April 2019

"Evidence for microbial iron reduction in the methanogenic sediments of the oligotrophic SE Mediterranean continental shelf" by Vigderovich et al.

Response to comments from anonymous reviewer #2 (our response in blue):

This manuscript presents pore-water data (S, CH_4, Fe^{2+}, H_2, $d^{13}C$-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 Fe^{2+} 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.

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 need careful checking and correction.

We thank the reviewer for the thorough and constructive review. We addressed and accepted all comments (see below) and revised the manuscript accordingly. In addition, we have edited and proofread the English.

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.

We accept this comment. In the revised version we have extended and strengthened the discussion (see below in the specific comments the clarifications and added calculations). We carefully analyzed the data, clarifying what is indicated directly and is supported by other publications (also listed below), and what is speculative.

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.

In the revised version we present and clarify the novel aspects of this paper:

a. **Combining** geochemical profiles, microbial profiles and incubation experiments to show evidence for microbial iron reduction in the deep methanic zone and the potential microbial population performing this reduction.
b. Showing that this deep iron reduction can occur even in sediments of oligotrophic seas, such as the **oligotrophic** SE Mediterranean. We suggest that the availability of iron minerals for reduction is linked to an intensive methane cycle (see below, addressing the comment on L. 111).

In the revised version we emphasize the connection between the deep iron reduction and the methane cycle more clearly.

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.

A map and a table were added to the revised manuscript.

4) Please, also add a table that gives the details of the sequential extractions performed in this study.

A table with the sequential extractions details was added to the revised manuscript.

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.

We thank the reviewer for this list. We added those references and several more. The following references were added (see below the specific places):

Boetius et al., 2000; Egger et al., 2017; Emerson et al., 1980; Hinrichs et al., 1999; Hoehler et al., 1994; Iversen and Jorgensen, 1985; Knittel and Boetius, 2009; Li et al., 2012; Lovley 1991; März et al., 2018; Milkov and Sassen, 2002; Milkov, 2004; Moutin and Raimbault, 2002; Niewöhner et al., 1998; Oni et al., 2015; Orphan et al., 2001; Paull et al., 2008; Riedinger et al., 2017; Wurgaft et al., 2019; Zhang and Lanoil, 2004.

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.

We accept this comment, and the results and discussion sections were properly separated, the data was presented first and then discussed. We also moved the references to the discussion section.

Specific comments:

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.

We completely agree with the reviewer that some of the methane in the pore-water originates from deeper sediments. This is indeed an important factor in this system that was discussed in our previous studies (which did not focus on iron - Sela-Adler et al., 2015; Wurgaft et al., 2019). We clarified this point in the revised version and included flux calculations (see below, addressing the comment on L. 111). As suggested, we rephrased the term to "methanic".

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

The microbial link between the iron and the methane cycles in marine sediments, either by competition between methanogens and iron reducing bacteria due to environmental

conditions, methanogens switching from methanogenesis to iron reduction metabolism or iron driven AOM. We explained this in the revised version.

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

Deleted.

L. 27: Do you mean Fe^{2+} concentrations in pore water?

Yes. Specified in the revised manuscript.

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.).

We added all the relevant references. In addition to the ones above, we cited Egger et al., 2016; Ettwig et al., 2016; Sivan et al., 2014; Slomp et al., 2013. These references support the fact that Fe(III) minerals have a key role in the biogeochemical cycles of carbon, sulfur, phosphorous and nitrogen.

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

The term infers that the methane diffuses away from the methanic zone to the SMTZ or deep layers. In the revised manuscript this was clarified.

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.

As we do not focus on sulfate-mediated AOM, we did not include most works on this topic. However, we agree with the reviewer that at least the key works should be included. We added thus these references, as well as Orphan et al., 2001; Knittle and Boetius 2009.

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.

Niewöhner et al. (1998) work from the west African margin was added.

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

Corrected.

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

The reference was added (Lovley 1991)

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

The sentence was rephrased as suggested.

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.

We accept this comment and have added these references (Marz et al. 2008; Oni et al., 2015; Egger et al., 2017) here as evidence for deep iron reduction.

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

Added, but in line 62 (in the original version), since the original line 68 is about freshwater sediments and Oni et al. (2015) studied the North Sea sediments. The original line 62 was rephrased to: "It was suggested through the modeling of geochemical profiles in deep sea sediments (Sivan et al., 2007; Marz et al., 2008; Riedinger et al., 2014), in microbial studies of marine sediments (Oni et al., 2015)…"

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

The sentence was rephrased to: "Whereas Fe(II) is highly soluble, Fe(III) that is the most abundant specie of iron under natural conditions, appears as low solubility minerals."

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.

The term "inactive" was changed to "of low reactivity" as suggested. We accept the comment and removed this word.

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

This sentence focuses on methanogenesis inhibition by iron reduction, and thus this reference was not added here. It can be found in other places in the revised manuscript (see above).

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"?

We infer that the iron oxides, which were not reduced in the upper sedimentary column by bacteria or archaea, are reduced in the deeper sediments, even though there is less energy for redox reactions. This suggests that there is some advantage at these depths that allows their reduction. Several processes may explain this reactivation: 1) Iron reducing bacteria succeed in outcompeting methanogens due to environmental changes, 2) the methanogens themselves switch to iron reduction due to some advantages (electron shuttling such methanophenazines?), or/and 3) the methane that is produced is more available for reduction than other organic substrates (Fe-mediated AOM). We clarified this point better in the revised version.

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

We infer a fundamental incubation experiment. We removed the word basic and rephrased it in the revised version to: " We show both geochemical pore-water profiles and microbial investigation at three different stations combined with a simple incubation experiment with slurry…"

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

Changed as suggested.

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.

To the best of our knowledge, the Levantine basin is considered an ultra-oligotrophic marine system. For example, Thingstad et al. (Science, 2005) discussed the phosphorus imitation in the "Ultraoligotrophic Eastern Mediterranean", as well as several other studies, which ranked the Mediterranean basin as oligotrophic to ultraoligotrophic based on nutrients, chlorophyll a and PP pools (Krom et al., 1991; Antoine et al., 1995; Siokou-Frangou et al., 2010; Kress et al., 2014; references in Herut et al., 2016 and more). However, we rephrased the sentence to: "The Levantine Basin of the SE Mediterranean Sea is an oligotrophic nutrient-poor marine system (Kress and Herut, 2001)."

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.

Indeed, a typo mistake. We corrected the sentence: "… the Levantine Basin have low TOC levels of ~1% (~0.5 – 1.4%; Sela-Adler et al., 2015; Astrahan et al., 2017)."

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 shallow 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.

As written above, we agree that some of the methane has migrated from deeper sources, at least in Station SG-1. However, our results indicate that part of the methane is also produced *in-situ* in the methanic zone (zone 3) based on our geochemical profiles mainly of $\delta^{13}C_{CH4}$ and $\delta^{13}C_{DIC}$ (Sela-Adler et al., 2015), and the mcrA profile (presented here). The geochemical profiles show the transition from sulfate reduction to methanogenesis, a clear SMTZ, very low carbon isotopic value of the methane (between -80 and -100‰) and classical "*in-situ*" diffusive $\delta^{13}C_{DIC}$ profiles with the significant increase in the isotopic values below the SMTZ in the methanogenic zone. The microbial profile shows that the mcrA gene copy number increases with depth and peaks below the SMTZ. All fits to *in-situ* biogenic methane production in zone 3, in addition to some migration. We clarified this in the text, writing clearly the two sources of methane and their supporting evidence.

As mentioned above, we discussed the migration of methane in our previous studies. In Wurgaft et al. (2019) that focused on sulfate reduction rates in the SMTZ based on alkalinity and DIC profiles, we wrote: "The similarity between sulfate reduction rates in the ultra-oligotrophic Southeastern Mediterranean and these eutrophic regions suggests that "external" methane, which is not the product of degradation of organic material originating in the water column but rather derives from deeper deposits, provides an important source of reducing power to the SMTZ. Such deep methane deposits and upward fluxes are common in many continental margins (e.g. (Milkov and Sassen, 2002; Milkov, 2004; Paull et al., 2008; Zhang and Lanoil, 2004))".

We agree with the reviewer that this source may explain our results of the low TOC. In the revised manuscript we suggested and clarified that this source of methane leads to intensive sulfate-mediated AOM in the SMTZ, and that this intensive process and

biomass may serve as additional substrate that “fuels” the deeper zone, activating the iron-oxides. We added to the text the following part with the calculation of the biomass that is produced from this source: “The importance of methane flux as a carbon source that supports the deep microbial community in the sediments of the SE Mediterranean can be inferred by comparing the organic carbon flux from the photic zone, with the flux of organic carbon that is oxidized by sulfate in the pore water. Using sediment traps, Moutin and Raimbault (2002) estimated an export flux of 7.4±6.3 mg C m^{-2} d^{-1}, which leaves the photic zone there. However, Wurgaft et al. (2019) estimated that the flux of DIC entering the SMTZ from sulfate reduction is equivalent to 8±3 mg C m^{-2} d^{-1}. While the difference between the two fluxes is statistically insignificant, it should be noted that the flux of organic material that survives aerobic oxidation in the water column and the upper part of the sediment column, as well as anaerobic oxidation by other electron acceptors with higher energy yield (Emerson et al., 1980; Froelich et al., 1979), is likely to be substantially smaller than the flux measured by Moutin and Raimbault (2002). Therefore, it is unlikely that export flux from the photic zone constitutes the sole source of carbon to the SMTZ. Wurgaft et al. (2019) suggested that methane originating from deep sediments and migrating upwards in the pore-fluids provides an important source of carbon to the SMTZ in SG-1. Methane sources of such are common along continental margins sediments (e.g. Milkov and Sasson, 2002; Milkov, 2994; Paull et al., 2008; Zhang and Lanoil, 2004). Here, we suggest that the supply of methane leads to intensive sulfate-mediated AOM in the SMTZ, and that this process produces(??) biomass which may serve as additional substrate. (New sentence) that “fuels” the deeper zone, activating the iron-oxides.”

