ‘The low number 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 where denitrification is reported to be dominant over anammox (Ward et al., 2009).’

ïČŸ ‘Sequences corresponding to sulfur reducers like Desulfobacterales (0.82%/1.64%) and Syntrophobacterales (0.76%/1.36%) was also recovered in sediments of the Black Sea sulfate-methane transition zone as well as in Arabian Sea





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OMZ water columns and sediments (Fernandes et al., 2018;Fuchs et al., 2005;Leloup et al., 2007).'

5. I would suggest a major rewriting of the manuscript with a clearer focus: It could be very interesting to see those sediment amplicon datasets compared to other OMZ sediment data and then to obtain information on a core benthic OMZ community versus a 'flexible' OMZ community.

→ Significant revisions were done as per referee #1 suggestions. Comparisons were made accordingly whose details are listed below.

ïČŸ "In our analysis, the 'nif gene' involved in nitrogen fixation was below detectable level in GS1A site, and only a few hits obtained from PS1B site, whose overall contribution is negligible. This is in accordance with the benthic OMZ nitrogen fixation reports from Peruvian OMZ and similar low oxygen environment, where the fixation rate was very less compared to well-oxygenated benthic ecosystems (Gier et al., 2016). "

ïČŸ "Our analysis identifies DNRA and denitrification as active benthic fluxes of northern Indian Ocean OMZ. In seasonally hypoxic Baltic Sea sediments, DNRA accounted for almost 75% of benthic nitrogen flux and remaining contributed by denitrification mediated nitrate reduction (Dale et al., 2011). Though the DNRA process is known to fuel anammox process (Jensen et al., 2011) here, such coupling is not expected as anammox hydrazine signatures were not recovered. As denitrification related and sulfite reductase genes were prevalent, we assume that the organic-rich sediments of northern Indian Ocean OMZ must have favoured heterotrophic denitrification (Arango et al., 2007) or sulfur driven autotrophic denitrification (Shao et al., 2010) over anammox."

ïČŸ "Assimilatory sulfate reduction was dominant over the dissimilatory reduction in the OMZ sediments analyzed, and dsrA and dsrB gene subunit responsible for encoding dissimilatory sulfite reductase enzymes were not at all detected. The absence of such genes does not signify the process is not active in benthic OMZ. Since we targeted surface sediments, we could have missed out such signatures, as in sub-sediment sample





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of the Arabian Sea their occurrence is confirmed through molecular and chemical signatures (Fernandes et al., 2018).

ïČŸ “Sulfur oxidizing protein, soxY was detected in high number, and probably the major contributors would be Sulfitobacter dubius (4.32%). All known species of genus Sulfitobacter were isolated from marine habitats and are known to perform sulfite oxidation (Sorokin, 1995;Long et al., 2011).”

6. This, rather than a PICRUST model, could then be used to explain the differences in benthic biogeochemistry and benthic-pelagic fluxes of different OMZ regions.

→ It is replaced with Piphillin model, which is more advanced and have fewer cons. For detailed investigation genes/enzymes pertaining to ‘nitrogen’ and ‘sulfur’ metabolic pathway were only targeted. "For the functional prediction of 16S rRNA amplicons, the OTUs were clustered at 97% sequence similarity. The OTU table and representative sequences fasta file were submitted to Piphillin server online (https://piphillin.secondgenome.com/). The KEGG reference database updated on Oct-2018 was used at 96% cutoff to predict functions (Narayan et al., 2020). The final output of this workflow was quantified in terms of predicted gene abundances. The relative distribution was checked between the samples for listed pathway in KEGG functional database: ko00910 (nitrogen metabolism), ko00920 (sulfur metabolism), ko00680 (methane metabolism) and ko00710 (carbon fixation in photosynthetic organisms). The genes significant in ‘nitrogen’ (M00175, M00528, M00529, M00530, M00531) and ‘sulfur’ (M00176, M00595, M00596) metabolism was considered for detailed investigation." “The predictive functional profiling of 16S rRNA sequences has identified a high proportion of genes involved in methane metabolism, followed by nitrogen and sulfur metabolism all contributing to non-photosynthetic carbon fixation, while the relative distribution of photosynthetic bacteria remains <20% (Fig. 5).” “The relative proportions of the identified genes were comparatively more in the PS1B site of BB-OMZ in correspondence to GS1A site of AS-OMZ (Table.3).”

7. I would further suggest to re-run the amplicon BLAST analysis on SILVA instead of Greengenes, SILVA is the only database constantly updated which will certainly give away more informative result.

→ Rerun in SILVA releases 132 databases. "The pairwise alignments were done with SILVA SSU database release 132 based on the RDP classifier method (ver.14) in mother pipeline (Im et al., 2012)." "The taxonomic identity of bacterial phylotypes clustered at 97% sequence similarity with an abundance cut-off of 1% was used for comparative analysis (Fig. 3). The complete details are presented as supplementary information, A1."

Specific comments

1) L. 37 I disagree with this statement

→ The referee pointing towards the statement '.....these were reported from other OMZs as well, suggesting their putative role in sediment biogeochemistry.' We understood the point, that 'mere presence of one taxonomic group' and 'whether it is functionally active or not' cannot assess solely based on DNA data, hence rephrased as follows.

"The predictive functional profiling of 16S rRNA amplicons pinpointed the occurrence of specific enzymes which are crucial in the cycling of nitrogen and sulfur compounds.

2) L. 38 not the site but the community is diverse, please rephrase

→ Rephrased as follows

"Bacterial diversity from the present study reveals that the bacterial community of off Paradip site of Bay of Bengal OMZ is highly diverse in comparison to the off Goa site of the Arabian Sea OMZ."

→And also likes to add one additional point in the abstract, which we feel very relevant to be highlighted as we assume 'shared OTUs' represents the OMZ microbiome core.

"Only 30% of OTUs were shared between the sites which makes up three-fourth of the Bay of Bengal OMZ microbiome, but only one-fourth of the Arabian Sea OMZ microbiome."

3) L. 39 it's diverse compared to off Goa, or it is unexplored as compared to off Goa?

→ It is 'diverse', as number of OTUs was almost double and more taxonomic groups were identified in PS1B site of Bay of Bengal. The term 'unexplored' used in a sense, as studies are scarce in Bay of Bengal OMZ in comparison to Arabian Sea OMZ, however, if it is confusing for the reader's point of view, we stick to one term only, i.e., 'diverse.' Also, one additional point we would like to add regarding the comment 'unexplored.'

".....is highly diverse in comparison to the off Goa site of the Arabian Sea OMZ, and this unexplored site of northern Indian Ocean demands more studies, which is at the tipping point."

