

Reviewer #1

General comments:

In this manuscript, Slomp et al. initially described the continental margin sediments (>100 m deep) of hypo- to anoxic nitrogenous basins as a niche for the development of cable bacteria (CB). Then, they estimated the abundance and diversity within the *Candidatus* Electrothrix lineage to provide an overview of the recently described diversity of this group, and to suggest a new genus adapted to these environmental conditions. The manuscript is fairly well structured, and its objectives are clear. The methodological approaches are comprehensive and aim to characterise the geochemical conditions of the bottom waters, the sedimentary compartment (pore-water and solid phases) and the microbial community concerned. The data sets are freely accessible on Zenodo. Despite the wide range and quality of the data, it is a snapshot in time, which limits the scope of the discussion on the factors controlling the CB dynamics in this potential niche. Overall, I think this is a robust and very interesting research paper which corresponds to the scope of Biogeosciences.

We thank the reviewer for the positive words.

Specific comments:

Line 104: you present bibliographical data on *Thioploca* in the Soledad basin, but nothing on the other groups presented in the introduction (*Beggiatoaceae* and *Thiomargarita*). Is there any information available on this subject in these basins? Similarly, in line 108, you present (admittedly old) data on the meiofauna and macrofauna of this same basin, but you say nothing about the other basins? Have no data been published?

Reply: We would be happy to include such data. However, to our knowledge there are no published studies on *Beggiatoaceae* or *Thiomargarita* for these five basins. The same holds for studies of the meiofauna and macrofauna in the basins other than Soledad.

Lines 133-135: only one O₂ profile was done in San Clemente? How were the pH and H₂S measurements set up to achieve complete microprofiling to a depth of over 3 cm in less than 15 minutes? I assume that the waiting and measurement times are particularly short. Is this relevant for this type of measurement?

Reply: Indeed, only one O₂ profile was collected in San Clemente basin. The measurement times for the pH and H₂S profiles were kept as short as possible to limit the potential for changes in the profiles following core collection. We will provide more information on this in the revised manuscript.

Line 160: one point I don't understand about the analysis of dissolved metals: why didn't you use the pore water Mn data from Bruggmann et al. (2023), as you did for Fe, in order to have the same vertical resolution for both? The vertical resolution in Bruggmann et al. (2023) is low and I find it relevant that you have carried out higher resolution analyses for Mn. But in that case, why didn't you do the same for Fe to get geochemistry within the same core?

Reply: Our study focuses on characterizing the geochemical environment of the cable bacteria in these sediments. This implies that the high resolution porewater data collected through centrifugation as presented in our manuscript are more relevant than the low-resolution profiles collected at the same sites (but from different multicores) through rhizon sampling by Bruggmann et al. (2023). Unfortunately, we do not have Fe data from the ICP-OES for our samples. This is why we chose to include the Bruggmann et al. (2023) Fe data. The main significance of the Fe porewater data is that they show evidence for Fe reduction throughout most of the profile, which is relevant since this is what would be expected in the absence of free sulfide in the porewater. Given that the Bruggmann et al. (2023) Mn profiles generally show similar depth trends and contrasts between stations as our high-resolution data, we do not see the added value of adding those data. The Mn data also are only provided as supporting data here and are not key to the discussion. We will modify the methods section and caption of figure 3 to indicate that the samples of Bruggmann et al. (2023) are from rhizon samplers and have a lower depth resolution.

Section 4.1: the densities observed in these hypo-anoxic basins are like those observed *in situ* on estuarine intertidal mudflats (oxygenated environment + sulphides) where CB could be particularly active (Daviray et al., 2024), or in the rhizosphere of aquatic plants (Scholz et al., 2019, 2021).

Reply: We are aware of the reviewer's excellent work on cable bacteria in estuarine intertidal mudflats (Daviray et al., 2024) and that of Scholz et al. (2019; 2021) on cable bacteria in the rhizosphere of plants. We prefer not to include references to the cable bacteria abundances in those studies since those sites are very different from the type of sites studied here. We will add in the text that we are comparing our cable bacteria to "submerged coastal sediments".