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.

We agree with the reviewer and clarified the sentence at the beginning of the study site section: " The bottom seawater across the continental shelf is well oxygenated and sulfate concentration in the water-sediment interface is ~30 mmol L^{-1} (Sela-Adler et al., 2015)."

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

Corrected as suggested.

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

We rephrased this sentence to: "These stations were previously investigated for other purposes…"

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

DIC- dissolved inorganic carbon. This abbreviation is explained in L 39.

L. 134: “at” -20◦C

Corrected.

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)?

We refer to the uppermost sediment of the piston core, which is indeed usually mixed with the top seawater entrapped between the surface sediment and the piston. To

avoid any disorder in the surface sediment, we have used a box corer sub-sampled by Perspex push cores for the top ~30 cm sediments. We revised therefore the sentence to: “The uppermost sediments were collected using a 0.0625 m^2 box corer (Ocean Instruments BX 700 AI). Two ~30 cm sediment cores were sub-sampled using Perspex tubes during the September 2015 and January 2017 cruises."

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

Revised, see above.

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?

We have measured the methane from the total wet sediment, by transferring the sediment sample immediately to a crimped bottle with 5 mL of NaOH and flushed with nitrogen. Then measured the methane in the headspace. We explained it in the revised version.

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?

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). Molybdate was not used in the experiment.

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

We agree with the comment and the figure (Fig 1) was referred in the revised MS.

L. 145: anoxic instead of anaerobic

Changed.

L. 147: anoxically instead of anaerobically

Changed.

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

We changed the units of the mineral content to grams in the revised MS and the final Fe(III) concentrations in mmol L^{-1} units in brackets.

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

The sediment was incubated only with synthetic sea water without sulfate (in a 1:1 sediment:water ratio) for three months prior to the experiment. The experiment then began with the division of the slurry to the 60 mL bottles, the addition of more synthetic water (final sediment:water ratio of 1:3) and some manipulations (addition of iron oxides and H_2 to some treatments). In the revised MS we clarified this point better.

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

Correct, ICP-AES measures total sulfur. Since sulfide was not detected in the samples (by Cline method) and these are marine samples, we assume that "total sulfur" here is actually sulfate. However, we changed the title to total sulfur and clarify its meaning in the revised version.

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.

Corrected.

Ls. 166 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.

The word "performed" was changed to "produced" in this context throughout the text. We agree with the reviewer that the other term is unclear, and it was removed.

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.

We thank the reviewer for the suggestion, in the revised version a detailed table was added with the specifics of the extractions. The extractions were conducted on dry sediment. In addition, the word "oxides" was changed to "minerals".

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

Rephrased to "produced".

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.

The reviewer is correct, ICP-AES measures total sulfur. We clarify this in the revised version as mentioned above.

L. 207: increase “with depth”

Corrected.

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.

We agree with the reviewer that the sentence is not clear, we also agree with the other comment and moved it to the discussion chapter. The sentence was rephrased to: "The maximum methane concentration was approximately 10 mmol L^{-1} at ~140 cm depth…"

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

We agree and moved it to the Discussion chapter.

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

We agree with the reviewer that the study sites sampling time was not clear in the previous version. The stations SG-1 and PC-3 were sampled three times each during different cruises and station PC-5 was sampled once. The text was clarified and a table with the specifics was added.

L. 238: Why are deviating points not discussed?

The few deviations are of only one data point each, and are probably due to an analytical error during the measurement/sampling process. We clarify it in the revised version.

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

Figure S1 can be found in the supplementary material, perhaps there was an error and the reviewer did not receive the file?

The word "concentrations" was changed to "content".

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

We agree, and moved part of it to the discussion, as well as the references.

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.

We are referring to station SG-1. This is clarified in the revised version. We agree that some of the methane migrated up from deeper subsurface (see above). We rephrased the sentence to: "At station SG-1 methane reaches higher concentrations, which leads to intensive methane oxidation by sulfate at the SMTZ…"

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

We agree and rephrased it to: "…causing it to occur at shallower depth and produce lower $\delta^{13}C_{DIC}$ values than the other two stations, as observed in previous studies (e.g. Sivan et al., 2007)."

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.

As mentioned above, we now refer to the two sources.

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

Please see above.

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.

We removed this sentence. The hypothetical environmental conditions are discussed by Sela-Adler et al., 2015 and Schattner et al., 2012, while not directly linked to this study.

L. 339: anoxic instead of anaerobic

Corrected.

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

The reviewer is correct, the matter is elaborated in the revised version in the methods chapter: "One mL of H_2 was added by gas tight syringe to two bottles with addition of hematite and two bottles with addition of magnetite (to final concentration of ~4% of the Head space volume)."

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

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 (Wurgaft et al., 2019). We clarify this point in the revised version.

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

As mentioned above, in the revised version we include the main literature on Fe-AOM.

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.

We agree with the reviewer and rephrased this sentence which now emphasizes just the general trend: " In our profiles AOM could be a valid option. As can be inferred from figure 5, some association was observed between the dissolved Fe(II) concentrations in zone 3 and the methane concentrations. It seems that at high concentrations of Fe(II), methane concentrations are low and vice versa."

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

We agree with the reviewer that the two sentences sound similar, however the first sentence is the key sentence of the paragraph, and the following three sentences are listing the main results of the study.

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.

Please see above.

Evidence for microbial iron reduction in the methan~~ogen~~ic sediments of the oligotrophic SE Mediterranean continental shelf

Hanni Vigderovich[1], Lewen Liang[2], Barak Herut[3], Fengping Wang[2], Eyal Wurgaft[1,4], Maxim Rubin-Blum[3] and Orit Sivan[1]

[1]The Department of Geological and Environmental Sciences, Ben-Gurion University of the Negev, Beer-Sheva, 8410501, Israel.
[2] School of Life Sciences and Biotechnology, Shanghai JiaoTong University, Shanghai, 200240, P.R.China.
[3] Israel Oceanographic and Limnological Research, Haifa, 31080, Israel.
[4]Currently: The Department of Marine Chemistry and Biochemistry, Woods-Hole Oceanographic Institution, Woods-Hole, USA
Correspondence to: Orit Sivan (oritsi@bgu.ac.il)

Abstract. Dissimilatory iron reduction is probably one of the ~~earliest~~oldest types of metabolisms~~, which~~ that still participate~~s~~ in important biogeochemical cycles, such as the carbon and sulfur cycles. It is one of the more energetically favorable anaerobic microbial respiration processes, and is usually coupled to the oxidation of organic matter. Traditionally~~,~~ this process is thought to be limited ~~in most aquatic systems~~ to the shallow part of the sedimentary column in most aquatic systems~~,~~ ~~as one of the energetically favorable anaerobic microbial respiration cascade, usually coupled to the oxidation of organic matter.~~ However, ~~in the last decade~~ iron reduction has also been ~~also~~ observed in the methan~~ogen~~ic ~~depth~~ zone ~~in~~ of many marine and freshwater sediments, well below its expected zone, occasionally accompanied by decreases in methane ~~decrease~~, suggesting a link between the iron and the methane cycles. Yet, the mechanistic nature of this link (competition, redox or other) has yet to be established, and has not been studied in oligotrophic shallow marine sediments. In this study we present combined geochemical and molecular evidences for microbial iron reduction in the methan~~ogen~~ic ~~depth~~ zone of the oligotrophic Southern Eastern (SE) Mediterranean continental shelf. Geochemical pore-water profiles indicate iron reduction in two zones, the traditional zone in the upper part of the sediment ~~cores~~ and in the deeper zone located in the enhanced methane concentration layer. Results from a slurry incubation experiment indicate that the deep iron reduction is microbial. ~~The Geochemical data~~The ~~sedimentary~~ profiles of microbial abundance and~~,~~ qPCR of the mcrA gene of the sediment, together with Spearman correlation between the microbial ~~abundance~~ data and Fe(II) concentrations in the pore-water, ~~as well as the qPCR analysis of the mcrA gene~~ point suggest types of~~to several~~ potential microorganism~~s~~ ~~s~~ that ~~could~~ may be involved in ~~in~~ th~~e~~is iron reduction via ~~three~~ several potential pathways: H_2 or ~~/~~organic matter oxidation, an active sulfur cycle or iron driven anaerobic oxidation of methane. We suggest that intensive upward migration of methane in the sedimentary column and its oxidation by sulfate may fuel deeper microbial activity that allows methanic iron reduction in sediments of the SE Mediterranean.