4) l. 41 the presence of genes is not necessarily related to their activity, so I feel this statement is too far-fetched.

→ Removed the statement because of same reason pointed out in the first specific comment, as there is no cent percent surety whether all microbes carry out the same function if subjected to the similar environment due to complexity of processes.

5) l. 64 This needs some re-thinking. There is a body of work now showing the plasticity of processes with overlaps between sulfur and nitrogen turnover

→ Earlier made a generalized statement 'sequence of electron acceptor utilization, generally follow the thermodynamic energy yield' but now added one more point which clarifies it cannot be true in all situations.

"However, recent studies support the possibility of co-occurrence of cryptic sulfur cycle along with nitrogen cycle challenging our understanding (Callbeck et al., 2018; Canfield et al., 2010)."

6) l. 65 Are these explanations relevant for the manuscript?

→ If NMZ and CMZ definitions are not fitting into the context, I withdrew the statement.

7) L. 67 what does this tell us in the context of the AS versus the BoB?

→ The d-Mn maxima region generally coincides with areas of SNM where severe oxygen depletion reported. Few more points added in the context of AS v/s BoB in the revised version.

“The dissolved Manganese (d-Mn) maxima regions localized within these oxygen minimum zones (OMZs) likely have an oxygen scavenging effect which intensifies them further. In AS-OMZ, d-Mn conc. of 3 to 8 nm coincides with areas of secondary nitrite maximum (SNM) where severe oxygen depletion is reported as the DO value falls below $\leq$ 2micro mol (Lewis and Luther III, 2000). In BB-OMZ d-Mn maxima are localized in shallow depths of 200m were 5 to 6 nm conc. observed (Vu and Sohrin, 2013).

8) l. 69 this is somewhat in contrast to water column studies from OMZs showing high abundances of aerobic ammonia oxidizers. Bristow et al showed for the BoB aerobic organisms existing in the OMZ

→Second generalized statement made ‘OMZs acts as a comfortable niche for microorganisms that can use alternative pathways of respiration.’ As pointed out by the referee, to show the dynamic nature of such systems, the following lines were added.

” In BB-OMZ aerobic communities have identified to be coexisting with anaerobic communities (Bristow et al., 2017), but preferably in separate micro-niches as identified in AS-OMZ (Pitcher et al., 2011).”

9) l. 74 This is the first time you are talking about benthic ecosystems. I think this should come straight away to better guide into the manuscript’s topic

→ The sentence shifted to the beginning of the second paragraph as suggested.

“Surface sediment underlying OMZs entraps all recent microbial signatures of the water





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column above (Gerdes et al., 2000); hence it is interesting to explore and compare such benthic OMZ ecosystems, especially those located in shallow zones."

10) l. 74-77 The sentences sound awkward, please rephrase.

→ The previous sentence {'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)'} rephrased as follows.

"Similar studies carried out in the eastern AS-OMZ sediments have identified Proteobacteria (52%) and Planctomycetes (12.7%) as the dominant phyla (Divya et al., 2011). Other integral phyla of soil/sediment habitat are Bacteroidetes, Acidobacteria, Actinobacteria, and Firmicutes (Lv et al., 2014)."

11) l. 82 explain

→ I think the question was to explain how 'PICRUSt' or similar algorithms like 'Piphillin' works?

"By utilizing algorithms leveraging functional databases, it is possible to predict putative functional ecology from 16S rRNA microbiome data (Iwai et al., 2016). For example, Piphillin algorithm utilizes nearest-neighbour matching 16S rRNA amplicons (or genomes) to predict the representative genomes. The normalized 16S rRNA copy number of each genome is inferred using gene content in a functional database like KEGG and BioCyc."

12) L 85/86/87 Please rephrase, sounds awkward

→ The previous sentence {'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.'} rephrased as follows.





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"The available data on the microbiome of the Northern Indian Ocean OMZ using such high-throughput sequencing techniques has chiefly been limited to the water column or restricted to some functionally significant groups rather than total bacterial community (Fernandes et al., 2018)". Fine details of OMZ sediment bacterial communities are lacking and needs special attention."

13) l. 92 This is not what PICRUSt does. You can predict functional diversity, you cannot say anything about genetic distributions

→ In the revised version PICRUSt is replaced with Piphillin model. As pointed out by referee #1, if such models are only useful for prediction of functional diversity, we stick to that purpose only.

14) l. 94 comparable to what?

→ The studied sites GS1A of AS-OMZ and PS1B of BB-OMZ had comparable depth, salinity and dissolved oxygen concentration.

15) l. 95 This sentence doesn't have any informative value.

→ Will remove the following sentence it is not much useful, 'Diversity studies are essential in understanding ecosystem processes and defining the role of microbes relevant to the study area.'

16) l. 101/ 102 What is GS1A, what is PS1B?

→ GS1A is the name of sampling site in the Arabian Sea, and PS1B is of Bay of Bengal located in OMZ of northern Indian Ocean.

17) l. 103 What does undisturbed sediment sample mean?

→ The term used to ensure that no mixing has happened to the 0-5 cm sediment sample during sampling and transport.

18) l. 109 please rephrase, sounds awkward





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→ The sentence '. . . . . .Dissolved oxygen (DO) sensor (RINKO from ALEC, Japan) was attached to the same' rephrased as follows.

"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, and dissolved oxygen (DO) sensor (RINKO from ALEC, Japan) was also attached to the CTD unit fitted with Niskin/ Go-Flo bottles."

19) l.113 explain C and N; Which standards did you use, how many blanks? Precision and detection limit?

→Few lines were added new to answer the questions raised in methodology section.

"Total carbon (TC) and nitrogen (TN) were analyzed in a CN analyzer (FISONS NA1500) using the method described in (Bhushan et al., 2001). The calibration of CN analyser was done using reference standard (NC-soil), and obtained recovery was 96% for TC and 99% for TN. The precision was monitored by carrying out replicates for both samples and was $\pm$1%."

20) l. 114 What means estimated here?

→ The term 'estimated' replaced with 'determined'. The total organic carbon (TOC) content was determined (estimated).

21) l. 117 reference?

→ Van Bemmelen's factor 1.724 (Heaton et al., 2016)

22) l. 119 This needs more explanation.

→ The sentence is elaborated by adding a few words which is underlined below, and we hope it is clear more.

"For CaCO3 calculation, TIC was multiplied with 8.33 to get the relative percentage by assuming all carbonate is calcium carbonate (Bernard et al., 1995)."

23) l. 123 did you do blank extractions? 'A Nanodrop. . .'