The presence of CB (DNA data) but the absence of activity also raises a hypothesis that is not discussed here: could it be the result of CB-enriched sediment transport into the basins? Do you have any information on the marine currents affecting these locations? In this case, it could be better to talk about a 'potential' niche.

Reply: There is no information on the potential occurrence of cable bacteria in the near-coastal regions adjacent to these basins. Importantly, however, these basins are characterized by generally low rates of detrital sediment input (e.g. van Geen et al., 2003) and, due to their offshore position, low rates of sedimentation overall (Table 1). If at Soledad basin, the site with the highest cable bacteria abundance and sedimentation rate, the cable bacteria had been transported long-distance through the water column (there is no evidence that that is possible over such long distances, even though it can happen over short distances; van Dijk et al., 2024), you would not expect the abundance to be as high as observed *in-situ* at sites like in the Baltic Sea because of dilution and degradation of the cable bacteria, also during long-term burial.

Furthermore, Soledad basin is approximately 85 km long and 35 km wide and has a flat bottom (Silverberg et al., 2004). Our samples were taken far away from the slopes. There are no rivers in the vicinity of the basin and there is no evidence for major lateral input of sediment material to this basin, also not from prior work. Silverberg et al. (2004), for example, attributed variations in input of lithogenic material in sediment traps in Soledad basin to variations in aeolian dust from the continent – other inputs were considered negligible. Van Geen (2003) and others have shown

that the sediment records from Soledad basin are highly suitable for detailed palaeoceanographic reconstructions. They also highlighted the similarity of many sediment records from different locations in Soledad basin, indicating lack of sediment heterogeneity, again confirming a limited role for lateral sediment input. To address the reviewer's point, we will expand the site description and we will mention in the text that lateral transfer of cable bacteria is unlikely to explain our results.

Section 4.2: The discussion of the sources of H₂S and its temporal dynamics is stimulating. However, it remains hypothetical and suffers from a lack of (temporal) data, in my opinion. Have you tested correlations between the various parameters (i.e., C_{org}, total S, Fe sulphides, etc.) to perhaps highlight this dynamic and support a periodic (seasonal?) increase in sulphate reduction?

Reply: The temporal dynamics in organic matter input in the basins along the Californian and Mexican margin is firmly grounded in prior work for this region. This was noted in the original manuscript with appropriate references (For example: "Seasonal and interannual variability of primary productivity and, hence, of organic matter supply to the sediment is a common feature of the basins along the Californian and Mexican margin (Thunell, 1998; Silverberg et al., 2004; Collins et al., 2011)." And "Increased coastal upwelling in the region in winter and spring not only enhances primary productivity but also can lead to periodic, sometimes even seasonal inflows of oxygen into the basins, as reported for the Santa Barbara basin (e.g. Bograd et al., 2002; Peng et al., 2024)". We will expand the information on the oceanographic setting in both the methods and the discussion section.

We will include that variations in input of organic matter on both seasonal and interannual time scales have been demonstrated with sediment trap studies (Silverberg, 2004). Generally, offshore Ekman transport and associated coastal upwelling are strongest in the winter and spring, with upwelling becoming less strong in summer and fall, but still supporting a high primary productivity (Tems and Tappa, 2024). Given the relatively shallow water depth at the Soledad and San Blas sites, these basins will be most affected by changes in the input of organic matter to the sediment. Paleo-oceanographic records confirm the variability of productivity in this setting (van Geen et al., 2003; Tems and Tappa, 2024).

Non-steady state features are evident from the C_{org} and total S profiles, as noted in the original manuscript. We will expand the corresponding section. Unfortunately, further correlation analysis is not expected to provide useful information when applied to non-steady state diagenesis at sites with a low rate of sedimentation when there is temporal variability – as is the case here. This is because of diagenetic overprinting. As noted in the manuscript, we do see a link between average Fe/S ratios and cable bacteria densities, indicating that, in basins where there is a higher supply of S relative to Fe, we see more cable bacteria. This supports a role for periodic enhanced sulfate reduction. We will expand this section to clarify this.

