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Interactive comment on "A comparison of bacterial communities from OMZ sediments in the Arabian Sea and the Bay of Bengal reveals major differences in nitrogen turnover and carbon recycling potential" *by* Jovitha Lincy and Cathrine Sumathi Manohar

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We express our gratitude to referee #2 for the valuable suggestions provided to overcome the sample limitations, and for the positive comments.

Please find below our point-to-point response to the review of referee #2.

Referee comments are numbered; our response to each comment is posted in the next

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paragraph, which is differentiated with an arrow symbol. Also, changes made in the manuscript are highlighted in a double inverted “comma”, and the previous statement is shown in single inverted ‘comma’.

There are three sections: General comments, Specific comments, and additional adjustment made.

General comments

1. The manuscript by Lincy and Sumathi Manohar, “A comparison of bacterial communities from OMZ sediments in the Arabian Sea and the Bay of Bengal reveals major differences in nitrogen turnover and carbon recycling potential” compares the bacterial diversity of surface sediments of two OMZ, in the Arabian Sea and in the Bay of Bengal based on 16S rRNA gene amplicon sequencing. The authors also use the 16S rRNA gene sequencing data to perform a predictive functional profiling to determine the metabolic potential of both sites in terms of nitrogen and carbon cycling.

→ Yes, this is exactly what we did.

2. The topic of this manuscript is very appealing as little is known regarding the bacterial diversity of OMZ sediments of those locations due to the difficulties for sampling on those regions. So far, what is known is mostly of pelagic samples in those OMZ and derived from studies performed approximately 10 years ago, so in most of the cases focused on specific microbial groups but not on the entire community. Notably, also no metagenomics studies are available.

→ Thanks for the positive comments. Yes, from Indian Ocean similar details are limited to pelagic zones, and rather restricted to some ecologically significant groups only. The sediments underlying OMZs are least explored, and especially the Bay of Bengal region is first time explored to understand the microbial ecology.

3. However, despite of the usefulness of these kind of data, the fact that the authors report only two samples (no replicates) total (one of each OMZ), no sequencing blanks,

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and that the total amount of 16S rRNA gene reads per sample is very low (barely 4000 reads in total), make the conclusions of this paper unsupported (i.e. in some cases, the discussion is based on microbial groups that represent barely 35 sequencing reads of the total). The authors acknowledge this limitation only at the end of the manuscript (i.e. line 398: "Despite the limitation of this study with regard to our sample number, we could contribute a first assessment of bacterial diversity and functionality in coastal sediments of the two Indian Ocean basins, as such, we hope to contribute to the general understanding of how these basins wok and why they are so different in their biogeochemistry") but even being this study limited, two samples, and without replication it is just too adventurous.

→ The read length of pyrosequencing is less compared to present day NGS techniques like Illumina or Ion-Torrent. The read number was limited to 4000-5000 due to strict QC (Quality filtering) measures. The data was generated in 2014, when I got offer for free analysis by ChunLab, South Korea, but the offer was limited to two samples only. I am a doctoral student from India, a developing country where students do not get much financial support, hence not able to include more samples. In the limitations, I hope I tried to extract maximum information possible. Regarding the sequencing blank, I am quite sure there is, but I got only the data pertaining to my samples, after QC. Though the limitations regarding the sample number are already highlighted in the concluding remarks, we will refer the same in the beginning itself as suggested to avoid any confusions.

4. The authors also put a lot of emphasis on the functional profiling approach which can be useful but it is of course highly speculative as the functionalities are inferred based on previously sequenced genomes which harbored those functions, but how about novel functions? The authors should have indicated more clearly the caveats of this approach and be very careful with the statements derived from it. As it is written now, the reader can even think this information is derived from metagenomics sequencing when it is not. You cannot simply say that "functional profiling of 16S rRNA amplicons

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pinpointed the occurrence of specific enzymes" but rather that the functional profiling pointed out to the presence of specific OTUs which have been previously described to harbor the activity X or the enzyme Y.

→ We agree, functional profiling could not be cent percent accurate, and it exclude the information's pertaining to many Candidate' communities; despite of the limitations such predictions are widely used to understand the microbial ecology, to understand the overview of microbial process governing. As suggested, we will re-write as the statement as 'functional profiling pointed out to the presence of specific OTUs which have been previously described to harbor certain activity' wherever applicable.