→ Yes, blank measurement done using elution buffer and purified DNA was quantified using Nanodrop 2000 spectrophotometer (ThermoScientific, USA).

24) l. 129 company's location?

→ Agilent, USA

25) l. 133 sequencing chemistry and sequencer type?

→ Sequencing was performed by Chunlab Inc. (Seoul, Korea) using the 454 GS FLX Titanium Sequencing system (Roche Branford, CT, USA) per the manufacturer's instructions. It is based on improved titanium chemistry; here Hydrogen bonds are formed between "reader" molecules and DNA base, whereas titanium–sulfur bonds are formed between "reader" molecules and titanium nitride electrodes. This electrode can generate distinct conductance for each DNA base, and thus we can identify easily.

26) l. 137 so you used the pre-analysis provided by the company?

→ Pyrosequencing and quality filtering were done at Chunlab Inc. (Seoul, Korea).

"Sequencing was performed by Chunlab Inc. (Seoul, Korea) using the 454 GS FLX Titanium Sequencing system (Roche Branford, CT, USA) per the manufacturer's instructions."

27) l. 146/147 How, which commands?

→ The richness, and diversity indices and rarefaction curve were calculated at 97% similarity using the Mothur platform v.1.43.0. (Schloss et al., 2009).

→ Command used were as follows:

- collect.single(list=combined_re.unique.filter.opti_mcc.list, label=unique-0.03) - rarefaction.shared(shared=combined_re.unique.filter.opti_mcc.shared, label=unique-0.03, iters=1000)

28) l. 154 Awkward sentence. Please replace. How did you decide on what genes are significant?

→ Yes, Thanks for pointing out awkwardness in the following sentence 'Only genes whose function which are ecologically significant were considered for a graphical representation.' It is rephrased as follows in the light of Piphillin algorithm analysis.

" The genes significant in 'nitrogen' (M00175, M00528, M00529, M00530, M00531) and 'sulfur' (M00176, M00595, M00596) metabolism was considered for detailed investigation represented in KEGG pathway module clustered at 96% identity cut-off."

29) l. 161 On what do you base this prediction?, The OM was highest- what does that mean, could there be a number?

→ The CTD cast 4m above the sediment, hence in the underlying sediment DO value is predicted to be lower than observed in the bottom water (2ïĂămicromol). Also, replaced with a number rather than telling 'highest' or 'lowest'.

"In the present study, both sampled sites showed intense oxygen depletion, i.e., near bottom DO was ∼2 micro Mol. The OM was 3.47% for GS1A and 2.24% for PS1B, and TN values were 0.28% and 0.16% for GS1A and PS1B respectively."

30) l. 162 The TOC/TN ratio was slightly high for PS1B sample- what does that mean?

→Replaced with a number for clarity rather than using the ambiguous term 'slightly.'

"The TOC/TN ratio was 8.28 for GS1A and 7.174 for PS1B."

31) How is the term 'significantly' explainable?

→ The term 'significantly' replaced with 'substantially'. It was used to highlight the observed differences in TIC between the two sites GS1A (8.11%) and PS1B (0.29%).

32) l. 168 This technical information could go into the methods part, where you should also describe the output of a rarefaction analysis.

→ Rarefaction analysis details re-allocated to method section.

“The richness, diversity indices and rarefaction curve were calculated at 97% similarity using the Mothur platform v.1.43.0. (Schloss et al., 2009). This richness estimation curve is a plot of the number of species as a function of the number of samples, and in the present study PS1B plot did not achieve saturation in comparison to GS1A.”

33) l. 170 Explain ACE

→ Described as below

“ACE (Abundance-based Coverage Estimator) separates the observed frequency in two groups: rare and abundant. It incorporates all data from all species with fewer than ten, and for abundant species, only presence/absence information recorded as they already are in considerable number. Here the accuracy of results strongly correlates with sample size."

34) l. 171 You have to convince with numbers and comparable numbers from other studies if you want to make such a statement.

→ Replaced with numbers, and comparable references were also added to make the statement more convincing.

“Out of 17,784 pyrosequenced reads, 43% reads were filtered out during quality processing step. After read pre-processing, 5944 reads for GS1A, and 4125 reads for PS1B were selected for further analysis. In marine sediments, pyrosequencing read number varied between 5,000 and 20,000 per sample (Zhu et al., 2013; Choi et al., 2016).”

35) l. 173 A rarefaction curve doesn’t give you information on the total diversity but on the sequencing saturation. As such you can only determine if your samples are quality-wise useable and if they can be compared. In your case you did not sequence in saturation and the saturation status between your two samples differs quite a bit. Thus it is questionable to compare the diversity.

→As per referee's suggestion Rarefaction curve not using for comparing the diversity of the two sites as sequence saturation is not obtained for PS1B sample. The Fig. 1 showing the rarefaction is shifted to the supplementary section in the revised manuscript.

36) I recommend a re-writing of l. 168-182. There is a lot of repetition and information that is not particularly useful the way it is presented.

→ A complete revision was suggested for section 3.2. It's re-written as follows.

"Out of 17,784 pyrosequenced reads, 43% reads were filtered out during quality processing step. After read pre-processing, 5944 reads for GS1A, and 4125 reads for PS1B were selected for further analysis. The OTU richness and diversity estimators indicate that the sampled sites are highly diverse. Between the studied sites, PS1B site of BB-OMZ is more diverse than GS1A site of AS-OMZ. The overall results are presented in Table 2. Simpson diversity index ranged between 0 and 1, where a value of '1' represents infinite diversity and '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 Jackknife suggests that the OTU richness can be 3 to 4 fold higher than observed. In rarefaction curve, though sequence saturation not obtained for PS1B sample, the Goods nonparametric coverage estimator indicates that the present, long read 454 pyrosequencing was successful in recovering ∼70% of bacterial phylotypes from PS1B and 90 % from GS1A."

37) l. 185 What does 'slightly different' refer to? To the diversity indices? This wouldn't be a surprise as those are two thing which you can't compare.

→ l.185 comparison is not about diversity indices. Since for taxonomic assignment,

the reads were aligned with two databases, Greengenes/SILVA (were upto genus-level classification is possible), and EzBioCloud (which claims to classify sequences upto species level) were compared initially. However, such comparison removed in the revised version, but the output of two different portals has given some additional insights; hence both data kept as supplementary information. The results obtained through the SILVA database was majorly presented and discussed in the revised version.

38) L. 187 I don't think Greengenes is reliable anymore. There are huge discrepancies to the SILVA database. I would recommend a re-mapping to SILVA.

→ Reanalysed in SILVA release 132 databases as suggested, and accordingly, changes are made in the manuscript.

