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# *Interactive comment on* "Cryptic role of tetrathionate in the sulfur cycle: A study from Arabian Sea sediments" *by* Subhrangshu Mandal et al.

**Subhrangshu Mandal et al.**

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Anonymous Referee #2

Referee's Comment: The study assesses the role of microbial populations in the sulfur cycle of sediments from the Indian Ocean OMZ and, in particular, the role of tetrathionate as a potentially cryptic intermediate in the inorganic sulfur cycle. The study brings together geochemical measurements of pore water concentrations of sulfur compounds, rates and concentrations of sulfur metabolism in sediment slurry incubations, isolation, phenotypiccharacterisation and genome sequencing of tetrathionate metabolising bacteria as wellas metagenomics, and transcriptomics of sediment mi-



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crobial communities.

Authors' Response: We thank the Reviewer for appreciating the phenomenon unearthed in this study.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: The key conclusions are that sediments of the Indian Ocean oxygen minimum zone are inhabited throughout the depth profile by bacteria that are able to metabolise tetrathionate in different ways (oxidation of thiosulfate to TT, reduction of TT to thiosulfate, oxidation of TT to sulfate). This is supported by abundance data based on annotation of metagenomic reads, assembly and functional annotation of metagenomes, as well as mapping of metagenome reads onto genome sequences of TT-metabolising bacteria isolated from the sediments. Furthermore, RNA sequencing of one depth horizon shows that some of these TT-metabolising bacteria appear actively transcribing genes of TT-metabolism in situ. While the diversity analysis of the sediments supports the presence of various bacteria implicated in TT metabolism, it is a pity that no 16S rRNA amplicon-based diversity analysis was carried out, since direct taxonomic annotationof reads is a relatively crude methodology. However, the key conclusions with regard to the potential role of the identified bacterial groups implicated in TT metabolism are supported even if the some of the taxonomic annotation may potentially be over simplified and crude.

Authors' Response: We thank the Reviewer again for endorsing that the diverse lines of culture-dependent and culture-independent data supported the presence and potential roles of the various bacteria implicated in tetrathionate metabolism.

As for the absence of 16S rRNA amplicon-based diversity analyses, we had already pointed out in our previous responses to the reviewers that such data for the SSK42 cores, including SSK42/5 and SSK42/6, have been published at length in our previous paper Fernandes et al., 2018, Enhanced carbon-sulfur cycling in the sediments of Arabian Sea oxygen minimum zone center (Sci. Rep. 8: 8665). In the current paper Man-





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dal et al., the genera which were identified in the various sediment-samples of SSK42/5 and SSK42/6 via taxonomic classification of protein-coding metagenomic reads were not only corroborated via manual scrutiny of the amplified 16S rRNA gene sequence-based diversity data of Fernandes et al. (2018) but also by searching the individual metagenomic sequence datasets against the 16S rRNA gene sequence database of the Ribosomal Database Project (using BlastN with minimum alignment length 50 bp, minimum identity cut-off 90% and maximum e-value cut-off 1e-5). These points are already mentioned in the existing Mandal et al. manuscript.

With respect to the Reviewer's reservations regarding direct taxonomic annotation of metagenomic reads (as being over simplified and crude), we are convinced that this approach is not fraught with major uncertainty in the present case because closely related organisms' genomes are available in the database and the parameters used in this paper to classify reads using the Best Hit Classification algorithm [BlastX search with minimum 45 nucleotides (15 amino acids) alignment and $\geq$60% identity, and maximum e-value allowed 1e–5] are stringent enough to assign taxonomic affiliation to homologs of metabolically diverse genes, irrespective of their intrinsic levels of conservation, in a reliable manner up to the genus level. This stringency level of search parameters is considered optimum across the literature because it neither exaggerates diversity not fails to resolve taxonomies for most categories of genes.

