

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

Anonymous Referee #2

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This manuscript presents pore-water data (S, CH₄, Fe²⁺, H₂, d¹³C-DIC), results of incubation experiments as well as data on the abundance and diversity of bacteria and archaea in sediments of the South Eastern Mediterranean continental shelf. Besides a typical zone of organoclastic iron reduction observed close to the sediment surface the authors report a second zone of enhanced Fe²⁺ pore-water concentrations within the methanic sediments below the sulfate/methane transition. Evidence for iron reduction in methanic subsurface sediments is commonly found in high accumulation continental shelf and margin sediments and a strong research interest currently exists in elucidating which (bio)geochemical pathways and potential microbial organisms mediate this “deep” iron reduction.

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In this respect, the paper focusses on an important and topical research question and is in principle suitable for Biogeosciences. However, I regret to say that the manuscript has numerous flaws and appears as if it has not been prepared with the required care. The manuscript thus needs a major overhaul before I can recommend publication. The English also requires quite some polishing and I would suggest to ask an English native speaker to proofread the manuscript. There are numerous typos (which I have not all corrected in detail) and the wording is imprecise in many places – all this needs careful checking and correction.

Several issues that need to be considered when preparing a revised version:

- 1) The most important point is that the discussion is not adequate as it stands, several assumptions are not supported by the data and many key publications have not been cited. Often statements occur in the form of single sentences without “really” discussing the data obtained in the framework this study.
- 2) It is not clear to me which novel findings your study contributes to the topic of deep iron reduction. This needs to be outlined precisely.
- 3) Please provide a map that shows the study area and the three sampling locations and a table that summarizes the dates, exact positions, precise names etc. of the samples used in this study.
- 4) Please, also add a table that gives the details of the sequential extractions performed in this study.
- 5) Referencing is not adequate – i.e. several relevant papers are missing. I have listed some publications below but a careful literature search should be performed.
- 6) Please, precisely distinguish between and separate Results and Discussion. The Results chapter already contains a lot of interpretation/discussion and several references, which is formally incorrect.

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Line 2 and throughout the manuscript: I do not like the term “methanogenic” very much because it implies that methane formation occurs in the respective sediment layer/interval. Based on your considerations on page 3 (lines 102 ff. and lines 112 ff.) concerning the current oligotrophic conditions in the study area as well as the deeper gas front detected based on seismic profiling, I suggest that it is likely that methane is diffusing/migrating up from deeper layers into the sediment depths investigated in this study. I would thus propose to speak of “methanic” sediments, which is more neutral.

L. 20: What exactly do you mean by “mechanistic” nature?

L. 25: delete “cores”; in the deeper methanic zone

L. 27: Do you mean Fe²⁺ concentrations in pore water?

L. 37: Li et al. (2012) is only one of a vast amount of literature on this topic – you may add a few other papers. So change to (e.g. Li et al., 2012; Riedinger et al., 2017 (Frontiers in Earth Science); März et al., 2018 (Mar. Geol.).

L. 45: What exactly do you mean with “outward” diffusing methane? This is not clear to me. Please, specify.

L. 47: Key papers on sulfate-mediated AOM are missing here: please add at least Hinrichs et al. (1999) and Boetius et al. (2000). . . . it should then read: (e.g. Hoehler et al., 1994; Hinrichs et al., 1999; Boetius et al., 2000) and you may of course add further papers.

L. 49: Also here Valentine (2002) is only one example of a vast amount of literature on this topic. You may also wish to cite Niewöhner et al. (1998), GCA, here.

L. 51: Has to be iron “reduction” (instead of oxidation)

Ls. 58/59: Please, give the respective references.

L. 60: Please rephrase to: . . . incubation of marine seep sediment . . .

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Ls. 61 ff.: Please also cite the following papers in this context: März et al. (2008), Oni et al. (2015), Egger et al. (2018), who have also presented evidence for Fe-coupled AOM in marine, coastal, and brackish sediments.

Ls. 68 ff.: Please, also cite Oni et al. (2015) here who have presented microbial studies for the methanic zone of North Sea sediments.

Ls. 74 ff.: This sentence is hard to follow and sounds a bit odd. Please, rephrase.

L. 79: I would not speak of “inactive” in this context but rather of “of low reactivity”. Furthermore, I do not find it surprising that reactive iron oxides are preserved and present below the SMT. This finding has already been explained by several studies/papers – amongst others by Riedinger et al. (2005), GCA, März et al. (2008), Mar. Geol., and März et al. (2018), Mar. Geol.

L. 87 ff.: You may also wish to cite Oni et al. (2015) here.

L. 92: What exactly do you mean with “reactivate” in this context? This is not clear to me – please specify. Were the Fe oxides “unreactive” before? By which process/condition have they been “reactivated”?

L. 97: What precisely is a “basic” incubation experiment?

L. 99: Please, rephrase to: . . . possible links between the cycling of iron and methane”.

L. 102: I find it hard to imagine that the Levantine Basin is really one of the most oligotrophic marine settings in the world. I thought that globally the most oligotrophic ocean area is the South Pacific Gyre?! Please, check carefully and rephrase accordingly.

L. 109: I do not believe that the TOC contents are/were really “zero”. I think this is an issue of the detection limit of the specific analytical method used. Please check.