39) l. 188 What means 'specific to both sites'? Present at both sites?

→ Yes, present at both studied sites of the northern Indian Ocean.

40) l. 192 I distrust both databases, as mentioned I suggest re-analyzing using SILVA

→ Reanalyzed in SILVA database, but we are still keeping EzBioCloud database information because it has given species-level information, which could be useful, if true. For example, the most dominant phylotype analysed through 'SILVA platform' was Paenisporosarcina sp. and as per EzBioCloud classification, it is Paenisporosarcina quisquiliarum," adding few more details.

41) l. 193 Which literature?

→ As said SILVA (though earlier we used GreenGenes database) is widely used platform for taxonomic affiliation of sequences the taxonomy obtained are comparable with most cited literature.

42) l. 202 It may result from the time of the last update of the databases

→ True, it can happen with different versions of the database, hence such an ambiguous statement removed in the revised version.

43) l. 204-208 I don't understand what you are trying to say with this sentence.

→ We are trying to tell about the taxonomic relatedness between the microbiome of two sites under study. At phylum level how much OTUs are shared to both sites and unique to GS1A/PS1B site, similarly for bacterial classes/orders/family/genus, everything depicted in Fig.2. The taxonomic profile generated through the EzBiocloud was represented in the previous version of the manuscript, now on the light of SILVA SSU database 132, Fig.2 re-drawn.

"The Venn diagram was used to represent the number of taxa that are unique to each site and common to both zones at different taxonomic level classified based on SILVA 132 database (Fig. 2)."

44) l. 211 How does this look compared to other OMZs?

→ These dominant taxa were not reported in other published reports of from OMZs, but common to soil/sediment habitat. Majority of the report available have detailed only up to Phyla or Family level.

45) l. 221 'Planococcaceae_uc' and similar expressions. Those are database output names, please directly replace with the proper taxonomy. What does identical mean here?

→ In Greengenes database the most abundant community in GS1A site was 'Planococcaceae_uc' (bacterial family), so after reanalyzing in SILVA database we were able to classify further as 'Paenisporosarcina sp.' (bacterial genera), and as per EzBioCloud upto species level classification made possible and majority of the hits were identical to Paenisporosarcina quisquiliarum.

46) l. 225 This doesn't emphasize spatial variability. It is one spot per ocean basin which has been sampled, here. To see variability two samples from very different regions are not enough.

→ We were comparing only two spots 'GS1A' of AS-OMZ and 'PS1B' of BB-OMZ under

study. Since such term like spatial variability is not appropriate as per referee's view, we removed the term and other related statements from the revised manuscript.

47) l. 220 -232 This is a pure listing, which would benefit from some context to guide the reader to what should be learned, here.

→ Yes, so in discussion section when we mention about this group, readers get more clarity.

48) l. 236 Which genome?

→ Sorry, here we intend to mention as 'microbiome', not genome which was derived from amplicon pyrosequencing of GS1A and PS1B sediment samples.

49) l. 237 Above you claimed a high percentage of known phyla.

→Yes we mentioned about 'Paenisporosarcina sp.', which is classified under phylum 'Firmicutes' which was the dominant phyla in GS1A site of AS-OMZ (33%).

50) l. 238 The statement about carbon fixation is neither clear nor well-founded, please rephrase or remove.

→We would like to remove the statement. After utilizing Piphillin algorithm (https://piphillin.secondgenome.com/) more specific information pertaining to enzymes and genes obtained and re-written with a more explicit focus on 'nitrogen' and 'sulfur' metabolic pathway.

51) l. 243 xenobiotic compound degradation pathways: What is that?

→ It was used as an example for pathways coming under the term 'unclassified energy metabolism'.

In the original version utilizing PICRUST analysis, all pathways relevant to 'microbial metabolism in the diverse environment' was listed, and discussion section written accordingly. After analyzing through piphillin algorithm, more detailed information on dif-

ferent biogeochemical pathway were obtained was obtained and presented.

52) l. 244 This sentence doesn't add any information and is not understandable.

→Sentence removed.

53) l. 250 What do the molarities refer to?

→ It refers to the average DO conc. in pelagic OMZs of Arabian Sea and Bay of Bengal.

54) l. 258 What does 'as well' refer to?

→ Our data also support the variability in AS-OMZ and BB-OMZ.

55) l. 261 What does early nitrogen burial mean? Rapid burial?

→Yes, Rapid burial.

56) l. 270 Remove this sentence. It doesn't add any information.

→Removed the sentence 'Pyrosequencing based 16S rRNA gene surveys are increasingly utilized to study bacterial community structure distribution (Youssef et al., 2009).'

57) l. 272 Pyrosequencing is not more useful, it has a lower coverage and depending on the pipeline way longer OTUs can be assembled from Illumina sequencing. In addition, V1-V3 is not a long fragment so this is no good point here and does anyway not contribute any information to the data discussion.

→ We understand, as pyrosequencing is already replaced with an advanced method, such statement has no value, hence removed in the revised version.

58) l. 273, 274 This statement is based on one publication which I don't agree with and which ignores a significant body of work saying exactly the opposite. In any case, you have been working on bacterial data which do only represent a subset of the total diversity so more cautiousness would be recommended, here.

→ The study by Walsh et al., 2015 compared bacterial community composition in the

photic zone, the sub-photic zone where OMZ was identified, surface sediments and 1.5m deep sub sediments. Found OMZ regions to be more diverse. As referee pointed out, pelagic OMZ comparisons and results based on the single study have no much value? the statement is removed in the revised version.

59) l. 280 what is now the H Index? Where does this come from?

→ Shannon diversity index (H) is commonly used to characterize species diversity in a community. It accounts for both abundance and evenness of the species present.

"The present study, as well as the similar available literature from the AS-OMZ surface sediments, has documented similar value, 4.4 for Shannon diversity index (H) (Divya et al., 2011). Reports are scarce from BB-OMZ benthic zones; however, in pelagic OMZ comparable value of 6.6±0.5 are recorded at 200m (Fernandes et al., 2019)."

60) This section contains only information which has been presented, before, and some evaluation of sequencing methods, which to me appear unnecessary and are partly incorrect.

→ Section 4.2. Discussion section mainly compares the diversity estimates between the sampled sites and also from the published literature. We have rephrased the entire section and will remove if still not satisfactory.

