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Interactive comment on "Biogeochemical Impact of Cable Bacteria on Coastal Black Sea Sediment" *by* Martijn Hermans et al.

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The manuscript represents a very comprehensive study of potential processes and effects of cable bacteria in sediments. Investigations on cable bacteria and their influence on biogeochemical processes are still in the beginning, but more and more studies show their importance for the element cycling; importance of cable bacteria activity on the oxygen demand in coastal sediments. In the present study, the authors used sediment cores from the coastal area of the Black Sea, which they homogenized and freed from macrofauna. This probably increased the availability of labile organic material and its distribution in deeper sediment layers. Furthermore, the sediment was anoxically stored until the experiment, during the experiment the overlying bottom water was saturated with oxygen so that a steady state must be established at the beginning

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of the incubations. This fact does not reduce the results of the experiment or the quality of the manuscript.

Reply: We thank Anonymous Referee #2 for reviewing our paper and the insightful and constructive feedback. Please find our replies to each comment below.

Comment #1 However, the authors should consider the study presented here as potential processes and not directly related to a coastal region (in this case the Black Sea). Therefore, I would strongly suggest to rewrite the manuscript and change the focus of the manuscript by concentrating on the "potential processes and bio geochemical impacts" rather than to directly relate it to coastal sediments of the Black Sea.

Reply: We are aware that the outcomes from our experiment are potential processes, which cannot be directly translated to the field site. This is why, in our title we specifically used the term "on coastal Black Sea sediment" instead of "in coastal Black Sea sediments". Other examples of sentences in our manuscript that indicate that we are not directly relating our results to a coastal region are:

1) Line No. 100: "In this study, we assess whether cable bacteria activity can establish in sediments that are relatively poor in FeS in an incubation experiment using siderite-bearing sediments from a coastal site in the Black Sea."

2) Line No. 552: "We can only speculate about the possible in-situ relevance of cable bacteria at the coastal site in the western Black Sea where the sediment for our incubation was collected."

3) Line No. 565-566: "Further field studies are required to assess the role of cable bacteria at our field site, preferably including an assessment of the burrow structures."

We will add the following additional text in the abstract, introduction and conclusion sections to further emphasise that we are referring to potential processes.

Abstract: "to determine the potential impact of their activity on the cycling of Fe, phosphorus (P) and sulphur (S)."

Introduction: “In this study, we assess whether cable bacteria activity can establish and thrive in sediments that are relatively poor in FeS. Although, this will be done in a controlled incubation experiment with siderite-bearing sediments from a coastal site in the Black Sea, our findings are relevant for natural environments populated by cable bacteria.”

Conclusion: “The results of our laboratory incubation (with a total duration of 621 days) show that cable bacteria can potentially strongly impact the Fe, Mn, P and S dynamics in coastal sediments. The strong acidity of the pore water associated with the activity of cable bacteria, which was monitored using microsensor profiling of the EP during the experiment, led to dissolution of FeS and siderite and formation of Fe and Mn oxides and Ca-P in mineral form near the sediment surface. Our experimental results provide conclusive evidence for siderite dissolution driven by cable bacteria activity as a source of Fe that can form an Fe oxide-enriched surface layer.”

Comment #2 The difference between the natural distribution of cable bacteria and the experiment is also evident when looking at Fig. 1c. The authors can use their main results as shown here, but the focus should be on the conditions used in their experiment, which are rather artificial, but very nicely show the potential of cable bacteria in the biogeochemical cycling.

Reply: See our reply to comment #1 and the associated changes in the text. We are aware that the outcomes of our experiment regarding the impact of cable bacteria are amplified when compared to field conditions and that we cannot directly link this to the field site. This is because we optimized conditions to sustain metabolic activity of cable bacteria, and their growth:

1) The sediment was homogenised, which is known to induce growth of cable bacteria.

2) There was no bioturbation by meiofauna and macrofauna.

3)The bottom water was continuously oxygenated.

This optimisation was deliberately done to study the effects of cable bacteria on sediment biogeochemistry. We note that the approach used is common in studies of the biogeochemical impact of cable bacteria (e.g. Risgaard-Petersen et al. 2012; Rao et al. 2016).