1 Introduction

Iron (Fe) is the fourth most abundant element in the Earth's crust. It appears as elemental Fe, Fe(II) and Fe(III), and has an important geobiological role in natural systems (Roden, 2006). Dissimilatory microbial iron reduction ~~is likely~~may be one of the first evolutionary metabolisms, and plays a key role in the reductive dissolution of Fe(III) minerals in the natural environment (Weber et al., 2006) and~~,~~ in the mineralization of organic matter in freshwater sediments (Roden and Wetzel, 2002). It also serves as a redox wheel that drives the biogeochemical cycles of carbon, nitrogen, sulfur and phosphorous (Li et al., 2012~~)~~ ; Slomp et al., 2013; Sivan et al., 2014; Egger et al., 2016; Ettwig et al., 2016; Riedinger et al., 2017; März et al., 2018).

Dissimilatory iron reduction is part of the anaerobic respiration cascade, in which different organic substrates are used for energy by microorganisms and oxidized to dissolved inorganic carbon (DIC). This is accomplished by reduction of electron acceptors, other than oxygen, according to their availability and energy yield. Denitrification is the first respiratory process in anoxic sediments, followed by manganese reduction, ~~and~~ iron reduction and then sulfate reduction. Methane (CH_4) production (methanogenesis) by archaeal methanogens is traditionally considered to be the terminal process of microbial organic matter mineralization in anoxic environments, after the other electron acceptors have been exhausted (Froelich et al., 1979). When the produced methane diffuses away from the methanic layer and ~~outward diffusing methane~~ meets an electron acceptor it can be consumed by microbial oxidation (methanotrophy). In ~~anoxic~~ marine sediments anaerobic oxidation of methane (AOM) ~~is~~ coupled ~~mainly~~ to sulfate reduction has been shown to occur ~~(~~Iversen and Jorgensen, 1985; Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001; Knittel and Boetius, 2009)~~)~~, and~~.~~ ~~This process~~ was found to consume up to 90 % of the methane that diffuses upwards to the sulfate methane transition zone (SMTZ) (Niewöhner et al., 1998; Valentine, 2002).

The classical process of dissimilatory iron reduction is coupled to the oxidation of organic matter (organoclastic iron ~~oxidation~~reduction) (Eq. 1, Lovley, 1991; Lovley et al., 1996). However, iron reduction can be coupled to other processes as well, such as hydrogen (H_2) oxidation (hydrogenotrophic iron reduction) (Eq. 1, Lovley, 1991). Besides ~~by H_2 oxidation~~, Fe(III) can be reduced microbially (and also abiotically) by pyrite oxidation (Eq. 2, Bottrell et al., 2000), leading to S intermediates, and followed by their disproportionation to sulfate and sulfide via a "cryptic" sulfur cycle (e.g. Holmkvist et al., 2011).

$$2Fe^{3+} + organic\ matter/H_2/humic\ acids \rightarrow 2Fe^{2+} + HCO_3^-/CO_2/2H^+ \quad (1)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (2)$$

Another recently discovered pathway of iron reduction is by AOM (Eq. 3).

$$CH_4 + 8Fe(OH)_3 + 15H^+ \rightarrow HCO_3^- + 8Fe^{2+} + 21H_2O \quad (3)$$

This process in marine sediments was evident through incubations of marine seeps (Beal et al., 2009; Sivan et al., 2014). It was suggested to exist mainly through the modeling of geochemical profiles in deep sea sediments (Sivan et al., 2007; März et al., 2008; Riedinger et al., 2014), and in brackish coastal sediments (Slomp et al., 2013; Segarra et al., 2013; Egger et al., 2015; Egger et al., 2016; Rooze et al.,

2016; (Egger et al., 2017) Rooze et al., 2016). In freshwater environments, it was suggested to occur in lakes (Crowe et al. 2011; Sivan et al., 2011; (Nordi et al., 2013)Nordi et al., 2013), and was shown in enriched, denitrifying cultures from sewage, where it was performed by methanogens (Ettwig et al., 2016). Iron-coupled AOM in natural lake sediments was indicated using isotope pore-water depth profiles (Sivan et al., 2011), rate modeling based on these profiles (Adler et al., 2011), microbial profiles (Bar-Or et al., 2015), and directly by from a set of sediment slurry incubation experiments using several methods (Bar-Or et al. 2017). The few microbial studies about on iron-coupled AOM (mainly in cultures) showed the involvement of methanogenic/methanotrophic archaea (Scheller et al., 2016; Ettwig et al., 2016; Rotaru and Thamdrup, 2016; Cai et al., 2018; Yan et al., 2018; Rotaru and Thamdrup, 2016) or cooperation between methanotrophic archaea and methanogensiron reducing bacteria (Bar-Or et al., 2017).

Whereas Fe(II) is highly soluble, Fe(III) that is the most abundant species of iron natural conditions, appears as low solubility oxidized minerals Fe(III) appears as low solubility minerals, and is the most abundant species of iron under natural conditions close to neutral pH. This makes iron usage a challenge to microorganisms, which need to respire low-solubility iron oxide minerals, thus rendering many of iron-oxide minerals effectively unavailable for reduction and leading to the dominance of sulfate reducing bacteria beyond a certain depth. Therefore, it is not trivial to findobserve the observation of iron reduction below its traditional depth, in the methanic zone, where iron-oxides are assumed to be of low reactivity is surprising. Moreover, this type of iron reduction is occasionally accompanied by depletion in methane concentrations, suggesting a possible link between the iron and the methane cycles. There are three potential mechanisms that can explain tThise coupling linkbetween the cycles: can be through 1) a competition between methanogens and iron reducing bacteria, 2) a metabolism switch of methanogens switching from methanogenesis to iron reduction metabolism, and/or 3) iron coupled AOM, as mentioned above. Previous observations in other environments demonstrated the inhibition of methanogenesis under iron-reducing conditions due to competition between methanogens and iron-reducing bacteria for the common acetate and hydrogen substrates (Lovley and Phillips, 1986; Roden and Wetzel, 1996;Conrad, 1999; Lovley and Phillips, 1986; Roden and Wetzel, 1996; Roden, 2003). Different methanogens can also utilize iron directly, by reducing Fe(III). This was shown in pure cultures with the amorphous Fe(III) oxyhydroxide (Bond and Lovley., 2002), in pure cultures close to natural sedimentary conditions (Sivan et al., 2016), in natural lake sediments with different iron oxides (i.e. amorphous iron, goethite, hematite and magnetite) (Bar-or et al., 2017), in anoxic ferruginous lake sediments enrichments (Bray et al., 2018), and in iron-rich clays (Liu et al., 2011; Zhang et al., 2012; Zhang et al., 2013).

Despite the above studies, the nature of the link between the iron and the methane cycles in marine methanogenic zone, which reactivates iron oxides and making them availablecreates suitable conditions for iron reduction, has not yet been determined yet. Furthermore, this microbial iron reduction in the methanogenic zones has not been shown in the sediments of oligotrophic shallow marine environments. In this study we report observations of microbial iron reduction in the methanogenic depth in marine sediments of from the oligotrophic SE Mediterranean continental shelf. This is by using both geochemical pore-water profiles and microbial investigation profiles at three different stations combined

with a ~~basic~~ simple slurry incubation experiment ~~with slurry~~ from the methan~~ogen~~ic zone. The slurries were amended with hematite and magnetite, as, given their low reactivity, these are the expected Fe(III) minerals to survive the sulfate zone (Canfield, 1989; Poulton et al., 2004). Furthermore, these minerals were found to ~~perform~~be ~~source to~~used for iron-coupled AOM in ~~our~~ lake sediments (Bar-Or et al., 2017). The profiles and the incubation experiment, including the related microorganisms, are discussed in terms of the possible links between the cycling of iron and methane.

2 Methods

2.1 Study site

The Levantine Basin of the SE Mediterranean Sea, including Israel's continental shelf, is an oligotrophic nutrient-poor marine system (Herut et al., 2000; Kress and Herut, 2001)~~The Levantine Basin of the SE Mediterranean Sea is one of the most oligotrophic nutrient-poor marine environment in the world (Kress and Herut, 2001), including the Israeli continental shelf (Herut et al., 2000)~~. The continental shelf narrows from south to north and ~~is built mainly~~compromises of Pliocene-Quaternary Nile-derived sediments~~,~~, ~~whose~~The ~~rate of~~ sedimentation rate decreases with increasing distance from the Nile Delta and from the shoreline (Nir, 1984; Sandler and Herut, 2000). ~~The sedimentation rate o~~Off the shore of Israel the sedimentation rate is a relatively high ~~sedimentation rate~~ ~~of~~at ~0.1 cm y^{-1} (Bareket et al., 2016). The bottom seawater ~~across~~along the continental shelf is well oxygenated and sulfate concentrations at ~~in~~ the water-sediment interface ~~is~~are ~30 mmol L^{-1} (Sela-Adler et al., 2015). While the highest levels of total organic carbon (TOC) (1 – 2%) in sediments were found in the Western Mediterranean Basin and offshore the Nile River delta, the central and eastern ~~deep water~~ regions of the Levantine Basin have relatively low TOC levels ~~(0-0.5%) of ~1%~~ (~0.~~5~~ 1 – 1.4%; Almogi-Labin et al., 2009; Sela-Adler et al., 2015; Astrahan et al., 2017). Along the Egyptian coast, ~~maximal contents of~~the TOC in surface sediments on the shelf ~~is up~~reaches maximum values of ~~to~~ 1.5% (Aly Salem et al., 2013)~~, while in the Israeli shelf sediments (< 100 m depth) the TOC levels vary between < 0.1 – 1%~~ (~~Almogi-Labin et al., 2009~~). The ~~discovery of~~finding of a 'gas front' ~~from~~ in seismic profiles within the sediments of the continental shelf of Israel (Schattner et al., 2012), led to the ~~findings~~ discovery of biogenic methane formation at some locations in shallow sediments (Sela-Adler et al., 2015). ~~The bottom seawater across the shelf is well oxygenated therefore sulfate concentration in the water-sediment interface is ~30 mmol L^{-1} ()~~