"In the present era of next-generation sequencing, immense data on the microbiome have accumulated, and hence diversity indices can provide much valuable information. In comparison to freshwater and intertidal sediments, microbial diversity is lower in marine sediments (Wang et al., 2012). The present study, as well as the similar available literature from the AS-OMZ surface sediments, has documented similar value, 4.4 for Shannon diversity index (H) (Divya et al., 2011). Reports are scarce from BB-OMZ benthic zones; however, in pelagic OMZ comparable value of 6.6±0.5 are recorded at 200m (Fernandes et al., 2019). This regional differences between AS and BB OMZ are not uncommon, e.g., in south and east China Sea sediment samples, Shannon





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index is 2.52 and 7.96 respectively (Zhu et al., 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 richness estimators suggest that sediment OMZ bacterial communities are even richer than coastal microbial mats (Bolhuis and Stal, 2011). In the pelagic BB-OMZ Chao 1 predictor has estimated 4697 OTUs (Fernandes et al., 2019) while in the benthic OMZ based on our study it was estimated as high as 7617, almost 0.6 fold higher, thus indicating that the sediment below BB-OMZ is more diverse than the sediment below AS-OMZ and pelagic OMZ of BB."

61) l. 305 First of all, what are those phyla? Second, how does their presence in the Pacific mean anything about their metabolic pathways?

→Those are Candidatus phyla which is having no cultivable representatives. Nevertheless, since they are detected in major OMZ including Indian and the Pacific Ocean, we speculate they have some pivotal role to play.

62) l. 308 13 phyla are not a vast diversity, but rather a very small diversity

→ When a total of 43-44 phyla are obtained altogether, 13 is not a small number. The significance of these 'microbial dark matter' is highlighted by Rinke et al. (2013) and attempts were made to study them utilizing single-cell genomic approach. Hence we believe it is important to mention their presence while commenting on total bacterial diversity of benthic OMZ.

63) l. 310 Remove, this doesn't add anything.

→ Removed the sentence {'The single-cell genomics approach is required to understand the coding potential of these "bacterial dark matter" in OMZ (Rinke et al., 2013)'}.

64) l. 312-317 Why is this reported if the clades are meaningless in the present dataset?

→ They are generally found in many other studied OMZ regions. Hence we thought if they are not reported in the Indian Ocean, it needs to be highlighted.





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"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). This suggests the variability between benthic and pelagic OMZ bacterial community structure."

65) l. 317 it's not the 454 sequencing but the selected regions that are possibly not well representative, please check the primer bias if you want to make such a statement

→ No specific published reports are available on primer bias of the studied primer set (9f/541r), but the possibility of primer bias cannot be neglected (Takahashi, 2014). As referee commented, when there is not enough representatives, it will not probably get detected, hence removed such speculations in the revised manuscript.

66) l. 320 Which ones?

→ Sulfate reducers identified in benthic OMZ microbiome from the northern Indian Ocean are Thermodesulfovibrio, Desulfobacterales, and Syntrophobacterales. These are discussed in details in section 4.4.

67) l. 325 What would ultra-low be?

→ In nano-molar concentration which is beyond the detection limit of the Winkler method, but we didn't have such high precision sensors.

68) l. 330 This would now give you a good chance to compare your data to data from the Eastern tropical Pacific and eastern tropical Atlantic OMZs, the Baltic and other environments (Gier et al, Bertics et al, work by Fullweiler and colleagues, by Jørgensen, Treude etc)

→We are extremely thankful to the referee for providing many useful references related to the benthic nitrogen cycle. Appropriate references where are incorporated were ever similar discussion came up. The details are incorporated under "General comment: 5".

69) l 348 'anammox bacteria', also introduce what this is

→ To introduce anammox bacteria, one line was added as follows.

“Anaerobic ammonia oxidizers, also known as anammox bacteria are a significant player in the removal of fixed nitrogen in OMZ. 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).”

70) l. 354 explain what those genes do

→ The nitrogen metabolic genes such as norB (nitric oxide reductase), nosZ (nitrous oxide reductase), nifD (nitrogen fixing), and nrfA (a formate-dependent pathway for nitrite reduction to ammonia) all involved in the nitrogen cycle. The relative distribution of such genes was tabulated at 96% cutoff and presented in Table.3.

71) l. 380 do you think they are active or are they detritus?

→Active or detritus is questionable, however in DNA based data, such questions always arise. We sampled from ∼240m depth where light penetration or negligible. As the presence of Chroococcales, a low-light adapted group in the surface sediments could be derived from water-column as 240m is not very deep.

72) l. 403 this is again a far-fetched statement

→The sentence {‘It is also interesting to note that even though the phylogenetic diversity was different, the relative contribution of functional genes was almost the same’}, removed as ‘PICRUSt’ / ‘Piphillin’ algorithms applicability only on predicting putative function and cannot be used to asses relative contribution.

73) l. 406 I disagree. This is pure speculation.

→The sentence {‘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.’} could be considered as speculation. We mentioned them to highlight the significance of these ’microbial dark matters’ whose coding potential is





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unknown. When reporting total bacterial diversity without mentioning their existence, the study will not be completed. The sentence is rephrased as follows and retained in conclusion without any claim.

". . .. . . . .. . . .. . .Also, 'Candidatus' phyla identified demands research focus as no ecological role could be predicted due to lack of cultivable representatives.

74) Also, the Conclusion part needs major re-writing

→Conclusion revised as follows, "The high throughput sequencing method was successful in revealing the majority of the sediment bacterial communities' residing at 200m water depth of the northern Indian Ocean OMZ. This comparative study has identified PS1B site of BB-OMZ to be more diverse than GS1A site of AS-OMZ, and the results were well supported by traditional diversity indices. Only less than one-third of the phylotypes were shared between the sites, which were also reported in other flexible benthic OMZs and form a significant proportion of BB-OMZ microbiome. Due to the dominance of certain phylotypes in the AS-OMZ microbiome, the actual community structure might be hidden, hence demands more study. The predictive functional profiling of amplicon dataset had identified many genes relevant to nitrogen and sulfur metabolic pathways like denitrification, dissimilatory nitrate reduction, assimilatory sulfate reduction, and sulfur oxidation. The genes responsible for anammox, nitrogen fixation and dissimilatory sulfur reduction were not well represented in the surface sediments. As the majority of the sequences recovered were environmental clusters, it is hard to come to any valid conclusion as only a subset of the population is taken for functional predictions. The novel phylotypes, sequence information could be utilized to target undiscovered bacterial communities. Also, 'Candidatus' phyla identified demands research focus as no ecological role could be predicted due to lack of cultivable representatives. We admit DNA based studies should be cautiously used as they could be active or detritus. This study signifies the northern Indian Ocean OMZ sediment communities are very versatile and diverse, and the processes governing in such ecosystem are highly complex"





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Technical corrections

1) L 31: I would rephrase by *high throughput sequencing', because it's not one technique → rephrased 'next generation sequencing' as 'high throughput sequencing'.

