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

Anonymous Referee #3

Received and published: 2 August 2019

The manuscript describes the analysis of inorganic sulfur compound cycling microbial populations in two sediment cores from the Indian Ocean. Particular focus is on populations driving the metabolism of thiosulfate and tetrathionate (thiosulfate reducing/tetrathionate forming, tetrathionate reducing, and tetrathionate oxidising groups). The study used a range of geochemical measurements, slurry incubations, microbiology (isolation of sulfur cycling microorganisms and assessment of their capabilities to transform thiosulfate and tetrathionate) as well as molecular biological approaches (metagenomics and metatranscriptomics).

The biogeochemistry of sulfur compounds in sediments is a complex web of chemical

and biological transformations, there is a need to better understand the role of individual species of inorganic sulfur compounds as well as the metabolic pathways and microbial groups involved in their transformations. As such, this is a topic of high interest, especially if, as the title implies, some of the transformations may be of a cryptic (not easily identified) nature.

My overarching impression of the manuscript is that it is not easy to follow the story and that it would benefit from revising the structure. It is lacking a clear approach to the analysis and presentation of the data. Even starting in the introduction, I would suggest that, given the focus on the various enzymes being instrumental in the transformations of thiosulfate and tetrathionate, the introduction should provide a brief overview of the most important enzymes involved (and their encoding genes) and perhaps contain a schematic conceptual overview illustrating the most important points.

It would be beneficial and aid readability, if a clear overview of the basic findings was shown perhaps as depth profiles showing key chemical parameters of the cores under investigation.

With a view of the diversity and metagenomics analysis, I have two key criticisms: (i) revolving around the specific use of metagenomics read data for taxonomic assignment and (ii) extrapolating from that assignment to physiological properties of entire genera of bacteria. In that context, I have to say that I think it is a pity the authors did not carry out a diversity analysis of the sediment samples based on pooled 16S rRNA amplicon sequencing in parallel to the metagenomics/metatranscriptomics, because the ribosomal RNA gene survey would provide a much better and more robust diversity analysis than the assignment of taxonomy based on random metagenomic reads. Although a taxonomic assignment of a random metagenomic read is possible, it is fraught with major uncertainty, unless a closely related organism's genome is available in a database. As that is not the case for the vast majority of microorganisms found in nature at present, the taxonomic assignment of metagenomic reads is a bound to provide unreliable/unresolved taxonomies and lead to poor estimates of the abundance of

specific types of bacteria. This affects data shown in Tables S8-13 as well as Fig 1 and 2.

My second criticism is the assumption in the paper that entire genera of bacteria always share specific physiological capacities with respect to the sulfur transformations of interest. While this is true for some genera (and a parameter used in systematics), for many genera this is not necessarily the case. Therefore, suggesting that genus A or B are tetrathionate producing bacteria or -oxidising bacteria, will likely overestimate the abundance of that specific metabolic type based on that assumption.

On the other hand, the metagenomics data can reveal the abundance of specific types of genes, as has been done here, and potentially identify the types of bacteria potentially contributing to the cycling of specific compounds. Too much of the discussion of inorganic sulfur metabolism in these sediments is based on the broad assumption of taxonomy, and too little is made of the specific genes found and listed in various supplementary tables. There are still limitations of our understanding of the genetics of sulfur transformations and it would be useful to perhaps illustrate whether the enzymes/genes driving specific transformations of sulfur compounds in some of the taxa mentioned (eg Salmonella) have actually been identified. Very little is done with the metatranscriptome data, it only gets a few mentions, but there is no clear overview of what has been found in which layer, how many reads were analysed and generally which bacteria were transcriptionally active with respect to sulfur cycling.

Regarding the transformations measured in slurry experiments, there needs to be a more complete reporting of the activities measured (or not) in all sediment samples. This should be shown comprehensively, not as currently done in Tables S14-S21, which suggest that only a few subsamples had certain activities. I am also not convinced that a 30-day incubation period of the slurries, some incubating sediments from a completely anoxic system under aerobic conditions (!), is providing the sort of activity data that would be supportive of suggesting that these key biological reactions are linked up in a cryptic cycle. The mentioning of alternative sources of oxygen from cryptic sources

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such as perchlorate is pointing to an unpublished study by the same authors. If this is crucial for the understanding of the functioning of this system and the microbiological activities required for the cryptic cycling of these compounds, these aspects should be incorporated here or the other study needs to be published. Alternatively, it is possible that the activities are due to facultative anaerobic bacteria in these sediments that have reverted back to an aerobic lifestyle given suitable incubation conditions and a 30-day period to wake up.

Specific comments

Introduction: The introduction would benefit from a description of relevant metabolic pathways and enzymes targeted by the analysis of this paper

Line 73: define mbsl

Line 75 what was the diameter of these cores?

Line 82: for ease of reading I suggest to always refer to both cores with the full abbreviation, not SSK42/5 and 6 but SSK42/5 and SSK42/6

Line 82: I find the description of the N2 shower lacking in detail and find it hard to understand how it would keep the exposed core adequately protected from oxygen.

Line 88: scraped

Line 145 define cmbsf , how thick was that sediment layer?

Line 147 and 190: pooling up, no 'up'

Line 213: kmer lenghts of . . .

Line 227: text string search?

Line 236/7: not every read would represent one of these functional groups. Please reword/revise or explain more clearly what you did. There are no 'genera' of this that and the other, they are genera that contain species some of which have certain physi-

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ological characteristics, but not necessarily all of them

Line 246 following: No context for why one would assess the aerobic metabolism of these compounds with samples from 275cm below the sediment surface where there is no oxygen.

Line 309: actually described below

Line 327: reword, genes do not have these activities, they encode enzymes that transform the compounds

Line 350: I do not think that all of marine Pseudomonas and Halomonas do that

Table S1 Please clarify what is meant with 1st or 2nd sample fraction

Table S4 Not a single DoxA encoding gene was identified in any of the samples. Should it perhaps not be listed in Table S4 accordingly? Please state what the significance of the yellow highlighting is in this and the other excel based supplementary tables

Table S6 Define what is meant with prevalence and how it is quantified Unclear what is meant with correlation of metabolic type with sediment depth, when depth is not quantified here

Tables S8 to S11 should have totals for the abundance of all types per depth

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