2.2 Sampling

Seven sediment cores (~5 – 6 m long) were collected using a Benthos 2175 piston corer, from the undisturbed seafloor sediments of the SE Mediterranean continental shelf of Israel at water depths of 81 – ~~88~~ 89 m from three stations; SG-1 ~~(32°57.82' N 34°55.30' E)~~, PC-3 ~~(32°55.30' N 34°54.14' E)~~ and PC-5 ~~(32°55.47' N 34°55.01' E)~~ (Fig 1). The cores were sampled ~~by~~ during cruises of~~the~~ R.V. *Shikmona* between 2013 to 2017, and by the R.V. *Bat-Galim* on January 2017 (Table 1). The sediment cores were sliced on board every 25 – 35 cm within minutes upon retrieval from the seafloor. This area was previously investigated ~~previously with~~ for other ~~other focuses~~purposes, such as the sulfate reduction in the SMTZ (Antler et al., 2015; Wurgaft et al., 2019~~., unpublished),~~.), and methanogenesis characteristics (Sela-Adler et al., 2015).

From each interval, a 2.5 mL of total sediment sample was collected and inserted immediately into an anaerobic 10 mL glass bottle filled with 5 mL NaOH 1.5 N for headspace measurements of methane concentration (after Nusslein et al, 2003). In addition, another 2.5 mL sediment sample was taken from each segment of the cores and transferred into a 20 mL glass bottle filled with NaCl saturated solution for H_2 concentrations measurements. Sediment samples from each segment of the cores were centrifuged on board if possible or in the lab within a day by Sorval centrifuge at 9500 RPM under 4 °C and Ar atmosphere in order to extract pore-water for chemical analysis. The supernatant was filtered (0.22 µm) and analyzed for Fe(II), sulfate, sulfide and the stable carbon isotope composition of the DIC ($\delta^{13}C_{DIC}$). After the pore-water extraction, the sediment was analyzed for the content of the different reactive iron minerals~~oxides~~ (Table 2). In addition, sediment sub-sample from each segment of the January 2017 core from SG-1 station was kept at~~in~~ -20 °C for molecular analysis. Due to high water content and movement in the uppermost part of the sediments, two ~30 cm sediment cores were also sub-sampled separately, using a 0.0625 m^2 box corer (Ocean Instruments BX 700 A1) and Perspex tubes during the September 2015 and January 2017 cruises.~~the top 20 cm of the piston cores pore-water measurements might be affected by sediment movement and mixing; to avoid this, two ~30 cm cores were collected during the September 2015 and January 2017 cruises via a 0.0625 m^2 box corer (Ocean Instruments BX 700 A1).~~ The short cores were stored at 4 °C, cut in the lab less than 24 hours after their collection and their results are presented for the top sediment (Fig. 2). ~~Sediment and pore-water samples were measured for CH_4, Fe(II), sulfate and $\delta^{13}C_{DIC}$ measurements.~~

2.3 Slurry incubation experiment

The experimental set-up consisted of 11 bottles with sediment from the methan~~ogen~~ic zone~~, (260~~ 265-285 cm ~~below sea floor level~~depth) from Station SG-1~~ station~~, where iron reduction was apparent in the pore-water profiles (Fig ~~1~~2). Prior to the beginning of the experiment, ~~The~~ sediment from the designated depth ~~was~~ had been homogenized in an anaerobic bag under N_2 atmosphere. It was then transferred under ~~anaerobic~~ anoxic conditions to a 250 mL glass bottle with the addition of synthetic sea water without sulfate to reach 1:1 sediment – water slurry ratio for 3 months incubation period. ~~Then~~ After the incubation period the slurry was sub-divided ~~anaerobically~~ anoxically to the 11 ~~60 mL~~ experiment bottles (60 mL each), and synthetic sea water was added for final sediment – water ratio of 1:3. The bottles were sealed with a crimp cap and were flushed with N_2 for 5 minutes, shaken vigorously and flushed again, (repeated 3 times). Three experimental bottles were autoclaved twice to serve as "killed" control for the experiment. The experimental bottles were amended with ~~iron oxides (~~1.6 g L^{-1} of hematite (Fe_2O_3) or 2.3 g L^{-1} of magnetite (Fe_3O_4) to reach Fe(III) final concentration of 10 mmol L^{-1} ~~with final concentration of 10 mmol L^{-1}~~. The three killed bottles were amended with the iron oxides after they cooled down to room temperature. ~~One~~H_2 was added to some treatments to test its potential as an electron donor.~~,~~ One mL of H_2~~It~~ was injected by gas tight syringe to the three killed bottles, to two bottles with the addition of hematite and to two bottles with the addition of magnetite ~~with/without hydrogen (H_2)~~ (to reach final concentration of ~4% of the h~~H~~ead space volume). The experimental bottles were sampled several times for dissolved Fe(II) concentrations during the 14 day experiment period.

2.4 Analytical methods

2.4.1 Pore-water analyses

Methane concentrations were analyzed by Focus Gas – Chromatograph (GC; Thermo) equipped with FID detector with detection limit of 50 µmol L^{-1}. H_2 concentrations were analyzed in a Reducing Compound Photometer Gas-Chromatograph (RCP-GC; Peak Laboratories). Dissolved Fe(II) concentrations were measured using the ferrozine method (Stookey, 1970) by a spectrophotometer at 562 nm wavelength with detection limit of 1 µmol L^{-1}. Sulfide was measured using the Cline (1969) method by a spectrophotometer at 665 nm wavelength with detection limit of 1 µmol L^{-1}. ~~S~~Total s~~u~~lfur~~fate~~ concentrations were measured in an inductively coupled plasma atomic emission spectrometer (ICP-AES), Perkin ~~Almer~~ Elmer Optima 3300, with an analytical error of ±1% (average deviations from repeated measurements of a seawater standard).~~–~~ The $\delta^{13}C_{DIC}$ values were measured on a DeltaV Advantage Thermo© isotope-ratio mass-spectrometer (IRMS) at a precision of ±0.1 ‰. Results are reported versus VPDB standard. Several pore-water profiles of dissolved total sulfur, CH_4, $\delta^{13}C_{DIC}$, Fe(II) and H_2 were ~~performed~~ produced during the study, and all of them are presented ~~(and not their average)~~. For each profile, the error bar is that of the average deviation of the mean of the duplicates, in cases where they were taken, otherwise it is that of the analytical error (if larger than the symbol).

2.4.2 Sediment analysis

Reactive Fe(III) in the sediments was measured according to Poulton and Canfield (2005) definition and sequential extraction procedure. The different reactive iron ~~oxides~~ minerals were separated to (1) carbonate-associated Fe; (2) easily reducible oxides; (3) reducible oxides and (4) magnetite. About 0.6 g dry sediment was inserted to a centrifuge tube with 10 ml of a specific extractant at every stage under oxic conditions and constant agitation (Table 2). The fluids were separated from the sediment by centrifugation and removed from the tube with Pasteur pipette after every extraction stage. At the end of each extraction stage, the extractant was transferred to a 15 mL falcon tube with 0.1 mL ascorbic acid and 0.1 mL ferrozine solution to reduce all the Fe(III) to Fe(II) and fix it, then it was measured spectrophotometrically. The results ~~are~~ presented as "total reactive Fe(III)"~~, which was~~ are the sum of the easily reducible oxides, reducible oxides and magnetite.~~-~~ Pyrite profile was produced by Wurgaft et al., (2019).

2.4.3 Quantitative PCR and 16S rRNA gene V4 amplicon pyrosequencing

DNA was extracted from the sediment core of station SG-1 from January 2017 using PowerSoil DNA Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's instructions. Copy numbers of selected genes were estimated with quantitative PCR (qPCR) as described previously (Niu et al., 2017) using specific primers: Uni519f/Arc908R and bac341f/519r for archaeal and bacterial 16S rRNA genes, respectively, and mlas/mcrA-rev for the *mcrA* gene, which encodes the α-subunit of methyl-coenzyme M reductase. The amplification efficiency was 94.5%, 106.3% and 92.4% for the archaeal 16S rRNA, bacterial 16S rRNA and the *mcrA* gene, respectively (the respective R^2 of the standard curve was 0.998, 0.998 and 0.995).