Comment #3 In a second step the transfer to coastal sediments and their biogeochemical conditions can be done. Here the manuscript lacks the coherence (hypoxia and oxy-gen depletion as mentioned in the Introduction). In a final paragraph the transfer of the laboratory experiment to natural sediments and possible variations in biogeochemical processes as well as the influence of macrofauna (bioturbation and bioirrigation) can be discussed.

Reply: Sections 4.1 to 4.4 focus only on the experiment. In sections 4.5 and 4.6 we discuss the implications for the field. We will add text to section 4.5 and 4.6 to clarify when we are referring to other laboratory experiments and results of field studies and the link with hypoxia.

1) Line 507-509: "This colour zonation is typical for sediments that have been geochemically affected by cable bacteria activity, as seen both in laboratory experiments (Nielsen and Risgaard-Petersen 2015) and at coastal field sites (Sulu-Gambari et al. 2016)"

2) Line 546-548: "may act as an additional sediment marker for present or recent cable bacteria activity, both in laboratory experiments and at field sites, also in cases where visual observations are not conclusive."

3) Line 548: "Macrofaunal activity within natural environments likely counteracts or prevents strong focusing of Fe oxides and associated P within such a thin subsurface layer."

4) Line 553-554: "At this site, which is in a region that is subject to seasonal hypoxia (Capet et al. 2013), both bivalves (up to ∼7200 ind. m-2) and polychaetes (up to

∼1700 ind. m-2). . .”

Comment #4 Is there any information about the organic carbon content of the sediment and how this changes over the incubation period? I would assume that this is the major driver for the development of biogeochemical zonation.

Reply: We now include the organic carbon content of the upper 0-10 cm of the sediment at the field site, as determined by Lenstra et al. (2019), in Table 1. We did not determine the change in organic carbon contents during the experiment because, at the typical rates of organic matter degradation expected here, only a small change in organic carbon content would be observed. Hence, we chose to focus on pore water NH4+ profiles to obtain insight in rates of (anaerobic) organic matter degradation.

Comment #5 How does the development of the oxic zone, as shown in the experiment, relate to natural variations in coastal sediments ?

Reply: The range in O2 penetration observed in the experiment is comparable to that observed in coastal systems with seasonal hypoxia (e.g. Seitaj et al. 2015). We will include this in the manuscript: Line No. 514-516: “During the experiment, O2 penetration varied within a narrow range and was initially fixed between 1 and 2 mm depth (Fig. 3A), with the layer highly enriched in Fe forming mostly at a depth of 2 mm (Fig. 8A). Such a range in O2 penetration is in accordance with observations in coastal sediments (e.g. Seitaj et al. 2015). The formation of the Fe-enriched layer can be explained by. . .”

Comment #6 How does the experiment relate to the development of hypoxia and depletion of oxygen in coastal areas ? The experiment shows the opposite reaction (from anoxic surface layer to an oxygenated layer).

Reply: As shown in previous work that we refer to in our manuscript (Seitaj et al. 2015; Sulu-Gambari et al. 2016), cable bacteria can induce formation of Fe and Mn oxides in seasonally hypoxic coastal systems during periods of oxygenated bottom waters in

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spring. As explained in line No. 41-44, the presence of these Fe and Mn oxides can delay the transition towards euxinia by removing hydrogen sulfide. Our experimental setting can be compared to the onset of bottom water re-oxygenation in spring in such seasonally hypoxic environments (i.e. the sediment was stored anoxically, and then exposed to oxygenated water). This allows study of the mineral dissolution and formation reactions in sediments populated by cable bacteria under such conditions, which is the goal of this work.

Comment #7 line2 121/122: .... with overlying water ....Was this bottom water taken from the site or artificial water, as used for the aquarium?

Reply: The 20 cm long cores (filled with 15 cm sediment) were gently submerged in the two aquaria. Hence, the overlying water in the cores at the start of each incubation was the same as the artificial water used in the aquaria.

Comment #8 line 153: .... core was place outside the aquarium .....Why was the core taken out ? was the incubation temperature maintained?

