

## ***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***

### **Anonymous Referee #2**

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The manuscript by Lincy and Sumanthi 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. The topic

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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. 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, 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 work 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 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 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. Other comments: - Formulate better the aim (both in the abstract and later in line 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

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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 - Line 33: substitute “high-throughput sequencing” for “16S rRNA gene amplicon sequencing” - 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 - Line 41Line 99-102: this part seems to imply that several sediment samples were collected while later in the Tables there are only TWO samples! Also, how do you define surface? - Line 128: Why were the samples extracted in triplicate and then pooled again for the analysis - 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

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