The V4 regions of bacterial and archaeal 16S rRNA genes were amplified using barcoded 515FB/806RB primers (Walters et al., 2015) and Arch519/Arch806 primers (Song et al., 2013), respectively. PCR mixture contained 6 – 10 ng total DNA, 5 µL 10× Ex Taq buffer, 4 µL 2.5 mmol L^{-1} dNTP mix, 1 µL of each primer, 0.25 µL Ex Taq polymerase (Ex-Taq; TaKaRa, Dalian, China) and 5 µL bovine serum albumin (25 mg mL^{-1}) in a total volume of 50 µL. DNA was sequenced as 2x150 bp reads using Illumina MiSeq platform (Illumina, USA). Sequence quality assessments, chimera detection and down-stream phylogenetic analyses were conducted in QIIME (Caporaso et al., 2010). Taxonomical assignments for each OTU were performed in QIIME using the BLAST method and the SILVA128 reference database. 24056 to 132042 high quality sequences were obtained per sample, with the proportion of high-quality sequence versus total sequence between 81.97 – 99.89%. Spearman correlation was performed using the online calculator (http://www.sthda.com/english/rsthda/correlation.php) to test the relevance of microbial abundance and communities with Fe(II) concentration along the depth of the sediment core from 185 cm to the bottom 575 cm, which is the methan~~ogen~~ic zone of the sediment core according to the geochemical profile (see the results below).

3 Results

3.1 Geochemical profiles

Geochemical pore-water profiles of several sediment cores from the three stations (SG-1, PC-3 and PC-5 (Fig. 1, Table 1)) were ~~performed~~ produced in order to ~~test~~ investigate ~~the possibility of~~the iron reduction process in the metha~~noge~~nic zone of the SE Mediterranean continental shelf and its potential sources. The p~~P~~ore-water ~~analyses~~ profiles ~~of~~ from ~~s~~Station SG-1 (Fig. 2) ~~of sulfate concentrations~~ show complete depletion of total sulfur at approximately 150 cm depth in all the station cores ~~at station SG-1 (Fig. 12, Supp XX)~~. Sulfide concentrations were below the detection limit in all cores~~-~~, indicating that the total sulfur is mostly sulfate. ~~and therefore are not presented~~. Methane concentrations ~~profiles~~ show an increase ~~in concentration~~ with depth immediately after the consumption of sulfate. The maximum~~al~~ methane concentration ~~was the saturation level (Sela-Adler et al., 2015) of about~~was approximately 10 mmol L^{-1} at ~140 cm depth in June 2015~~. (station SG-1), probably due to intensive methane production at the exact location of the core collected at that time.~~ The other methane depth profiles show ~~high~~ an increase in the concentrations of methane ~~of~~ to approximately 2 mmol L^{-1} and then leveling off ~~all the way to~~throughout the bottom of the cores (~600 cm). Detected dissolved Fe(II) concentrations were found in the traditional iron reduction zone in the upper part of the ~~cores~~ sediment (between 30 – 90 cm depth) ~~However~~, and a second peak was found in the deeper part of the sediment, at the metha~~noge~~nic zone (below 180 cm depth). Maximum dissolved Fe(II) concentrations reached 84 µmol L^{-1} in the traditional iron reduction zone of the sediment cores, and 65 µmol L^{-1} in the metha~~noge~~nic zone ~~(Fig. 1). It should be noted that iron species are highly sensitive to environmental changes such as shifts in local pH, the different types of electron shuttles, and organic compounds that are present in the surroundings. These changes affect the net dissolved Fe(II) observed; consequently the dissolved Fe(II) results show variability between the cores that were extracted and analyzed from the same station.~~ The $\delta^{13}C_{DIC}$ values were the lowest (-35 ‰) as expected at the SMTZ depth, ~~as expected from the intensive sulfate-coupled AOM process there, which uses the isotopically light carbon of the methane as a carbon source with only~~

small fractionation. The $\delta^{13}C_{DIC}$ values wereand the highest in the methanogenic zone, . as expected (the carbon source for the methane comes from the CO_2, leaving the residual DIC heavier by about 60 ‰ (Whiticar, 1999)). Fitting the intensive methane profile from June 2015, the $\delta^{13}C_{DIC}$ showed the most dramatic decrease and increase that date as well. The SMTZ was also the shallowest in this core because of the intensive methane oxidation, thus the traditional iron reduction is missing in the sampled pore-water. H_2 concentrations decreased to a minimum peak of 0.017 µnmol L^{-1} at 155 cm depth, and then increased to a maximum of 0.147 µnmol L^{-1} at 485 cm depth.

Pore-water analyses profiles from station Station PC-3 (Fig. 2) on all three sampling dates show similar patterns to Station SG-1 with less lower methane concentrationsactivity (Fig 12). Sulfate Total sulfur was completely depleted within the upper 300 cm depth. Sulfide concentrations were below the detection limit at this station as well. MThe methane profiles show an increase in methane concentration immediately after the consumption of sulfate. The maximum methane concentration reached 0.8 mmol L^{-1} at 450 cm depth in the Aug-13 core. The Fe(II) profiles show two peaks also here, one in the upper part of the cores sediment with maximum of 32 µmol L^{-1} at 177 cm depth, and another one with maximum of 64 µmol L^{-1} at 390 cm depth at the methanogenic depth. The $\delta^{13}C_{DIC}$ values decreased from approximately -10 ‰ at the water-sediment interface to -20 ‰ at the SMTZ. Below that zone there was an increase in $\delta^{13}C_{DIC}$ values to about -5 ‰ due to methanogenesis. H_2 concentrations remained around 2 µmol L^{-1} along the core. The threefew deviating points that do not fit a the clear pattern are attributed to an analytical or sampling errorand therefore not discussed. The H_2 concentrations at the PC-3 station are higher by one order of magnitude than the concentrations at the SG-1 station. This is probably due to the more intensive methanogenesis process at SG-1 station, as shown by the higher methane concentrations than those at PC-3 station.

Pore-water analyses profiles from the core collected at station Station PC-5 (Fig. S1) resembles the profiles of Station PC-3 station. Sulfate Total sulfur was depleted at approximately 300 cm, and methane concentrations increased below that depth to 0.3 mmol L^{-1}. The Fe(II) profile shows two peaks in this core as well, one in the upper part sediment of 20 µM at 150 cm depth and the second of 30 µmol L^{-1} in the methanogenic zone. The $\delta^{13}C_{DIC}$ value decreased from -5 ‰ at the water-sediment interface to -25 ‰ at the SMTZ, and below that depth increased δ^{13}C values increased to -17 ‰ at the methanic zone.

In addition to the dissolved constituents profiles, reactive iron minerals were extracted from the sediment collected on September 2015, and iron minerals profiles forfrom sStations SG-1 and PC-3 were produced (Fig. 2). In Station SG-1 there appears to be a slight variability in the content of the iron minerals. The Fe-carbonate minerals (i.e. siderite and ankerite) content in the upper part of the sediment was 0.22 dry wt%, increased to ~0.45 dry wt % at 103 cm depth and then remained constant. The iron (hydr)oxides (i.e. ferrihydrite and lepidocrocite) content was 0.49 dry wt % in the upper part of the sediment, it peaksed at 203 cm depth withto 0.64 dry wt % and then decreased to 0.50 dry wt % at the bottom of the core. The reducible oxides (i.e. hematite, goethite and akageneite) content was 2.15 dry wt % in the upper part of the sediment, decreased to 1.03 dry wt % at 312 cm depth, and then it increased to 1.55 dry wt % at 427 cm depth. Magnetite content was 0.34 dry wt % atin the upper part of the sediment, decreased to 0.32 dry wt % at 153 cm depth, increased to 0.35 at 253 cm depth, decreased to 0.23 dry wt % at 312 cm

depth, and increased again to 0.35 dry wt % ~~the~~ at the bottom. A ~~P~~pyrite content profile from ~~s~~Station SG-1 was also produced ~~as well~~ from the September 2015 cruise data and shows two peaks; the first of 1.10 wt % at 153 cm depth, and the second of 1.80 wt % at 312 cm depth. The total reactive Fe(III) oxides profile ~~from SG-1 (Fig. S1)~~ showed~~s~~ a general decrease from 3.00 dry wt % at 13 cm depth to 2.~~3~~27 dry wt % at 507 cm depth, with two minimum peaks of 2.42 dry wt % at 103 cm and of 1.~~9~~88 dry wt % at 312 cm.

In Station PC-3 ~~station~~ there appeared to be no significant changes in the different reactive oxides with depth (Fig. 2). The Fe-carbonate minerals content in the upper part of the sediment was 0.50 dry wt % and reached 0.69 dry wt % in the deep sediment. The iron (hydr)oxides concentrations were approximately 1.00 dry wt % throughout the sediment column. The reducible oxides concentrations were 0.78 dry wt % in the upper part of the sediment, increased to 0.89 dry wt % at 167 cm depth and then decreased to 0.76 dry wt % at 495 cm depth. Magnetite concentration was 0.83 dry wt % in the upper part of the sediment, increased to 0.89 dry wt % at 167 cm, and then ~~it~~ decreased again to 0.75 dry wt % at 495 cm depth. The total reactive Fe(III) oxides content var~~y~~ied between 2.10 dry wt %~~, which is the maximum point~~ (at 167 cm depth)~~,~~ and 1.76 dry wt %~~, which is the minimum point~~ (at 137 cm depth).