Reply: The entire experiment was carried out at in a temperature controlled laboratory at 20 °C. We will make explicit that the flux incubations also took place at 20 °C: "At each time point, one core was placed outside the aquarium at 20 °C..."

Comment #9 Was the overlying water during the 24-hour incubation for the solute flux measurements stirred to avoid stratification? This could have influenced the flux across the sediment-water interface because stagnant waters lead to an increase of the Diffusive Boundary Layer, which controls the solute exchange.

Reply: The overlying water was mixed continuously by bubbling it continuously with air. The airflow was set in such a way that the water was effectively mixed, while the surface layer of the sediment was left undisturbed. We will make this more explicit in the methods: "At each time point, one core was placed outside the aquarium at 20 °C, and the isolated volume of overlying water in the core was continuously aerated. Potential stratification of the overlying water was prevented by actively bubbling it. Parafilm was wrapped on top of the cores to prevent evaporation."

Comment #10 Pore water profiles (especially Fig 1a, Fig 2) are very small and it is difficult to recognize the different profiles (O2, pH, H2S) different; graphs should be enlarged.

Reply: We enlarged these graphs and we increased the font sizes to improve the readability of the figures.

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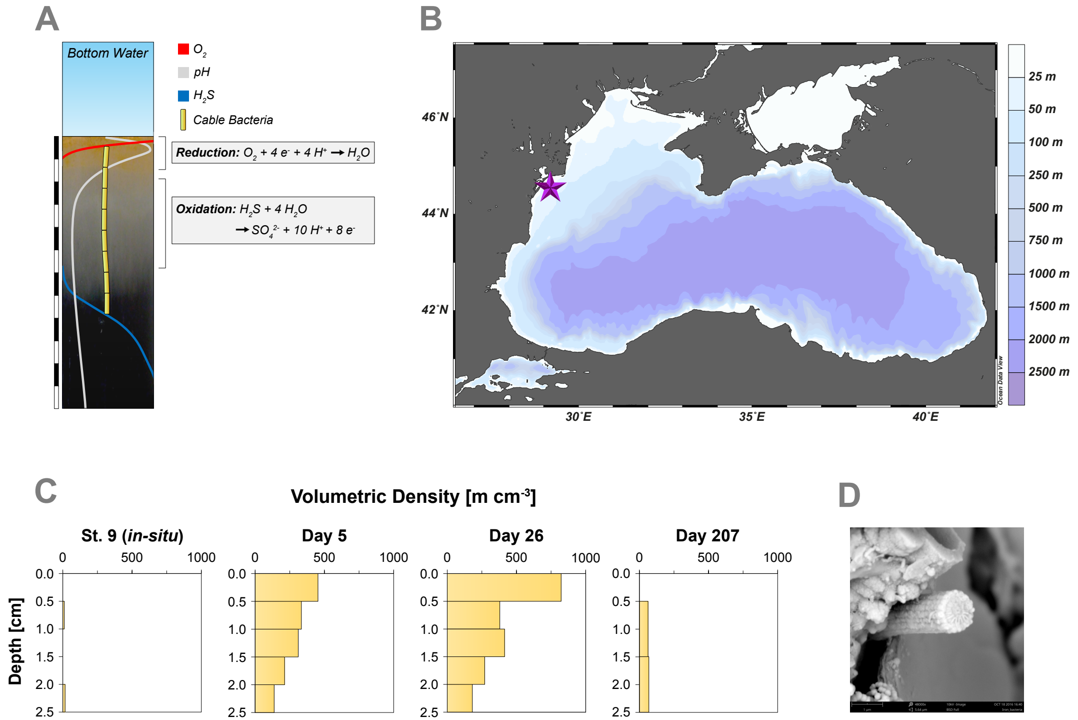

