

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 thankful to referee #2 for reviewing our manuscript. We can only partly agree with the comments provided, and necessary changes were made accordingly in the manuscript. Please find below our point-to-point response to the review of referee #2.

Referee comments are numbered; our response to each comment is written in the next paragraph, which is differentiated with a symbol. Also, changes made in the manuscript are highlighted in a double inverted comma.

General comments:

1. The study of Lincy and Manohar investigated the microbial community between two sediment samples under the Oxygen Minimum Zones in the Northern Indian Ocean. The authors used 16S rRNA pyrosequencing data to assess the diversity and infer the functional potential of the two investigated sites.

⇒ Yes, this is what we intended to convey.

2. First of all, I would like to point out those accurate calculations, and predictions about the diversity of such a highly complex ecosystem are hardly possible based on the obtained sequencing depth (10,069 reads for both samples) and sample numbers.

⇒ Compared to the traditional Sanger sequencing method, the obtained reads using the 454 pyrosequencing method was many folds higher. However, in comparison to current next-generation sequencing methods and shotgun genomics, reads might be less but not small enough to carry out diversity estimates.

⇒ We agree that the sample number could have improved. The opportunistic samples we got from two core OMZ locations had comparable near-bottom dissolved oxygen levels (~2 micromol) and sampling depth (~200 m). The results are impressive, and we believe it has to be communicated without further delay.

3. The authors also only focused on the bacterial community, not taking into account that archaea would play an important role for ecosystem functions in such environments. This was also not mentioned in the discussion.

⇒ We aimed to focus only on the bacterial community though it makes up only a subset of the microbial population. Archaea, also an exciting group, since we used universal bacterial primer, it is not possible to get any information on Archaea from the generated dataset.

4. Furthermore, the authors assessed the functional potential of the microbial community based on the amplified 16S rRNA genes. First of all, I see such an analysis highly critical since the 16S rRNA information cannot be translated into functional po-

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tential.

⇒ We cannot entirely agree. Many models, like PICRUSt, Tax4Fun, Piphillin, PUMA, are already in use, which can predict functional potential from 16S rRNA amplicon dataset. We admit that whole-genome information is not available for many bacteria, but we could attempt to understand the conventional role of a particular group if information about one or two members is available.

5. In this study, the 16S rRNA genes were not extracted from a metagenome but were amplified with universal primers. Taking into account the PCR bias, the low sequencing depth, and the number of samples (one for each site), the assessment of the functional potential is impossible.

⇒ It is a metagenomic data only, total DNA extracted from sediment and later targeted using bacterial specific primers. Sequences filtered out are those with ambiguous bases, homopolymers of ≥ 6 bp, and sequence length of ≤ 300 bp; reducing sequencing artifact to a greater extent. Yes, I agree there is only one sample per site, but while DNA extraction, triplicates were prepared and pooled together before subjecting to pyrosequencing.

6. Also, as mentioned before, an assessment of the functional potential is not possible without taking into account the archaeal population.

⇒ I am afraid we have to disagree, as already mentioned, many models were successfully applied, and in comparison to archaea, the bacterial database is more updated.

7. For potential functional assessments, the authors should have performed metagenome sequencing and analysis.

⇒ We think the referee is pointing towards 'Shotgun metagenomic sequencing'. Sorry, we have performed only 454 pyrosequencing where a universal bacterial primer was used to target the v1-v3 hypervariable region of 16S rRNA amplified. Phylogenetic analysis was carried out further. As per our understanding, the 'metagenomics' term is

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applicable for 'cultivation-independent genome-level characterization,' hence the term is applicable to present amplicon data set.

8. All in all, the conclusions of this study are very farfetched based on the obtained data.

⇒ We agree, and the same thing was pointed out by referee #1. The conclusion is re-written 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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9. I suggest to either change the scope of the study or to obtain more data (e.g., metagenomics) to assess the questions raised in this study.

⇒ The objective of our work was ‘to compare the surface sediment bacterial diversity within two major OMZs in the northern Indian Ocean utilizing 454 pyrosequencing technique.’ Based on this objective, the complete analysis conducted, and the manuscript written. At present, no more funds are available for this project to carry Shotgun metagenomic sequencing or similar high-throughput techniques. If the term is not apt in the context as pointed out by the referee, we will stick to ‘16S amplicon sequencing’ rather than ‘metagenomics’ wherever applicable.

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