Lines 434-447: in my opinion, this paragraph lacks any link with the biogeochemical data to explain the diversity observed.

Reply: In this section of the text, we provide details on the metabolic pathways of micro-organisms in the sediments at our sites that directly link with the data on, for example, the

presence or absence of oxygen and ammonium since these are substrates for the microbes that we are discussing. We will expand the text to clarify this.

Line 441: what electron acceptor do the Bacteroidota use?

Reply: Bacteroidota defy simple characterization in terms of their electron acceptor use, with members capable of aerobic respiration, various anaerobic respiration pathways, as well as fermentation. We will add in the text that they have a broad metabolic capability.

Line 453: it's a shame that we don't have this data, as it would have helped to underpin the discussion on interspecific competition.

Reply: We agree. This should be a target for further research.

Line 461: any suggestions on these factors (bioturbation or others generating sediment heterogeneity, Fe curtain, etc.)?

Reply: We will modify the text to clarify that with “different factors” we were referring to other factors other than oxygen availability, i.e. we were referring to the preceding sentence in which we were discussing published work for the Santa Barbara basin. We have also added a sentence indicating that bioturbation is expected to hinder both cable bacteria and *Thioploca*. It is not clear to us what “Fe curtain” refers to in this context. The sediment heterogeneity in Soledad Basin is not high, see reply to an earlier comment above.

Lines 475-476: out of curiosity, do you have any idea what these benefits might be? The same for the selective pressures mentioned line 489. The section 4.4 is very interesting and frustrating: we want to know more!

Reply: Based on the line numbers, we infer that this statement refers to the co-existence of multiple species (Line 475), and the phenomenon of gigantism (paragraph starting Line 477). Regarding co-existence of multiple species, we agree this is intriguing and worthy of further investigation. Regarding the occurrence of gigantism in cable bacteria, we are uncertain if there are selective pressures driven by top-down forces (e.g., predation) or bottom-up forces (e.g. resource acquisition). Indeed, we would also like to know more and with the current text we are providing direction for future studies.

Section 4.5: this section could perhaps be further summarised and incorporated into the second paragraph of the conclusion.

Reply: While we appreciate the reviewer's comment, we prefer to keep this as an outlook section since we are introducing additional points of discussion and additional references and suggestions for further research that are not appropriate in a conclusion section.

Figures:

Figure 1: the blue colour contrast is poor. Would it be possible to improve it like in Bruggmann et al., 2023?

Reply: For reference and easy comparison, we repeat the Bruggmann et al. 2023 figure (top) and our figure (bottom) below. While we appreciate the reviewer's comment, we do not see the benefit of replacing our figure with that of Bruggmann et al.. The figure would not provide additional insight in the bathymetry relevant to the relatively shallow sites of our study and in fact would lead to less information since the water depth differences between the stations are less well visible and the figure lacks a legend. We also note that our color scheme is the ODV standard and cannot be changed. Detailed information on the water depths is given in Table 1. We will add this information in the figure caption.