Fig. 1. Figure 1

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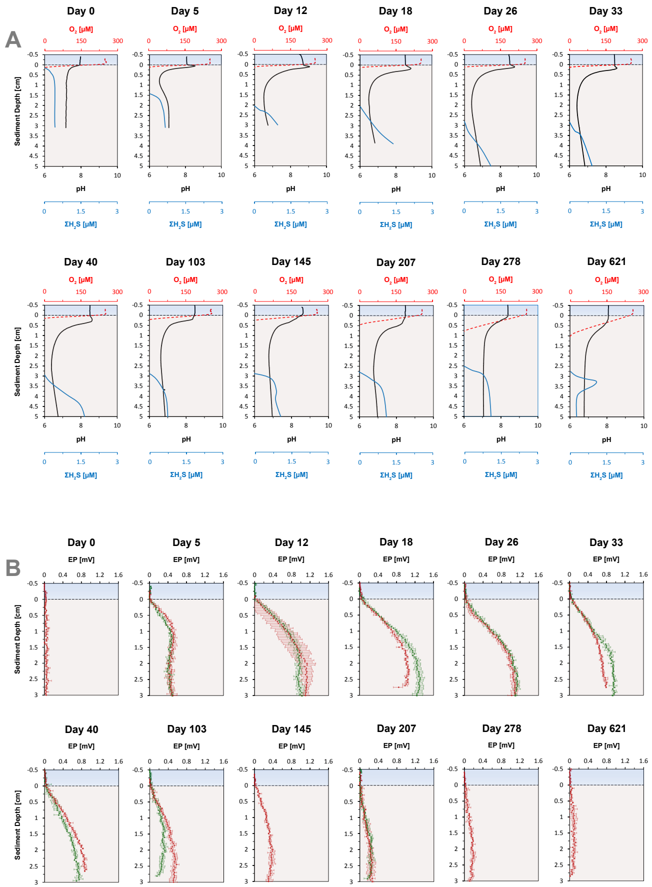

Fig. 2. Figure 2

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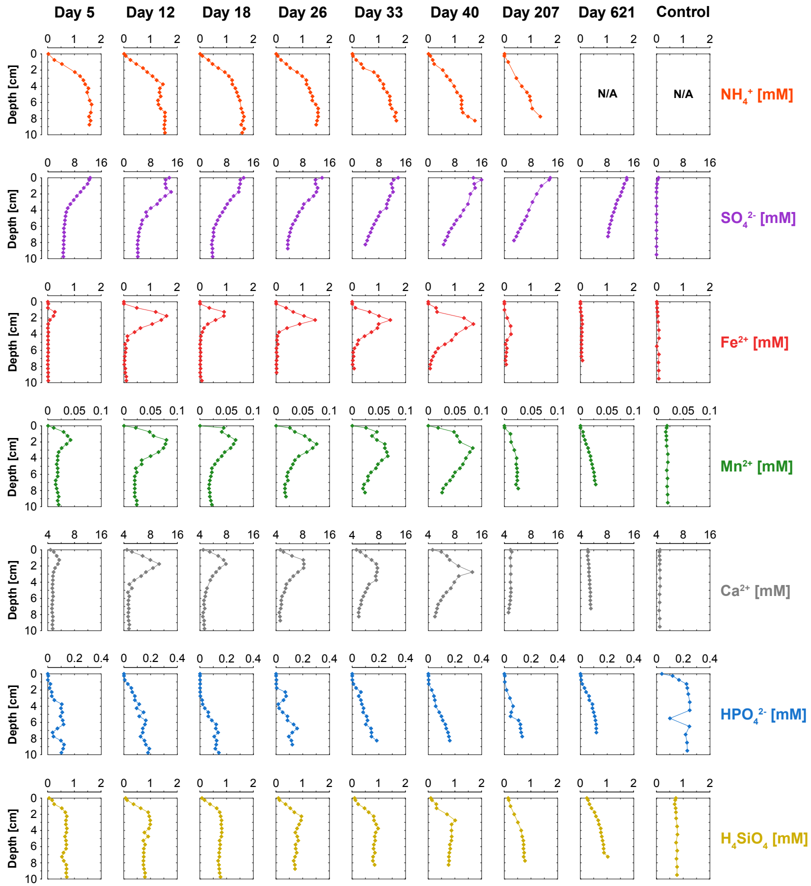

Fig. 3. Figure 5

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