2) L. 34 replace 'from' with 'in' → corrected.

3) l. 35 'indicated', 'the bacterial diversity' → corrected

4) l. 41 'at both sites' → corrected

5) l. 58 'consists' or 'is comprised' → corrected as 'consists.'

6) l. 73 'hence it is interesting' → corrected

7) l. 78 replace 'a database' with 'data' → rephrased as 'data.'

8) l. 79 'helped' → corrected

9) l. 83 ' the Northern Indian Ocean' → corrected

10) l. 84 'techniques', replace 'restricted' by 'been limited', replace 'pelagic OMZs' by 'the pelagic realm' or 'the water column' → corrected

11) L 89 here and elsewhere, please remove 'technique'. It is not a technique unless you refer to pyrosequencing, but even then you wouldn't say it like this. → The term 'technique' was removed from eight positions, wherever the term 'high throughput' was mentioned.

12) l. 90 replace 'long-read 454 pyro-sequencing technology' with 'pyrosequencing'. Also 'the bacterial community composition' → corrected

13) l. 90 'the 16S ribosomal RNA' → corrected

14) l. 91 'in a perennial OMZ location' → corrected

15) l. 92 replace 'utilizing' by 'using the' → corrected

16) l. 101/ 102 replace ‘region of’ by ‘in the’→ corrected

17) l. 103 replace ‘and corresponding water column depth’ by ‘in waters with a depth of’. ‘A box corer’→ corrected

18) l. 104 ‘liners’ → corrected

19) l. 106 ‘containers’, replace ‘aseptically’ with ‘sterile’ → corrected

20) l.113 ‘a CN analyzer’ and also ‘described in’,→ corrected

21) l. 129 Replace ‘are’ with ‘were’ → corrected

22) l. 141 ‘the EzBioCloud 16S. . .’ → corrected

23) L. 150 ‘The PICRUS. . .’ → corrected.

24) l. 163 remove comma after ‘water’ → comma removed.

25) l. 197 ‘contribute’, ‘the total bacterial. . .’ → corrected

26) l. 203 ‘refer to’ → corrected

27) l. 210 ‘at the two sites’ → corrected

28) l. 219 ‘orders’ → corrected

29) l. 220 Bacteria, which. . . → corrected

30) l. 237 ’Firmicutes were’ → corrected

31) l. 238 number are commonly spelled out up to twelve → corrected 4 as four.

32) l. 240 at the GS1A site → corrected

33) l. 241 remove ‘the’ → corrected

34) l. 242 replace ‘too’ with ’to’ → corrected

35) l. 256 replace ‘its’ by ‘it is’. ‘Also, the BB. . .the AS. . .’ (also in L. 266) → corrected

36) l. 297 ecosystems → corrected

37) l. 298, replace ‘utilizing’ with ‘using’, again this is not a technique → corrected

38) l. 303 replace ‘alter’ with ‘determine’ → corrected

39) l. 350 ‘are known’ → corrected

40) l. 372 ‘the Black Sea’ → corrected

41) l. 382 ‘Chroococcales are’, ‘a low-light. . .’ → corrected

Additional adjustments:

We would like to shift Fig.1. Rarefaction curve to supplementary section, and accordingly the numbering will change.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-330/bg-2019-330-AC2-supplement.pdf

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-330, 2019.



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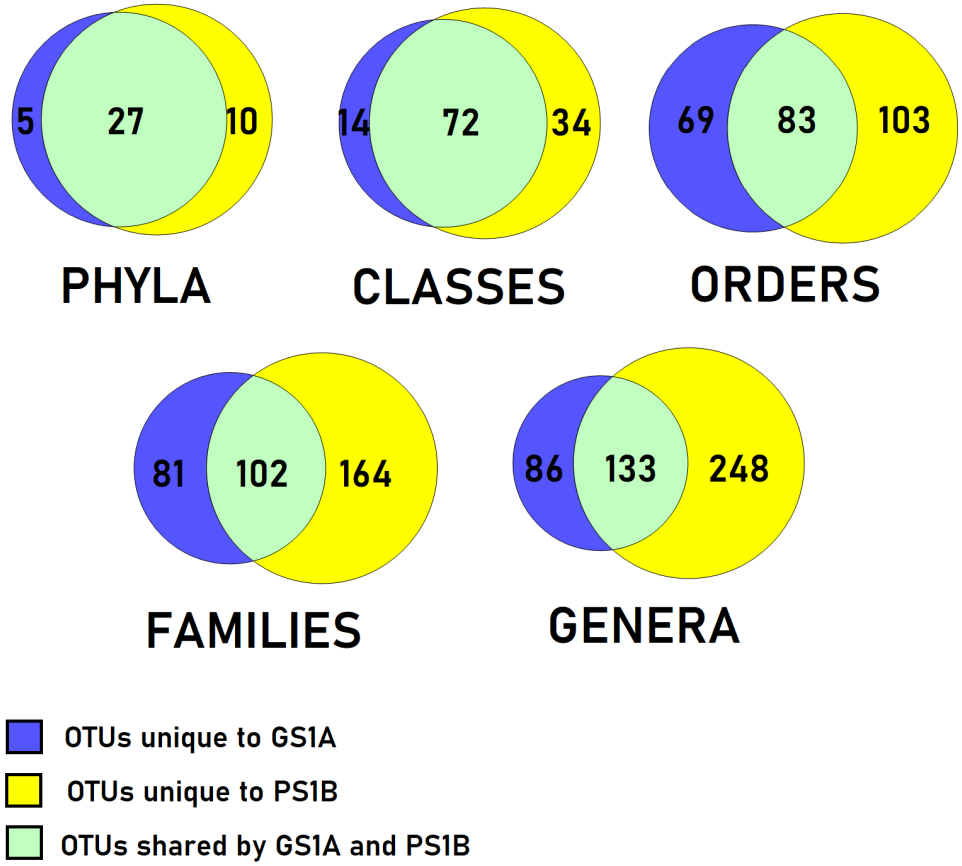


