

Interactive comment on “Evidence for microbial iron reduction in the methanogenic sediments of the oligotrophic SE Mediterranean continental shelf” by Hanni Vigderovich et al.

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Response to comments from anonymous reviewer #1 (our response is below each comment)

This manuscript reports the first evidence for microbial iron reduction in a methanogenic zone in an oligotrophic shallow marine environment. The subject matter is within the scope of BG. The title is appropriate, the abstract is concise and complete, and the paper is well structured. Below I highlight comments in order to further strengthen the manuscript.

C1

We thank the reviewer for the positive and constructive review. We addressed and accepted almost all comments (see below) and revised the manuscript accordingly.

1. More information is needed about the rationale for the incubation experimental design. There is no mention in the methods of how the killed-control incubations were “killed”. Was Fe added to the incubations? Was this before or after “killing”? How are the authors certain that the killing mechanism did not change the Fe(III) mineralogy? Please also clarify why hematite and magnetite were added instead of the more commonly used ferrihydrite and goethite; would these forms be more environmentally relevant in the methanogenic zone? Please state explicitly with citations if that is the rationale. Please provide citations for hydrogen utilization as an electron donor for hematite and magnetite reduction to justify this experiment.

We agree with the reviewer that additional details regarding the incubation experimental design were needed. The revised manuscript includes the specific information regarding the “killed” bottles (sediment killing via autoclave) and the addition of the hematite/magnetite to those bottles (after autoclaving). We believe the mineralogy of the Fe(III) that we added was not changed in the killed control since we added it after the autoclave stage when the killed bottles were already cooled down. The rationale of adding hematite and magnetite is: a. These minerals are expected to survive the sulfate zone (amorphous iron or goethite are expected to be used first) and to remain in the methanogenic zone. These minerals were indeed found in our methanogenic sediments (see the supplementary figure). b. These were the minerals that were reactive in Fe-AOM in our previous work in lake sediments (Bar-Or et al., 2017) and seeps (Sivan et al., 2014). c. Magnetite is a conductive mineral, and has been shown to be preferred in some microbial processes due to this characteristic (e.g. Cruz et al., 2014; Tang et al., 2016; Rotaru et al., 2018). d. As these were the tested minerals, we added also H₂, a known electron donor for iron reduction, to these experiments. H₂ was shown also to reduce magnetite by *S. putrefaciens* (Kostka and Nealson, 1995). All of the above is explained and clarified in the revised version.

C2

2. The data for the Fe extracts seem to be missing. The methods state (lines 171-172): “The different reactive iron oxides were separated to (1) carbonate associated Fe; (2) easily reducible oxides; (3) reducible oxides and (4) magnetite.” Yet the only data for iron speciation has all four of these clumped together. It would be much more informative to know the depth profiles of the four individual species instead of the sum. Why wasn't this reported?

We decided it would be more relevant to show the whole amount of reactive Fe(III) in the sediments in order to show generally how much reactive Fe(III) minerals are in our sediments, considering that all four types of oxides can act as a potential electron acceptor. Nevertheless, we agree that it would be more informative to show the depth profiles of the four separately. Thus, In the revised manuscript we present a different figure containing all four types of Fe oxides.

Specific comments:

1. Line 86-90: also shown in anoxic ferruginous lake sediment enrichments, Bray et al 2018 *Geobiology* “Shifting microbial communities sustain multiyear iron reduction and methanogenesis in ferruginous sediment incubation”

We thank the reviewer for this comment, and we will add the citation (Bray et al., 2016).

2. Line 248: therefore *are* not discussed. . . Line 333: regardless *of* the area's.

We accept the reviewer's correction; and the revised manuscript is corrected.

3. Line 342: “The H₂ levels at stations SG-1 and PC-3 (Fig. 1) are relatively high (Lilley et al., 1982; Novelli et al., 1987), suggesting that there is enough H₂ to sustain the iron reduction process.” Need to clarify that H₂ is two orders of magnitude higher at SG-1 vs. PG-3 and implications of that finding in relation to other findings. More text is needed explaining the references. Are these cited to show that H₂ has been historically high at the site? Need a reference for the second half of the statement about the H₂ concentrations needed for reduction of these Fe(III) minerals.

C3

We thank the reviewer for pointing out that this paragraph needs elaboration. We believe that the H₂ concentrations at PC-3 station is higher than at SG-1 station because the gas front is shallower at SG-1, which causes metabolic processes to be more intense and less amounts of H₂ can accumulate in the sediments. The text is revised to clarify this subject. The cited papers are there to show that the known H₂ concentrations in marine environments are lower than what we found in both stations. Reference for the second part of the sentence was added.

4. Line 351-357: any evidence for or against this hypothesis in this study?

Cryptic sulfur cycle is observed more and more in marine sediments (e.g. Holmkvist et al., 2011; Brunner et al., 2016). It seems that this cycle is possible here based on the microbial populations that contain those that may be involved in sulfur cycling (from 16S analysis). Also, pyrite was found in the methanogenic zone. We add and clarify this point in the revised version.

5. Line 396: positive or negative correlation? Need to cite that supp material where this data is shown.

We agree with the reviewer, the type of correlation and the cite to the supplementary material should have been mentioned. It is a positive correlation and is mentioned now in the revised manuscript.

6. Figure 1: Add zeroes before the decimal place on the x-axis. Increase the interval if it doesn't fit as is.

We accept the reviewer's comment, and the figure in the revised manuscript is adjusted accordingly.

7. Figure 2 and Figure S1: Move these to be included in Fig 1. Would enable easier comparison to have all the data together and will fit on a full-page graph oriented horizontally on page. Please add zones 1, 2, 3 like in Fig 1 to data in Figure 2 and Figure S1. Could move PG-5 depth profiles to supplementary.

C4

We accept the reviewer's comment, and the figure in the revised manuscript is adjusted accordingly.

8. Figure 3: This color scheme and stretched out horizontal lines with white gaps in between is extremely hard to look at. Please fix to make it easier to see.

We accept the reviewer's comment, and the figure in the revised manuscript is adjusted accordingly.

9. Figure 5: I don't see a clear inverse relationship like the figure caption states. I think you can say that there is more variability in methane at low Fe(II) and that methane is low for the few data points at the highest Fe(II).

We agree with this comment and change the term "inverse correlation" to this more accurate sentence in the revised version.

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