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Interactive comment

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

# **Anonymous Referee #2**

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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 porewater concentrations of sulfur compounds, rates and concentrations of sulfur metabolism in sediment slurry incubations, isolation, phenotypic characterisation and genome sequencing of tetrathionate metabolising bacteria as well as metagenomics, and transcriptomics of sediment microbial communities.

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 tetrathion-

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ate 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 annotation of 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 oversimplified and crude.

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).

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.

### Specific comments

- The manuscript is complex due to the large number of analyses carried out, with a

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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 it is currently, it is difficult to read and follow.

- A schematic overview of relevant pathways of TT metabolism would be beneficial for context and should be presented in the introduction.
- 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?

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