**Fig. 1.** Fig.2. Venn diagram showing unique and shared OTUs at different taxonomic level



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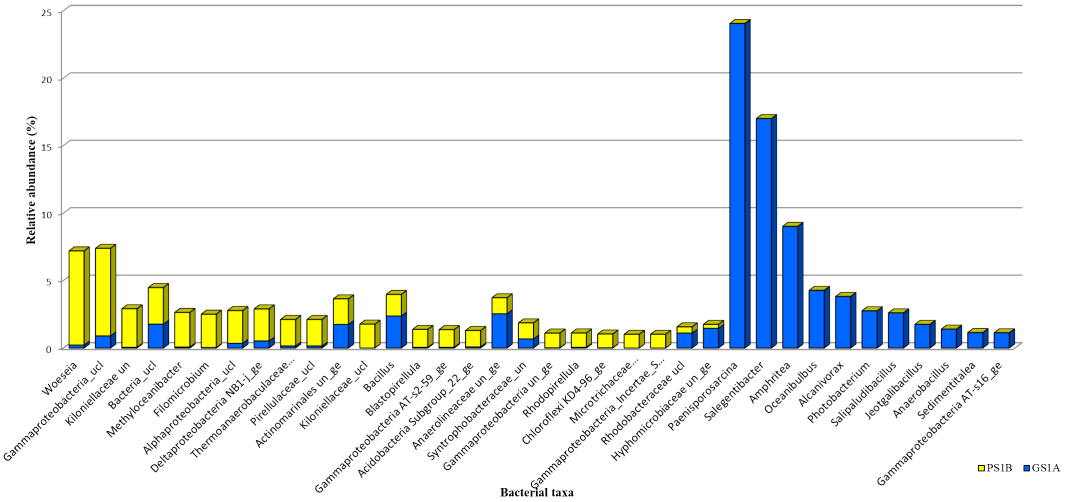


**Fig. 2.** Fig.3. Dominant bacterial taxa retrieved at 1% cut-off from the surface sediments underlying northern Indian Ocean OMZ



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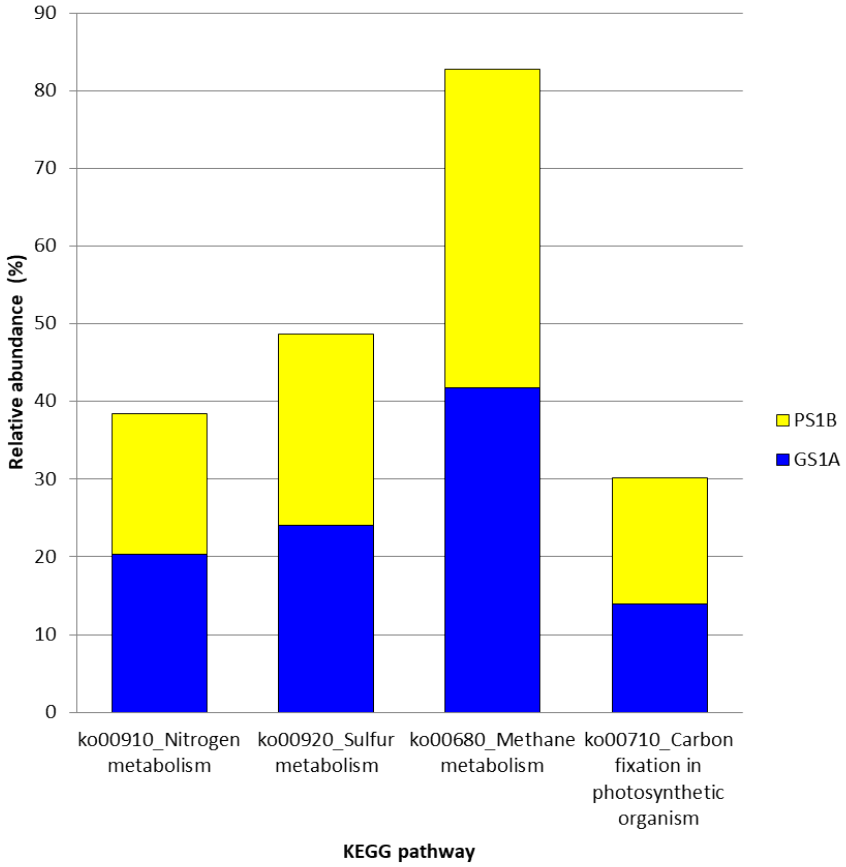


**Fig. 3.** Fig.5. Relative distribution of selected KEGG pathways inferred from 16S rRNA amplicon dataset at 96% similarity.