Specific comments (Other comments):

1. Formulate better the aim (both in the abstract and later in lane 84): the aim is not the comparison between the bacterial diversity between these two sites. The aim should go beyond the comparison, as far I can tell what the authors are aiming is to better understand how the differences between the functional diversity of these two sites might explain differences in the N and C cycle in these two OMZ systems

Abstract: 'High-throughput sequencing was used to understand the total bacterial diversity in, the surface sediment off Goa within the OMZ of the Arabian Sea, and from off Paradip within the OMZ of the Bay of Bengal.'

Ln 84: 'The objective of our work was to compare the surface sediment bacterial diversity within two major OMZs in the northern Indian Ocean, the Arabian Sea (AS) and the Bay of Bengal (BoB), using NGS on the v1- v3 hypervariable region of the 16S rRNA gene. Based on this high throughput sequencing dataset, we predicted the metabolic potential present at both sites, the AS and the BoB with a key focus on genes relevant for nitrogen and sulfur turnover.'

⇒ Some additions were made as follows, in the abstract and in the objective:

Abstract:

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"16S rRNA gene amplicon sequencing was used to understand the total bacterial diversity in, the surface sediment off Goa within the OMZ of the Arabian Sea, and from off Paradip within the OMZ of the Bay of Bengal. Functional profiling was carried out to pinpoint the occurrence of specific OTUs which have been previously described to harbor certain genes/enzymes relevant to biogeochemical cycling of carbon, nitrogen and sulfur compounds."

Objective:

"The objective of our work was to compare the surface sediment bacterial taxonomic and functional diversity within two major OMZs in the northern Indian Ocean, the Arabian Sea (AS) and the Bay of Bengal (BoB), using NGS on the v1- v3 hypervariable region of the 16S rRNA gene. Based on this high throughput sequencing dataset, we predicted the metabolic potential present at both sites, the AS, and the BoB with a key focus on genes relevant for nitrogen and sulfur turnover, and many fermentative pathways to understand the bacterial role in carbonate precipitation, a possibility least explored.

2. Line 33: substitute "high-throughput sequencing" for "16S rRNA gene amplicon sequencing"

⇒ Changed to "16S rRNA gene amplicon sequencing."

3. Line 37: considering that the authors did not cover the entire diversity of these microbial communities with the depth of sequencing applied, statements regarding the shared OTUs between the sites should be avoided

→ We indeed didn't cover the entire community diversity, but the most abundant clades were resolved. Fig.2 which shows the shared communities at different taxonomic level, will clearly give an idea that BoB site is much more diverse than AS sampled of the northern Indian Ocean OMZ, hence would like to retain it.

4. Line 41: Line 99-102: this part seems to imply that several sediment samples were

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collected while later in the Tables there are only TWO samples! Also, how do you define surface?

'Sampling at both stations covered surface sediments below a ∼200m deep water column, underlying OMZ waters. Though both areas experience intense oxygen depletion with the core of the OMZ located between 150 and 500 m, the maximum NOx values are twice as high in the AS as compared to the BoB, with a prominent secondary nitrite maxima (SNM).'

→Surface sediment here means the first 0-5 cm section of the sampled core, which are in direct contact with water-column above.

To familiarize the situation pertaining to the sampling sites an additional figure (Fig-1B) is added. The supporting data was obtained from other cruise, but the location is remarkably close by, and sampling time also coincide very well.

In BoB cruise, due to heavy cyclones, we were not able to perform detailed sampling.

5. Line 128: Why were the samples extracted in triplicate and then pooled again for the analysis

→To get maximum representative communities we pooled the sample, and per the funding's available only two samples were given for outsourcing.

6. Line 131: add a reference regarding the primers used. Are 9F/541R universal or bacterial? If they are universal, didn't you encounter any archaeal 16S rRNA gene sequences? On base of this point I would suggest to change the title and throughout the text from bacterial to microbial or prokaryotal.

→ The primer set used 9F (AGAGTTTGATCMTGGCTCAG) and 541R (ATTACCGCGGCTGCTGG) are remarkably similar primer-pair as 27F (GAGTTTGATCMTGGCTCAG) and 518R (WTTACCGCGGCTGCTGG). Since 454 was performed by Chun-Lab, Korea and the reference paper given utilized 9F/541R, hence the confusion arise. However, we got only bacterial sequences, suggesting here the pyrosequencing was

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carried out using bacterial specific primer: 27F/518R (Okubo et al., 2009), rather than universal primer.

Additional Adjustments:

The current C/N ratio shows wt/wt., we would like to convert it into molar ratio by multiplying with a factor 1.167 derived from atomic weights of Carbon and Nitrogen (i.e., 14/12). Accordingly, some shift in values, AS becomes 8.372 (7.174) and BoB – 9.662 (8.279); the old values shown in bracket.

x————————-x

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