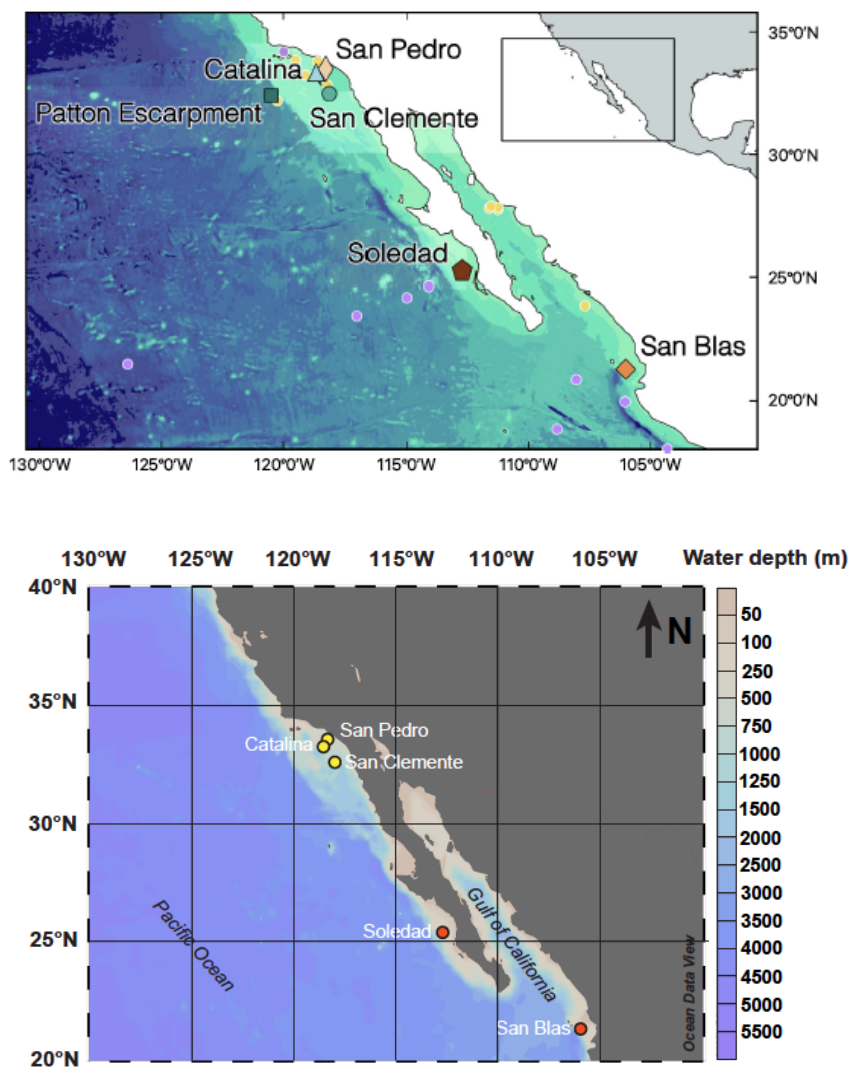


Figure 2: you write that triplicates were achieved for pH and H₂S μ profiles. Is the absence of standard deviation on these profiles justified by their high reproducibility? I suppose so for H₂S (because there isn't any), but what about pH?

Reply: Thank you for this comment. We indeed report only the first profile and will remove the mention of triplicates for pH and H₂S from the manuscript. While these triplicates were taken, movements of the ship interfered with the measurements after the first profile at each location, hence these were considered unreliable.

Figures 2, 3 and 4: please, put the unit of the vertical axis in cm.

Reply: We will change “cmbsf”, which refers to “cm below seafloor”, by “cm” as suggested.

References: please see below and in the original manuscript

Daviray, M., Geslin, E., Risgaard-Petersen, N., Scholz, V. V., Fouet, M., and Metzger, E.: Potential impacts of cable bacteria activity on hard-shelled benthic foraminifera: implications for their interpretation as bioindicators or paleoproxies, *Biogeosciences*, 21, 911–928, <https://doi.org/10.5194/bg-21-911-2024>, 2024.

Scholz VV, Muller H, Koren K, Nielsen LP, Meckenstock RU. 2019. The rhizosphere of aquatic plants is a habitat for cable bacteria. *FEMS Microbiology Ecology* 95: fiz062.

Scholz, V.V., Martin, B.C., Meyer, R., Schramm, A., Fraser, M.W., Nielsen, L.P., Kendrick, G.A., Risgaard-Petersen, N., Burdorf, L.D.W. and Marshall, I.P.G. (2021), Cable bacteria at oxygen-releasing roots of aquatic plants: a widespread and diverse plant–microbe association. *New Phytol*, 232: 2138-2151. <https://doi.org/10.1111/nph.17415>

Tems, C.E.; Tappa, E. Regional Fluctuations in the Eastern Tropical North Pacific Oxygen Minimum Zone during the Late Holocene. *Oceans* 2024, 5, 352–367.

van Dijk, J.R., Geelhoed, J.S., Ley, P., Hidalgo-Martinez, S., Portillo-Estrada, M., Verbruggen, E. et al. Cable bacteria colonise new sediment environments through water column dispersal. *Environmental Microbiology*, 26(10), e16694, 2024