Furthermore, to add robustness to our inferences regarding the widespread distribution of tetrathionate-metabolizing bacteria across SSK42/5 and SSK42/6, we have subsequently carried out whole genome sequencing and annotation for the three tetrathionate-forming isolates, the two tetrathionate-oxidizing isolates, and the lone tetrathionate-reducing isolate. Following this, we have mapped the available metagenomic sequence data from the 25 distinct sample-sites of SSK42/5 and SSK42/6 separately onto each of the de novo sequenced genomes: remarkably, in those analyses, significant percentages of the metagenomic read-sets were found to match sequences from the individual genomes, thereby giving a clear picture of the relative abundances





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of the tetrathionate-metabolizing strains in each of the 25 distinct sediment-samples.

Authors' Changes in Manuscript: Whilst the above explanations were already there in the previously revised manuscript, we have now elaborated them a little more so as to make the narrative more clear-cut and lucid.

Referee's Comment: The work addresses an aspect of sediment sulfur cycle that has not been widely studied and therefore breaks new ground in demonstrating that TT cycling in such sediments is likely to be a significant but overlooked process, despite not presenting in situ rates of TT metabolism (all rates are based on long slurry incubations and are thus demonstrating potential).

Authors' Response: We agree that determination of in situ rates of tetrathionate formation, oxidation and reduction would have added to the robustness of the study and would attempt the same when fresh sampling is conducted again in this area. This said, we are thankful to the reviewer for appreciating the overall adequacy of the current data in demonstrating the potential roles of tetrathionate metabolisms in marine sedimentary sulfur cycle.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: The metatranscriptome data have been presented in more detail in this revised version. However, for Table S25 for instance, a relative expression based on mapping onto reference genomes is but a crude estimate/proxy for showing the involvement or in situ activity of these genes in the sediment. I would suggest to report mapping rates for housekeeping genes alongside those of sulfur metabolism and to report the taxonomic affiliation of those sulfur cycling genes identified in the assembled metatranscriptome as well.

Authors' Response: We agree and have now reported the mapping rates for representative house-keeping genes (namely those involved in transcription, translation, DNA replication, ABC-type membrane transport, phosphotransferase system, bacterial se-





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cretion systems and cell cycle) alongside those of sulfur-metabolism-related genes (please see the revised Table S25).

Taxonomic affiliations of the sulfur cycling genes identified in the assembled metatranscriptome were already given in Table S25 and Table S20.

Authors' Changes in Manuscript: Table S25 has now been upgraded by incorporating the mapping rates for a large number of housekeeping genes alongside those already there for the sulphur-metabolism-related genes.

Specific comments

Referee's Comment: The manuscript is complex due to the large number of analyses carried out, with a plethora of acronyms which makes it difficult to follow in some sections. I would suggest that any effort to make it more readable and digestible would be well expended. As itis currently, it is difficult to read and follow.

Authors' Response: We agree that the manuscript is complex due to the large number of distinct analyses carried out, so have now appended a separate key for all the acronyms and tried to use full-forms in the text itself wherever the same did not hamper reading of the sentence. Overall, we have again overhauled the entire text by fragmenting complex sentences into simple ones, removing extraneous details, and making the language lucid.

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

Referee's Comment: A schematic overview of relevant pathways of TT metabolism would be beneficial for context and should be presented in the introduction.

Authors' Response: We agree with your concerns, so during the previous revision had already added an overview of tetrathionate-metabolism pathways in the Introduction (this included all the enzymes and genes which are known to be instrumental in the formation and transformation of tetrathionate). A schematic illustration of these pathways, in the context of the present data, is also there in Figure 6.





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Authors' Changes in Manuscript: In the current re-revised manuscript (Discussion section) we have now added a few, previously-missed, information on the potential abiotic mechanisms of tetrathionate transformation.

Referee's Comment: Were abiotic control incubations carried out with slurries (poisoned, autoclaved) that would account for chemical conversion of thiosulfate, tetrathionate and sulfate? If so, how high were chemical conversion rates?

Authors' Response: We apologize for forgetting to include these control data in the previous manuscript amidst the complex web of results and arguments. Indeed, abiotic control incubations involving autoclaved sediment-samples were always carried out alongside the slurry incubation experiments and abiotic chemical-conversion rates for thiosulfate to tetrathionate, tetrathionate to sulfate and tetrathionate to thiosulfate/sulfide, for all the sediment-samples were found to be negligible.

Authors' Changes in Manuscript: We have now added these data to the re-revised manuscript.

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