~~The reactive Fe(III) oxide profile from SG-1 (Fig. S1) shows a general decrease from 3 dry wt % at 13 cm depth to 2.3 dry wt % at 507 cm depth, with two minimum peaks of 2.4 dry wt % at 103 cm and of 1.9 dry wt % at 312 cm. PC-3 profile shows no significant trends in the reactive Fe(III) concentrations. The values vary between 2.1 dry wt %, which is the maximum point at 167 cm, and 1.8 dry wt %, which is the minimum point at 137 cm depth.~~

3.2 Abundance and diversity of bacteria and archaea

The qPCR of bacterial and archaeal 16S rRNA genes from the SG-1 core (collected on January 2017) revealed ~~that the~~an abundance of bacterial genes ~~was~~ between $1.46 – 9.45 \times 10^6$ copies per g wet sediment, while that of archaea was between $8.15 \times 10^5 – 2.25 \times 10^7$ copies per g wet sediment (Fig. ~~2~~3). The abundance of bacteria and archaea decreased gradually in the top 95 cm, increased sharply at 125 cm depth within the SMTZ, remained relatively stable with high abundance at 185 – 245 cm (the top layer of the metha~~noge~~nic zone), and then decreased. Notably, the abundance of both bacteria and archaea peaked within the metha~~noge~~nic zone at 245 cm in correspondence with a Fe(II) concentration peak. However, it is not feasible to compare the abundance of archaea and bacteria by this method due to bias caused by the PCR primers used (Buongiorno et al., 2017). The abundance of the *mcrA* gene increased sharply from the surface layer to the SMTZ, peaked at 155 cm and remained stable at 155 – 245 cm, indicative of active anaerobic methane metabolism in the SMTZ and an active methan~~ogen~~ic zone (Fig. 2). Spearman correlation test (Table S2) shows that the abundance of the bacteria and archaea 16S rRNA genes and *mcrA* genes correlated with Fe(II) concentration in the metha~~noge~~nic zone, where *mcrA* gene correlated the most significantly ($r = 0.5429$, p value = 0.04789).

Illumina-sequencing of the 16S rRNA gene revealed diverse bacterial and archaeal communities throughout the SG-1 core (Fig. 4). Although no clear plateau was observed on species rarefaction curve for the current sequencing depth (Fig. S2), Shannon diversity indices reached stable values, indicating

that those sequences well covered the diversity of bacterial and archaeal populations in the samples (Fig. S3). Shannon index, based on 16S rRNA gene sequences, showsed higher diversity in the top layers of the sediment along with similar values through the core using the bacterial primers, while for sequences using archaeal primers, the values varied in different layers (Table S1). The bacterial sequences were affiliated with the following phyla: Planctomycetes (25.7%), Chloroflexi (23.2 %), Proteobacteria (12.9%), Deinococcus-Thermus (9.9 %), Acidobacteria (3.5%), Aminicenantes (3.3 %), Spirochaetes (2.3%), Deferribacteres (1.7%), Elusimicrobia (1.6%), Aerophobetes (1.6%), Nitrospirae (1.4%), Firmicutes (1.4 %), Actinobacteria (1.4 %), TM6 (Dependentiae) (1.2%), Marinimicrobia (SAR406 clade) (1.0%), and other taxa with less than 1% of the bacterial communities (Fig. 3a4a). Bathyarchaeota were the predominant archaea in all the sediment layers, based on the high relative abundance of their 16S rRNA gene sequences (91.0%). The remaining archaeal phyla comprised Euryarchaeota (3.2%), Thaumarchaeota (2.4%), Lokiarchaeota (1.0%), and other phyla with less than 1% of the archaeal communities (Fig. 3b4b).

Spearman correlation analysis (Fig. XXTable S2) revealed that uncultured SBR1093 (r = 0.6176, p value = 0.01859) from bacterial Candidate Phylum SBR1093, subgroup 26 of Acidobacteria (r = 0.5841, p value = 0.02828), the uncultured bacterium from TK10 Class of Chloroflexi phylum (r = 0.5297, p value = 0.0544) and *uncultured Bathyarchaeota sp*. (archaea) (r = 0.5516, p value = 0.04388) correlated significantly with Fe(II) concentration.

3.3 Incubation experiment

Sediment from the observed deep iron reduction zone of Station SG-1 from January 2017 core was used for a basicsimple short-term (few couple of weeks) slurry incubation experiment in order to characterize the iron reduction process in the methanogenic zone. The slurries were amended with hHematite and magnetite, which were expected to remain throughsurvive the sulfate zone, and were shown to be a source for AOM in lake sediments, were added to the slurries, which were expected to survive the sulfate zone, and were shown to perform AOM in lake sediments. Indeed, the iron oxide profiles (Fig. 12) confirm that hematite and magnetite wewre abundant in the methanic zone in this core. Hydrogen was added as well to some of the slurriesbottles.

The results of the experiment are shown in figure 45. Dissolved Fe(II) concentrations show significant increase from 11 µmol L^{-1} to approximately 90 µmol L^{-1} during the first three days in all the experimental bottles, except for the killed bottles, implying that the reduction is microbially mediated. Another observation was that the microorganisms were able to reduce both hematite and magnetite to the same extent. In addition, no difference in the Fe(II) concentrations between bottles with and without the addition of H_2 was observed.

4 Discussion

4.1 General

This study was performed in the SE Mediterranean in the area of the recently discovered 'gas front' (Schattner et al., 2012), where biogenic methane was found at some locations in shallow sediments with

low TOC content (Sela-Adler et al., 2015). Station SG-1 is located at the center of this area, while PC-3 and PC-5 stations at the edges, and indeed methane involved related processes seem were more intensive at this sStation SG-1 (Fig. 1), linking, the shallow sediment processes to this reservoir. Our results suggest that there are two sources for methane in the sediment: the first is from migration of methane from thise gas front area (Wurgaft et al., 2019), and the second is from *in-situ* formation. *In-situ* methanogenesis in the shallow shelf sediments is evident by the geochemical profiles of $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CH4}$ (Sela-Adler et al., 2015) and by the microbial profiles of population and functional *mcrA* gene (Figs. 3 and 4, and is(further (discussed further below)).

CThe omparingcomparison between the sites shows that, methane reaches higher highest concentrations at Station SG-1 (up to the saturation level (Sela-Adler et al., 2015)), specifically in the June 2015 profile., and the intensive methanogenesis alsowhichThis leads to intensive methane oxidationAOM by sulfate at the SMTZ, causing it to occur at shallower depth with and to produce lower $\delta^{13}C_{DIC}$ values than the other two stations. The relation between the upward fluxes of methane, and the SMTZ depth and the $\delta^{13}C_{DIC}$, as observed invalues fits previous studies (e.g. Sivan et al., 2007). Fitting the intensive methane profile from June 2015, the $\delta^{13}C_{DIC}$ showed the most dramatic decrease and increase that date as well. The higher methane concentrations in the June 2015 profile is presumably due to intensive migration of methane from the deeper sediments and/or more intensive methane production at the exact location of the core collected at that time. The shallower SMTZ values in June 2015 also interfered with the ability to observe the traditional iron reduction zone in our SG-1 sampling resolution (Fig 2). The H_2 concentrations at the PC-3Station SG-1 station arewere higherlower by onetwo orders of magnitude than the concentrations at the Station SG-1PC-3 station. This is probablypossibly due to the more intensive methanogenesishydrogen consuming processes at SG-1 station, as shown by the higher methane concentrations than those at PC-3 station.. Fe(II) profiles show also some variability between the cores within the same station. This is reasonable as iIron species arereduction is sensitive to environmental changes such as shifts in local pH, the different types of electron shuttles, and organic compounds that are present in the surroundings.

It should be noted that iron species are highly sensitive to environmental changes such as shifts in local pH, the different types of electron shuttles, and organic compounds that are present in the surroundings. These changes affect the net dissolved Fe(II) observed; consequently the dissolved Fe(II) results show variability between the cores that were extracted and analyzed from the same station.

Despite the pore-water profiles variability between the stations, they show a resemblance in their trends. All geochemical pore-water and reactive Fe(III) profiles suggest that the sediments in this area of the SE Mediterranean shelf can be classified into three general depth-zones (Fig. 12): **zone 1** is the upper part of the sediment, where the traditional classical iron reduction occurs, probably coupled to organic matter oxidation, with sulfate reduction below it; **zone 2** is the SMT depthZ, where methane starts to increase with depth, sulfate is completely depleted, sulfide is absent and Fe(II) is either present in low concentrations or absent as well (probably due to the precipitation of iron-sulfide minerals). In addition, the $\delta^{13}C_{DIC}$ values are the lowest in this zone, as expected from the intensive sulfate-coupled AOM process there, which uses the isotopically light carbon of the methane as a carbon source with a small

fractionation (Whiticar, 1999);~~;~~ **zone 3** is the methan~~ogen~~ic zone, where methane concentrations increased to the highest values in all stations, as did the $\delta^{13}C_{DIC}$ since the carbon source for the methane comes mainly from ~~the~~ CO_2, leaving the residual DIC heavier by about 60 ‰ (Whiticar, 1999).~~.~~ At this zone, local maxima of Fe(II) concentrations in the pore-water were found in all cores, indicating reduction of iron oxides~~ reactivation and reduction~~. ~~–~~The slurry experiment results ~~of the slurry experiment~~ show only a slight increase in Fe(II) concentrations in the killed bottles compared to their significant increase in the non-killed bottles, ~~indicating~~ ~~inferring~~inferring that ~~most of~~ the iron reduction in zone 3 is microbial (Fig. ~~4~~5).

