

Authors' Response to Anonymous Referee # 2

General Comments

Referee's Comment: In this manuscript, Bhattacharya et al., investigate and compare microbial community structure, metagenomes and metatranscriptomes in sediments below perennial and seasonal oxygen minimum zones in the Arabian Sea. They report the difference in community structure between the two as well as varying biogeochemical processes determined by -omics data and physiochemical and isotope data. Specifically, the perennial OMZ sediments exhibited cryptic methane cycle and high abundance of methanogens, ANME, SRB, and acetogens in surface sediments. Authors also incorporated physiochemical and geochemical data to help explain these processes and main environmental drivers of the community differences and I really appreciate this approach.

Authors' Response: We thank the Reviewer for appreciating the phenomenology and scientific approaches underlying the study.

Authors' Changes in Manuscript: Not applicable

Sampling and methodological approach were rigorous, well executed, and generated a plethora of thought-provoking and really valuable data. However, the manuscript is way too long and thus very difficult to get through. It also includes a lot of redundant information between the methods, results, and discussion sections (results in discussion/discussion in results). The main findings are difficult to follow due to the length of text and not very informative data presentation. Additionally, extensive lists of taxa make the manuscript really long and disturb the flow (without really bringing much valuable information). Seems like metatranscriptome results (arguably one of the most interesting parts of this study) are buried in text and not represented in figures. On the other hand, diversity data, which is valuable but not as captivating, is discussed at lengths and displayed in a large panel figure. I think the manuscript needs to be condensed and only the really interesting, major findings should be discussed and highlighted. I understand that sifting through so much data and determining what really matters is difficult, but I think it is a necessity so that this manuscript is comprehensible and thought provoking.

Figures should also be improved and better represent the compelling data the authors have here, but these data are buried in text.

Authors' Response: We agree with the concerns, so have now dealt with each of them in the revised manuscript.

Authors' Changes in Manuscript: Over the revision we have now

- reduced the length of the manuscript considerably,
- removed redundancy of information,
- moved all collateral information such as the lists of taxa to the SI,
- incorporated new figures for better visualization of metatranscriptomic and slurry incubation results,
- deleted the detailed diversity data, keeping only what is central to demonstrate that the microbiome profiles of the two sediment systems are different (notably Figure 2 is central in this regard, because only this kind of an all-encompassing heat map can comprehensively and quantitatively illustrate that the microbiome profiles of the perennial and seasonal OMZ sediments are distinct, which in turn is one of the main discoveries of this paper),
- removed the detailed values for the geochemical data,
- and, overall, streamlined the results and discussions as per the objectives spelt out in Introduction.

Specific Comments

Line 53-58: please shorten this sentence or break it up, it is hard to follow

Authors' Response: We agree, now fixed.

Authors' Changes in Manuscript: The sentence has been simplified.

Line 67: maybe co-exist instead of co-founded?

Authors' Response: The idea here is to convey the sense that microbial communities get established (founded) in the sediments after getting deposited alongside organic matter. The word "exist" would not exactly convey this sense.

Authors' Changes in Manuscript: Not applicable.

Line 78-81: I am not sure this statement is true; I feel like there has been plenty of research done on this globally

Authors' Response: Flux and composition of the organic matter and microflora deposited has been shown to influence the carbon remineralization/sequestration dynamics of a sediment system (Kristensen et al., 1995; Parkes et al., 2000; Burdige, 2007; LaRowe et al., 2020). Water-column oxygenation level is also known to impact the preservation of labile (biochemically reactive) organic matter, as well as the composition of the microbial community in the seafloor (Jessen et al. 2017).

From this perspective, however, the following issues are almost unexplored,

- how microbiome profile changes in the age-depth context of a diagenetically maturing sediment package (Kallmeyer et al., 2012; Orsi et al., 2017),
- how differential bottom-water DO concentration, sedimentation rate, and flux and nature of the deposited organic matter, as often encountered along water-depth transects across continental margins (Middelburg, 2019a, 2019b), shape the microbiome profile of marine sediments.

Authors' Changes in Manuscript: The text has now been edited by incorporating new reference in such a way as to explain the above point more objectively.