Ls. 111 ff.: I do not understand the argumentation in this sentence. How can you conclude that methane found in shallow sediments is of biogenic origin if a deep gas front has been detected by seismics? Are you sure that the methane found in the shallo

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sediments investigated here really formed in situ. I guess it is much more plausible – I particular given the current oligotrophic conditions and low TOC contents discussed above – that methane has migrated up from deeper sources.

Ls. 114 ff.: Also the argumentation in this sentence is odd. Even if waters are anoxic they almost always have the typical marine sulfate concentration of 28-30 mmol/l. Thus, anoxia does not necessarily lead to sulfate reduction.

Ls. 120 ff: The cores were sampled during cruises of R.V. Shikmona ...

Ls. 122 ff.; This sentence sounds odd. Please, rephrase.

L. 132: ... the “stable carbon” isotopic composition ... explain the abbreviation DIC

L. 134: “at” -20°C

L. 136: The wording in this sentence is a bit odd. Do you mean that the surface sediment has been lost during sampling (which is usually the case during gravity or piston coring)?

L. 137: Does it mean that you have sub-sampled the box corer by means of push cores? If yes, please say so.

L. 139: Does it mean that you have determined methane both in pore-water as well as sediment samples? How precisely and how have the pore water and solid phase been separated?

Ls. 141 and 289: Some details of how precisely these incubation experiments have been performed are missing. How were the respective experiments/bottles killed? Did you use molybdate to inhibit sulfate reduction?

L. 143: Refer to the respective figure with pore-water profiles here.

L. 145: anoxic instead of anaerobic

L. 147: anoxically instead of anaerobically

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L. 151: You are talking about mineral contents here – so the unit (mmol L⁻¹) is not correct.

L. 152: In line 146 you have stated that incubations lasted for 3 months. Here you speak about 14 days?!

L. 161: It has to be “total sulfur” instead of sulfate. Sulfate can’t be measured by ICP-AES

L. 162: has to be “inductively” and Perkin “Elmer”

At this point I stopped to correct typos and odd wording – there are just too many.

Ls. 16 ff.: a pore-water profile can’t be “performed”; please also state which parameters have been analysed and in which figures they are shown; what do you mean with “and not their average”? This is absolutely unclear to me.

Ls. 170 ff.: I would suggest to insert a table, which gives the details of the extraction used – including reagents, solid-phase/reagent ratios, shaking times, etc.; please, also state whether the extractions has been performed on dry or wet sediment samples; if you used wet samples, how has porosity been determined? By the way, carbonate-associated Fe is not an “iron oxide” as stated at the beginning of this sentence.

Ls. 202 ff.: Again: pore-water profiles can’t be performed. Please, rephrase.

Ls. 204 ff.: As also stated above you have not determined sulfate but total sulfur. So, rephrase accordingly and also correct this in Fig. 1 and throughout the manuscript.

L. 207: increase “with depth”

Ls. 207 ff.: I do not fully understand this sentence. Moreover, part of this sentence is interpretation/discussion and should thus not be part of the Results chapter.

Ls. 215 ff.: Large parts of this is discussion/interpretation.

Ls. 229: I found this sentence confusing because from the chapter 2.2 “Sampling” it

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was not clear to me that the sites have been sampled three times. Please clarify and give a table summarizing the dates, exact positions, precise names etc. of the samples used in this study. What is the “Aug-13 core”? Where is it shown in Fig. 1? A legend and/or respective explanations in the figure caption are missing

L. 238: Why are deviating points not discussed?

Ls. 248 ff.: I can't find Fig. S1; solid-phase values are “contents” (not concentrations)

Ls. 257 ff.: A lot of this is already interpretation/discussion. Moreover, papers should not be cited in the Results chapter.

L. 303: Which station precisely do you refer to here? “at this station”? How do you know that intensive methanogenesis occurs in the respective sediment layer? Due to the fact that TOC contents in the shallow sediments are low and free gas is detected in deeper layers, I would rather suggest that methane is migrating up from the deeper subsurface. Please discuss and consider this carefully.

Ls. 305 ff.; This sentence needs to be rephrased.

Ls. 314 ff. and 331 ff.: As already stated above I do not agree that methanogenesis necessarily occurs in the respective sediment zone. To me it seems more likely that methane has migrated up from deeper layers.

Ls. 317, 351 and throughout the manuscript: What do you mean with iron oxide “reactivation”? This is odd.

Ls. 334 ff.: I do not understand at all how the findings link or relate to the Last Glacial Maximum?! How can the current environmental conditions be attributed to the Last Glacial Maximum or Mid-Pleistocene? You need to much more carefully discuss this.

L. 339: anoxic instead of anaerobic

Ls. 346 ff.: This has not been described in the respective methods chapter.

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Ls. 351 ff.: And how does all of this relate to your data?

Ls. 358 ff.: Numerous papers that have discussed and presented evidence for Fe-mediated AOM in natural aquatic sediments have not been cited here.

Ls. 363 ff.: I would not overinterpret methane concentrations, which have been determined ex situ because methane typically suffers from strong degassing during core retrieval.

Ls. 412-415: These two sentences more or less say the same.

From the discussion, as it is presented, it is not clear to me at all which novel findings your study and data contribute to the discussion on and research topic of potential drivers of deep iron reduction.

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