4.2 Potential methanic iron reduction pathways

The observed intensive iron reduction in the metha~~noge~~nic sediments is the first in the SE Mediterranean shelf. The phenomenon of iron reduction in the metha~~noge~~nic depth has been observed before in other marine provinces (~~Egger et al., 2016;~~ Jorgensen et al., 2004; März et al., 2008; Slomp et al., 2013; Riedinger et al., 2014; ~~Slomp et al., 2013;~~ Treude et al., 2014; Egger et al., 2016). Yet,~~,~~ ~~however,~~ the type of link to the methane cycle ~~is complex~~is not well understood. Usually, iron reduction is coupled to oxidation of organic matter (Lovley and Phillips, 1988) and is performed by iron reducing bacteria, which is probably the case in zone 1. It is however questionable if this also stands for zone 3 and if not, what process is responsible for the ~~reactivation of iron oxides~~iron reduction at this depth and its relation to methane. The iron reduction in zone 3 can occur via four potential pathways: 1) oxidation of organic matter arriving from the SMTZ~~, and fueled~~ produced by the upward migrating methane, 2) oxidation of the methane itself, 3) H_2 oxidation or 4) oxidation through sulfur cryptic cycle.

The oligotrophic nature of the studied area would suggest that intensive bacterial iron reduction coupled simply to the oxidation of organic matter in zone 3 is less likely. The ~~present~~ low nutrient and low chlorophyll concentrations in the water column results in low ~~amount of~~ TOC amounts ~~in the sediments, reaching up to only ~1%~~in the sediments (<1%) (Sela-Adler et al., 2015). ~~However~~Nevertheless, we observe high methane concentrations in zone 3 in all three stations, where ~~that~~ part of it is from upward migration. This indicates that regardless of the area's present oligotrophic nature, the TOC substrate may be enough to sustain all the microbial activity and to take part in the iron reduction process in the methanic zone, just from biomass production in the SMTZ and its fast use below (so the TOC content seems still low).

~~This indicates that regardless the area's present oligotrophic nature, the TOC substrate is enough to sustain all the microbial activity up to methanogenesis. These environmental conditions are hypothetically attributed to the Last Glacial Maximum or Mid-Pleistocene sources (Schattner et al., 2012). At the methanogenic zone and below, it might be that the microbial communities present at these depths are used as a food source.~~ The importance of the methane flux as a carbon source that supports the deep microbial community in the sediments of the SE Mediterranean can be illustrated by comparing the organic carbon flux from the photic zone, with the flux of organic carbon that is oxidized by sulfate in the pore-water. Using traps, Moutin and Raimbault (2002) estimated an export flux of 7.4±6.3 mgC m^{-2} d^{-1}, which leaves the photic zone. However, Wurgaft et al. (2019) estimated that the flux of DIC toward the SMTZ from sulfate reduction is equivalent to 8±3 mgC m^{-2} d^{-1}. Whereas the difference

between the two fluxes is statistically insignificant, it should be noted that the flux of organic material that survives aerobic oxidation in the water column and the upper part of the sediment column, as well as anaerobic oxidation by other electron acceptors with higher energy yield (Froelich et al., 1979; Emerson et al., 1980), is likely to be substantially smaller than the flux measured by Moutin and Raimbault (2002). Therefore, it is unlikely that export flux from the photic zone constitutes the sole source of carbon to the SMTZ. Wurgaft et al. (2019) suggested that "external" methane, originates in deeper portions of the sediments, provides important source of carbon to the SMTZ in Station SG-1. Such fluxes of "external" methane are common along continental margins sediments (e.g. Milkov and Sassen, 2002; Milkov, 2004; Zhang and Lanoil, 2004; Paull et al., 2008). Here, we suggest that this supply of methane, leads to intensive sulfate-mediated AOM in the SMTZ, and that this intensive process and biomass may serve as an additional substrate that "fuels" the deeper zone, activating the iron-oxides.

The recently discovered iron-coupled AOM process (Eq. 3) is the second potential process that can involve iron oxide~~s~~ reduction in the deep methanic zone (Sivan et al., 2011: Segarra et al., 2013; ~~Riedinger et al., 2014;~~ Slomp et al., 2013; Riedinger et al., 2014; Egger et al., 2015~~; Egger et al., 2017~~; Rooze et al., 2016; Egger et al., 2017; Bar-Or et al., 2017). Fe(III) as an electron acceptor for AOM provides a greater free energy yield than sulfate (Zehnder and Brock, 1980), and its global importance was emphasized (Sivan et al., 2011: Segarra et al., 2013; Sivan et al., 2014). Two of the main environmental conditions for iron-coupled AOM to occur are high dissolved methane concentrations and abundant reducible iron oxides (Egger et al., 2017). Thus, from our profiles it seems that AOM could be a valid option, considering the high methane concentrations ~~that~~ and the high sedimentation rates (0.1 cm y^{-1} (Bareket et al., 2016)), which allow the iron oxides to survive the sulfidic zone and reach the methanic zone (Egger et al., 2017). ~~It~~ This can also be inferred from figure ~~5~~6, where some association was observed between the dissolved Fe(II) concentrations and the methane concentrations in zone 3. It seems that at high concentrations of Fe(II) methane concentrations are low and vice versa. This could be a result of iron-coupled AOM that uses methane to reduce Fe(III)-oxides, releasing dissolved Fe(II) to the pore-water. ~~This~~ It can also suggest a type of competitive relationship between methanogenesis and microbial iron reduction, or microbial population switching from methanogenesis to iron reduction metabolism (e.g. Sivan et al., 2016). It should be noted that our experiment was not designed to test AOM due to its short time scale of a few weeks, hence another long experiment with the addition of the ^{13}C-labeled methane will enable us to shed more light on this association.

~~Another~~ The third potential process that can be coupled to iron reduction in the metha~~noge~~nic zone is H_2 oxidation. H_2 is an important intermediate in ~~anaerobic~~ anoxic aquatic sediments. In this type of environment, it is produced mainly by fermentation of organic matter (Chen et al., 2006), and can be involved in different microbial processes~~;~~ where each process would need a certain amount of H_2 in order to occur (Lovley and Goodwin, 1988). The H_2 levels at SG-1 and PC-3 stations (Fig. ~~1~~2) are relatively high ~~compared~~in comparison to other marine environments (Lilley et al., 1982; Novelli et al., 1987), suggesting that there is enough H_2 to sustain the iron reduction process. The increase in H_2 concentration profile at the metha~~noge~~nic zone in SG-1 station could be explained by the occurrence of fermentation processes, which enables H_2 to accumulate (Chen et al., 2006). The H_2 involvement was tested by

injecting 1 mL of this gas to the experimental bottles in the methanogenic iron reduction process (Fig. 5). We observed that the increase of Fe(II) concentration was similar in the bottles with H_2 addition compared to the bottles without H_2. This could mean that either there is enough H_2 in the sediments as it is, as implied by the H_2 pore-water profiles, or that at the methanogenic depth H_2 is not involved in the iron reduction process.

The fourth potential way to reduce iron in zone 3 is by an active sulfur cycle. The pyrite profile supports this possibility~~nother A different way to reactivate the iron reduction process in zone 3 is to have an active sulfur cycle at this depth.~~ by showing two peaks, one in zone 2 of ~1 wt% and the second in zone 3 of ~2 wt% at about 300 cm depth (Fig. 2). The peak at 300 cm depth indicates possible active sulfur cycle, ~~at some point at this depth~~ even though sulfate is already undetected at 200 cm. Thus, a possible scenario ~~beis,~~ that Fe(III) is reduced by pyrite oxidation (Eq. 3) (Bottrell et al., 2000), which triggers the ~~sulfur~~ '~~"~~cryptic'~~"~~ sulfur cycle, as observed in other marine sediments (Holmkvist et al., 2011; ~~(~~ Brunner et al., 2016~~)~~; Egger et al., 2016). In this cycle, elemental sulfur, and eventually by disproportionation also~~,~~ sulfide and sulfate, are produced.~~,~~ T~~t~~he sulfide reacts with iron-oxide and precipitates as FeS or as pyrite (Holmkvist et al., 2011). The sulfate can inhibit methanogenesis (Mountfort et al., 1980; Mountfort and Asher, 1981), which can result in the enhancement of the iron reduction process due to competition for substrate with the methanogenesis process. Another indication for an active sulfur cryptic cycle comes from the 16S analysis (Fig. 4), which shows that Proteobacteria, a potential sulfur related bacteria phylum, is one of the most abundant phyla in the sediments. Moreover, the increase in the abundance of Sva0485 order of the deltaproteobacteria class, a known sulfate reducer (Tan et al., 2019) with depth, supports an active sulfur cycle in zone 3 as well.

