Response to reviewer #2

Review of Slomp et al. A niche for diverse cable bacteria in continental margin sediments overlain by oxygen-deficient waters.

The manuscript of Slomp et al. describes a study where activity and abundance of Cabel bacteria were addressed in sediments from 5 hypoxic basins along the coast of Mexico and California, together with a multitude of geochemical features (O_2, pH, Fe, Mn, Nox, Al) and microbial populations. In general cable bacteria abundance were low and activity were below detection limit. Phylogenetic analysis revealed that the Cable bacteria belonged to the Candidatus Electrotrix lineage and included specimens affiliated with *Electrotrix gigas*. In addition several sequences were affiliated with a sister clade to Electrotrix and it is suggested that these represent a novel genius.

In general, the manuscript adds novel information about the biogeography and diversity of cable bacteria in marine environments and has therefore merits justifying publication.

Reply: We thank the reviewer for the positive words

I recommend however that the authors put somewhat more effort in discussing the vast amount of geochemical data and provide the motivation behind the analysis these features. There are severall fractions of solidphase and dissolved compounds that is not (to my knowledge)really linked to cable bacteria (e.g. Al, Mn, NH4): Why is this relevant in the given context?; What was the hypothesis?

Reply: Indeed, we present a wealth of geochemical data. These data are relevant to understanding the factors controlling the electron acceptor and donor availability for cable bacteria in this setting (i.e. the availability of oxygen, nitrate and sulfide). Regarding the elements mentioned: the dynamics of Fe and Mn are closely linked to those of sulfide and oxygen. Aluminum is used to normalize Mn and Fe to assess for dilution of reactive Fe and Mn by detrital components. Checking for such dilution is standard in geochemistry. Fe/Al ratios are valuable as a redox proxy. Porewater NH_4 is an excellent indicator of the rate of degradation of organic matter – more so than the organic carbon records, which just reflect the proportion of the organic matter that has not degraded yet. We also note that geochemical records can carry signatures of cable bacteria activity (Risgaard-Petersen et al., 2012), i.e. we know that cable bacteria can impact Mn, Fe and S dynamics. We will add text in the methods and discussion to further clarify the points above.

In addition the motivation behind the analysis of the general microbial community should be stated more clear. What is the relevance of this analysis for the target features: cable bacteria?

Thank you for your question and the opportunity to elaborate. By analyzing cable bacteria together with the broader microbial community, we gain a better understanding of the factors governing cable bacteria distribution and activity, providing a more comprehensive understanding of their biogeochemical role. We report on the microbial community composition in our results and supplemental material and discuss the composition in some detail (section 4.3), because it provides insight into the biogeochemical functioning of the study sites, which sets up the context for interpreting the niche of Electrothrix. The microbial community composition, characterized by highly structured beta diversity patterns (Fig. 6), and displayed with taxonomic resolution in the supplemental material, enables inferences about the metabolic processes occurring across our sampling sites. These biogeochemical conditions effectively define the niche parameters for the cable bacteria. By combining the microbial community data with the comprehensive biogeochemical measurements, we can formulate testable hypotheses regarding the physiological capabilities and adaptations of cable bacteria in these poorly understood environments. We will add text in the manuscript to make this more explicit.

In my view these data should be more integrated in the overall framework of the study:

Would it e.g. be possible to apply e.g. network analysis to identify specific cablebacteria – microbe associations?

Reply: Unfortunately, this dataset is not well-suited to network analysis due to the sample number and sparsity of the cable bacteria. Liu et al. 2021 (ISME) was able to successfully examine the networks of cable bacteria (in their system), but this was from a laboratory experiment with a total of 60 samples. In our case, this approach would not have sufficient replication to be robust.

Minor:

L 32: remove the Nielsen et al . 2010 reference. This paper says nothing about cable bacteria. The authors suggest nanowires or conductive minerals as mediators of the electric currents that runs through the sediment (exactly like in the Revil et al. (2010) paper!) The discovery of cable bacteria was published in 2012. i.e. two years after the Nielsen et al. paper.

Reply: We will make the change as suggested.

L 34: Remove the reference Nielsen and Risgaard-Petersen 2015 paper: This is a review paper that amongst other review the Risgaard-Petersen et al. 2012 paper on how cable bacteria influence the biogeochemistry.

Reply: We will make the change as suggested.

L 49. The Damgaard et al 2014 paper describes the construction and application of the silver silver chloride electrode used for electric potential measurements. The focus in this paper is not relationships between cable bacteria activity and the electric potential. Such is more thoroughly described in Risgaard-Petersen et al. (2012) and in Risgaard-Petersen et al. (2014). Since both papers precedes the Damgaard et al. paper I suggest that that the authors include those here instead of the Damgaard et al. paper.

Reply: We will make the change as suggested.

L 133. Please spell out "EP": Electric potential.

Reply: EP is spelled out at its first occurrence in line 115 (original manuscript).

L150: What sediment volume was collected for FISH: 0.5 ml?? Please specify.

Reply: We will add that this was a volume of 0.5 mL in the text.

L 195. The method description for quantitative FISH analysis is a bit odd. What is the rational for not using direct methods like those described in e.g. Schauer et al. (2014)?. Why use Nycodens extractions? There is to my experience a great risk of loosing cells with this method. Do the authors have any data on the cell recovery efficacy ?

Reply: The density gradient methods described have been used in several previous field and laboratory studies investigating cable bacteria (e.g, Malkin et al. 2022, Liau et al. 2022). Trojan et al. 2016 (AEM), is the first study we are aware of that used this method with Nycodenz, based on the procedure published by Kallmeyer et al. (2008). Trojan et al. 2016 specifically used this method on samples they obtained from Aarhus Bay. In our revision, we will add that reference to the text. Our rationale was based on our laboratory experience: we have found that we generally achieve the same or higher counts with this method, over the less labor-intensive method of simply adding sediment to a slide. This is effective presumably because it removes particles that impede the view of cable bacteria. We note that a critical step is that we collect the supernatant on a filter after each wash, minimizing cell loss between washing steps. Although that information is stated in Malkin et al. 2022, and Kallmeyer et al. 2008, we will repeat that information in this manuscript for clarity.

It apereas that Sybr Green I staining were used for Cable bacteria quantification : Why this step when you have FISH stained filaments already?

Reply: Thank you for this comment. We indeed did not additionally use Sybr Green. All samples examined for this study were FISH-stained and we will alter this in the text.

References in replies above: please see the original manuscript.

References

Revil, A., Mendonca, C.A., Atekwana, E.A., Kulessa, B., Hubbard, S.S., Bohlen, K.J., 2010. Understanding biogeobatteries: Where geophysics meets microbiology. J. Geophys. Res. (Biogeosci.) 115, G00G02.

Risgaard-Petersen, N., Damgaard, L.R., Revil, A., Nielsen, L.P., 2014. Mapping electron sources and sinks in a marine biogeobattery. J. Geophys. Res. (Biogeosci.) 119 1475–1486.

Risgaard-Petersen , N., Revil, A., Meister, P., Nielsen, L.P., 2012. Sulfur, iron-, and calcium cycling associated with natural electric currents running through marine sediment. Geochimica Et Cosmochimica Acta 92, 1-13.

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