Line 160: "DNA for metagenomic analysis was extracted"

Authors' Response: We agree and have now edited the text as suggested.

Authors' Changes in Manuscript: "Metagenomes were extracted" has been replaced with "DNA for metagenomic analysis was extracted".

Line: 225-250: This is so much text and it's hard to follow/keep track of. Maybe present in a table in SI? The manuscript is already so voluminous...

Authors' Response: We agree and have now moved these lists of taxa to the SI.

Authors' Changes in Manuscript: As mentioned in the authors' response.

Line 270-280: I don't think it is necessary to describe and discussion diversity indices at such length.

Authors' Response: We agree, so have now shifted the mathematical details to the SI and provided only the conceptual explanations in the main text.

Authors' Changes in Manuscript: As mentioned in the authors' response.

Line 312: have you checked GTDB for ANME?

Authors' Response: We have now upgraded all the metagenomic and metatranscriptomic data pertaining to ANME with analyses based on the searching of our sequence datasets against a comprehensive database of ANME genomes (MAGs) curated non-redundantly from NCBI and GTDB.

Authors' Changes in Manuscript: As mentioned in the authors' response.

Lines 497-508: At this point I am lost in the jargon and equations.

Authors' Response: We agree, so have conceptually elaborated and explained the mathematical terminologies by adding simpler descriptions.

In this context it is noteworthy that the probability density functions (equation fitting) determined and presented for the individual data series critically authenticate the phenomenon "synchronous population fluctuation of multiple metabolic types along the perennial OMZ cores", a main finding of this paper.

If we don't check the population fluctuation trends for potential equations and interpret them mathematically, people would not accept what we are calling increases, decreases or a bell-shaped (Gaussian) distributions are indeed so. From a statistical point of view, people would argue that if still finer resolution data are generated then what is the probability that the trends we are seeing now would still remain so. To answer this it is indispensable to check out probability density functions for the trends observed; if the equations were not obtainable even after carrying out the statistical tests then the envisioned phenomenon of synchronous population fluctuation for the different anaerobic metabolic types along the perennial OMZ sediment cores would have been completely on shaky ground.

Authors' Changes in Manuscript: We have now conceptually elaborated and explained the mathematical terminologies by adding simpler descriptions.

Line 612: The discussion specifically needs to be shortened and to the point. I think it will greatly improve the manuscript.

Authors' Response: We agree, and have now considerably streamlined the discussion section as per the objectives spelt out in the Introduction

Authors' Changes in Manuscript: As stated in the authors' response.

Fig 3: See my general comments above. Is this figure necessary in the main text?

Diversity is important but with the amount of data in this study I think transcriptome results or a map showing metabolic pathways would be much better to display here.

Authors' Response: We thank the reviewer for appreciating the significance of our metatranscriptomic data.

The following is a key phenomenological finding of this study. As a characteristic feature of the perennial-OMZ, overall microbial diversity peaks in the upper sediment-layer and then declines with increasing sediment-depth; microbiome profile is reverse in the seasonal-OMZ sediment horizon (this is stated in the Abstract also). Fig. 2 depicts this phenomenon in terms of the microbiome profile (community composition) along the three cores, while Fig. 3 presents the quantitative interpretation of microbial diversity along the three cores (i.e., in terms of Simpson and Shannon–Wiener indices calculated based on the phylum-level community composition data depicted in Fig. 2). Articulation of the aforesaid phenomenon without showing Figures 2 and 3 would make the inference sound baseless and subjective.

Authors' Changes in Manuscript: We have now incorporated new figure for the visualization of the results pertaining to the metatranscriptome analyses.

Fig 5: I am not sure this is the best way to represent your data

Authors' Response: We agree that this MATLAB-derived representation of the population fluctuation trends is atypical as the data is also unusual. However, it is the only way we can comprehensively compare population fluctuation patterns across the different metabolic categories as well as the distinct sediment cores.

Authors' Changes in Manuscript: Not applicable

Fig 6: Thank you for including geochemical data!

Authors' Response: We thank the Reviewer for appreciating the cross-disciplinary depth of our study.

Authors' Changes in Manuscript: Not applicable