| NITROGEN METABOLISM | KEGG code | GS1A | PS1B |
|---|---|---|---|
| **nitrogen fixation, nitrogen => ammonia** | | | |
| anfG; nitrogenase delta subunit [EC:1.18.6.1] | K00531 | 0.00 | 0.18 |
| nifD; nitrogenase molybdenum-iron protein alpha chain [EC:1.18.6.1] | K02586 | 0.00 | 16.52 |
| nifH; nitrogenase iron protein NifH | K02588 | 0.00 | 8.66 |
| nifK; nitrogenase molybdenum-iron protein beta chain [EC:1.18.6.1] | K02591 | 0.00 | 16.38 |
| vnfD; vanadium-dependent nitrogenase alpha chain [EC:1.18.6.2] | K22896 | 0.00 | 0.00 |
| vnfK; vanadium-dependent nitrogenase beta chain [EC:1.18.6.2] | K22897 | 0.00 | 0.00 |
| vnfG; vanadium nitrogenase delta subunit [EC:1.18.6.2] | K22898 | 0.00 | 0.00 |
| vnfH; vanadium nitrogenase iron protein | K22899 | 0.00 | 0.00 |
| **Assimilatory nitrate reduction, nitrate => ammonia** | | | |
| narB; ferredoxin-nitrate reductase [EC:1.7.7.2] | K00367 | 0.75 | 54.50 |
| NR; nitrate reductase (NAD(P)H) [EC:1.7.1.1 1.7.1.2 1.7.1.3] | K10534 | 1.00 | 7.14 |
| nasB; assimilatory nitrate reductase electron transfer subunit [EC:1.7.99.-] | K00360 | 0.00 | 2.53 |
| nirA; ferredoxin-nitrite reductase [EC:1.7.7.1] | K00366 | 36.42 | 176.34 |
| nasA; assimilatory nitrate reductase catalytic subunit [EC:1.7.99.-] | K00372 | 48.93 | 122.54 |
| NIT-6; nitrite reductase (NAD(P)H) [EC:1.7.1.4] | K17877 | 0.00 | 0.00 |
| **Dissimilatory nitrate reduction, nitrate => ammonia** | | | |
| narG, narZ, nxrA; nitrate reductase / nitrite oxidoreductase, alpha subunit [EC:1.7.5.1 1.7.99.-] | K00370 | 35.29 | 121.06 |
| narH, narY, nxrB; nitrate reductase / nitrite oxidoreductase, beta subunit [EC:1.7.5.1 1.7.99.-] | K00371 | 35.29 | 120.72 |
| narI, narV; nitrate reductase gamma subunit [EC:1.7.5.1 1.7.99.-] | K00374 | 35.29 | 120.72 |
| napA; nitrate reductase (cytochrome) [EC:1.9.6.1] | K02567 | 15.47 | 1.57 |
| napB; nitrate reductase (cytochrome), electron transfer subunit | K02568 | 22.93 | 0.72 |
| nirB; nitrite reductase (NADH) large subunit [EC:1.7.1.15] | K00362 | 59.85 | 126.85 |
| nirD; nitrite reductase (NADH) small subunit [EC:1.7.1.15] | K00363 | 24.68 | 9.93 |
| nrfA; nitrite reductase (cytochrome c-552) [EC:1.7.2.2] | K03385 | 10.97 | 1.16 |
| nrfH; cytochrome c nitrite reductase small subunit | K15876 | 0.50 | 0.50 |
| **Denitrification, nitrate => nitrogen** | | | |
| nirK; nitrite reductase (NO-forming) [EC:1.7.2.1] | K00368 | 2.33 | 2.03 |
| narG, narZ, nxrA; nitrate reductase / nitrite oxidoreductase, alpha subunit [EC:1.7.5.1 1.7.99.-] | K00370 | 35.29 | 121.06 |
| narH, narY, nxrB; nitrate reductase / nitrite oxidoreductase, beta subunit [EC:1.7.5.1 1.7.99.-] | K00371 | 35.29 | 120.72 |
| narI, narV; nitrate reductase gamma subunit [EC:1.7.5.1 1.7.99.-] | K00374 | 35.29 | 120.72 |
| napA; nitrate reductase (cytochrome) [EC:1.9.6.1] | K02567 | 15.47 | 1.57 |
| napB; nitrate reductase (cytochrome), electron transfer subunit | K02568 | 22.93 | 0.72 |
| nirS; nitrite reductase (NO-forming) / hydroxylamine reductase [EC:1.7.2.1 1.7.99.1] | K15864 | 5.00 | 5.00 |
| norB; nitric oxide reductase subunit B [EC:1.7.2.5] | K04561 | 74.50 | 71.60 |
| norC; nitric oxide reductase subunit C | K02305 | 10.80 | 5.00 |
| nrfH; cytochrome c nitrite reductase small subunit | K00376 | 12.47 | 5.20 |
| **Nitrification, ammonia => nitrite => nitrate** | | | |
| hao, hydroxylamine dehydrogenase [EC:1.7.2.6] | K10535 | 5.00 | 1.10 |
| pmoA-amoA, methane/ammonia monooxygenase subunit A [EC:1.14.18.3 1.14.99.39] | K10944 | 0.00 | 0.00 |
| pmoB-amoB, methane/ammonia monooxygenase subunit B | K10945 | 0.00 | 0.00 |
| pmoC-amoC, methane/ammonia monooxygenase subunit C | K10946 | 0.00 | 0.00 |
| **anammox, nitrite => nitrogen** | | | |
| hzsC, hydrazine synthase subunit [EC:1.7.2.7] | K20932 | 0.00 | 0.00 |
| hzsB, hydrazine synthase subunit [EC:1.7.2.7] | K20933 | 0.00 | 0.00 |
| hzsA, hydrazine synthase subunit [EC:1.7.2.7] | K20934 | 0.00 | 0.00 |
| hdh, hydrazine dehydrogenase [EC:1.7.2.8] | K20935 | 0.00 | 0.00 |
| **SULFUR METABOLISM** | **KEGG code** | **GS1A** | **PS1B** |
| **assimilatory pathway, sulfate => H2S** | | | |
| PAPSS, 3'-phosphoadenosine 5'-phosphosulfate synthase [EC:2.7.7.4 2.7.1.25] | K13811 | 0.00 | 0.00 |
| sat, sulfate adenylyltransferase [EC:2.7.7.4] | K00958 | 9.38 | 69.22 |
| cysC, adenylylsulfate kinase [EC:2.7.1.25] | K00860 | 12.51 | 63.08 |
| cysNC, bifunctional enzyme CysN/CysC [EC:2.7.7.4 2.7.1.25] | K00955 | 70.00 | 234.00 |
| cysD, sulfate adenylyltransferase subunit 2 [EC:2.7.7.4] | K00957 | 115.47 | 352.68 |
| cysN, sulfate adenylyltransferase subunit 1 [EC:2.7.7.4] | K00956 | 10.47 | 2.93 |
| cysH, phosphoadenosine phosphosulfate reductase [EC:1.8.4.8 1.8.4.10] | K00390 | 49.84 | 190.20 |
| cysJ, sulfite reductase (NADPH) flavoprotein alpha-component [EC:1.8.1.2] | K00380 | 86.59 | 231.32 |
| cysI, sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2] | K00381 | 88.43 | 242.43 |
| sir, sulfite reductase (ferredoxin) [EC:1.8.7.1] | K00392 | 0.75 | 57.87 |
| **dissimilatory pathway, sulfate => H2S** | | | |
| sat, sulfate adenylyltransferase [EC:2.7.7.4] | K00958 | 9.38 | 69.22 |
| aprA, adenylylsulfate reductase, subunit A | K00394 | 0.00 | 6.07 |
| aprB, adenylylsulfate reductase, subunit B | K00395 | 0.00 | 6.07 |
| dsrA, dissimilatory sulfite reductase alpha subunit | K11180 | 0.00 | 0.00 |
| dsrB, dissimilatory sulfite reductase beta subunit | K11181 | 0.00 | 0.00 |
| **sulfur oxidation, thiosulfate => sulfate** | | | |
| soxA, L-cysteine S-thiosulfotransferase [EC:2.8.5.2] | K17222 | 2.00 | 8.20 |
| soxX, L-cysteine S-thiosulfotransferase [EC:2.8.5.2] | K17223 | 2.00 | 8.20 |
| soxB, S-sulfosulfanyl-L-cysteine sulfohydrolase [EC:3.1.6.20] | K17224 | 2.00 | 7.20 |
| soxC, sulfane dehydrogenase subunit SoxC | K17225 | 7.00 | 9.20 |
| soxY, sulfur-oxidizing protein SoxY | K17226 | 72.00 | 243.20 |
| soxZ, sulfur-oxidizing protein SoxZ | K17227 | 2.00 | 9.20 |
| soxD, S-disulfanyl-L-cysteine oxidoreductase SoxD [EC:1.8.2.6] | K22622 | 7.00 | 9.20 |

**Fig. 4.** Table.3. Distribution of genes relevant in cycling of 'nitrogen' and 'sulfur' compounds in benthic OMZs