4.3 Potential microbial players

Our data profiles and incubations indicate that the observed iron reduction in the methanic zone of the SE Mediterranean shelf is performed by microbial activity. The microbial results show first that the abundances of the bacteria and archaea (Fig. 4) are typical to oligotrophic marine sediments (e.g. South China Sea that contains ~0.5 – 1 % TOC (Yu et al., 2018)). Second, even though potential bacterial iron reducers, such as *Alicyclobacillus*, *Sulfobacillusin*, *Desulfotomaculum* genera (Firmicutes), *Acidiphilium* (Alphaproteobacteria), *Desulfobulbus*, *Desulfuromonas*, *Geobacter*, *Geothermobacter, Anaeromyxobacter* (Deltaproteobacteria) and *Shewanella* (Gammaproteobacteria) (Weber et al., 2006) comprise less than 0.1% of bacteria detected in the methanic zone (from 185 cm and below), it appears that both the microbial abundance and the Fe(II) concentration peaked at this zone. Cultivation efforts indicated that archaeal methanogens may also play a role in iron reduction within sediments (Sivan et al., 2016). Moreover, the relative abundance of methane-metabolizing archaea was shown to correlate with Fe(II) concentrations in Helgoland muds from the North Sea, where microbial abundance and the Fe(II) concentrations peaked at the methanic zone (Oni et al., 2015), similarly to the Mediterranean sediments. It is possible that methane-metabolizing archaea were involved in the iron reduction in the Mediterranean sediments, as the highest *mcrA* gene copies per gram wet sediment were detected in the SMTZ and in the top of the methanic zone where the Fe(II) concentrations are high. Methanotrophs, such as ANMEs, were found to be involved in iron coupled AOM in marine and freshwater cultures (Scheller et al., 2016;

McGlynn et al., 2015; Ettwig et al., 2016; Cai et al., 2018). ANMEs were found here with relatively low frequencies (ANME1, below 1% in most samples, circa 5% in the 185 cm layer), and their role in iron reduction within the Mediterranean sediments remains to be tested.

It should be noted that even though the microbial population was tested only on one sediment core that was extracted on January 2017 at Station SG-1, we believe that it represents the general microbial population abundance in the SE Mediterranean continental shelf. In our study, Spearman correlation analysis (Table S2) revealed that bacterial phyla SBR1093 (candidate Phylum), Acidobacteria and Chloroflexi, as well as archaeal Phylum Bathyarchaeota showed significant positive correlation with a Fe(II) concentration in the methan~~ogen~~ic zone. The Candidate Phylum SBR1093 was firstly identified in phosphate-removing activated sludge from a sequencing batch reactor (Bond et al., 1995), and continuously detected in a short-chain fatty acid rich environment such as wastewater treatment, and marine sediments (Wang et al., 2014). It was thought to be capable of growing autotrophically, but the metabolic capabilities related to iron reduction remain unclear. Strains of Acidobacteria and Chloroflexi phylum were found to be capable of iron reduction (Kawaichi et al., 2013; Kulichevskaya et al., 2014). In addition, members of Acidobacteria were found in iron-coupled AOM enrichment (Beal et al., 2009). The metabolic properties of Subgroup 26 from Acidobacteria and TK10 Class of Chloroflexi are still not known. Bathyarchaeota are globally distributed and account for a considerable fraction of the archaeal communities in the marine sediments, particularly, in the Mediterranean Pleistocene sapropels (Coolen et al., 2002; Zhou et al., 2018). While Bathyarchaeota have diverse metabolic capabilities (Lloyd et al., 2013; Meng et al., 2014; Evans et al., 2015; He et al., 2016; Yu et al., 2018; Feng et al., 2019), their role in iron reduction warrants further studies, as suggested from their high abundance here. Therefore, iron reduction and methane cycling within the deep methan~~ogen~~ic zone may be facilitated by an interplay among bacterial and archaeal groups, whose physiology and functions needs further investigation.

5 Conclusions

~~This~~Our study uses combined ~~The~~ geochemical and microbial ~~data from the~~ profiles together ~~and the~~with slurry incubation experiment ~~suggest for the first time that deep~~to show microbial iron reduction ~~is occurring~~ in ~~the~~ methanic ~~depth~~sediments, ~~of the SE Mediterranean continental shelf,~~ and the potential microbial population performing this reduction. ~~that both bacteria and archaea can be involved in the process. The geochemical profiles show Fe(II) peaks in the deep part of the sediments, indicating iron reduction. The iron reduction was shown also in the incubation experiment, where microbial involvement was evident. T~~The Spearman ~~correlation~~ analysis ~~pointed~~ points out several potential microbial players (both bacterial and archaeal) that correlate to the dissolved Fe(II) profiles (e.g. Bathyarchaeota, Acidobacteria and Chloroflexi). Moreover, ~~O~~our study emphasizes that this methanic iron reduction can occur even in sediments of oligotrophic seas such as the SE Mediterranean. We suggest that the availability of iron minerals for reduction is linked to intensive upward fluxes of methane and high sulfate-AOM rates that may produce available biomass or/and hydrogen, which fuel~~s~~ deeper microbial processes~~. T~~. The deep iron reduction may also be linked ~~also~~ to a cryptic sulfur cycle and iron-coupled AOM. ~~The geochemical conditions lead to three possible main microbial iron reduction pathways: a) H_2 or organic carbon oxidation, b) an active sulfur cycle, or c) iron driven AOM. To verify the main iron~~

~~reduction process at the methanogenic depth of the Mediterranean shelf sediments further incubations and microbial work are needed.~~

5 Author contribution

H.V and O.S designed research; B.H and O.S. were the PIs of the cruises; H.V, E.W and L.L performed research and analyzed the data; H.V, O.S, B.H, F.W, M.RB and L.L synthesized the data and wrote the paper.

The authors declare that they have no conflict of interest.

6 Acknowledgments

We thank the captain and crew of the R/V Shikmona and R/V Bat Galim from the Israel Oceanographic and Limnological Research for all their help during field sampling. Many thanks to E. Eliani-Russak for her technical assistance in the lab and to V. Boyko for her help with the reactive iron speciation procedure. We also thank all of Orit's lab member for their help. We would like to thank also to the anonymous reviewers for their helpful and constructive comments. This study was supported by the joint grant of Israel Science Foundation and the National Natural Science Foundation of China (ISF-NSFC) [grant number 31661143022 (FW) and 2561/16 (OS)]. Funding was provided to H. Vigderovich by the Mediterranean Sea Research Center of Israel.

References

Adler, M., Eckert, W. and Sivan, O.: Quantifying rates of methanogenesis and methanotrophy in Lake Kinneret sediments (Israel) using pore-water profiles, Limnol. Oceanogr., 56(4), 1525–1535, doi:10.4319/lo.2011.56.4.1525, 2011.

Almogi-Labin, A., Herut, B., Sandler, A., Gelman, F.: Fast changes at the Israeli continental shelf attributed to the damming of the Nile River., 2009.

Antler, G., Turchyn, A. V, Herut, B. and Sivan, O.: A unique isotopic fingerprint of sulfate-driven anaerobic oxidation of methane, , 43(7), 1–4, doi:10.1130/G36688.1, 2015.

Astrahan, P., Silverman, J., Gertner, Y. and Herut, B.: Spatial distribution and sources of organic matter and pollutants in the SE Mediterranean (Levantine basin) deep water sediments, Mar. Pollut. Bull., 116(1–2), 521–527, doi:10.1016/j.marpolbul.2017.01.006, 2017.

Bar-Or, I., Ben-Dov, E., Kushmaro, A., Eckert, W. and Sivan, O.: Methane-related changes in prokaryotes along geochemical profiles in sediments of Lake Kinneret (Israel), Biogeosciences, 12, 2847–2860, doi:10.5194/bg-12-2847-2015, 2015.

Bar-Or, I., Elvert, M., Eckert, W., Kushmaro, A., Vigderovich, H., Zhu, Q., Ben-Dov, E. and Sivan, O.: Iron-Coupled Anaerobic Oxidation of Methane Performed by a Mixed Bacterial-Archaeal Community Based on Poorly Reactive Minerals, Environ. Sci. Technol., 51, 12293–12301, doi:10.1021/acs.est.7b03126, 2017.

613 Bareket, M. M., Bookman, R., Katsman, R., de Stigter, H. and Herut, B.: The role of transport
614 processes of particulate mercury in modifying marine anthropogenic secondary sources, the case of
615 Haifa bay, Israel, Mar. Pollut. Bull., 105(1), 286–291, doi:10.1016/j.marpolbul.2016.02.014, 2016.

616 Beal, E. J., House, C. H. and Orphan, V. J.: Manganese-and Iron-Dependent Marine Methane
617 Oxidation, Science, 325(5937), 184–187, doi:10.1126/science.1169984, 2009.

618 Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R.,
619 Jùrgensen, B. B., Witte, U. and Pfannkuche, O.: A marine microbial consortium apparently mediating
620 anaerobic oxidation of methane, Lett. to Nat., 407(October), 623–626, 2000.

621 Bond, D. R., Lovley, D. R.: Reduction of Fe (III) oxide by methanogens in the presence and absence
622 of extracellular quinones, Environ. Microbiol., 4(2), 115–124,doi: 10.1046/j.1462-2920.2002.00279.x,
623 2002.

624 Bond, P. L., Hugenholtz, P., Keller, J. and Blackall, L. L.: Bacterial Community Structures of
625 Phosphate-Removing and Non-Phosphate-Removing Activated Sludges from Sequencing Batch
626 Reactors, , 61(5), 1910–1916, 1995.

627 Bottrell, S. H., Parkes, R. J., Cragg, B. a. and Raiswell, R.: Isotopic evidence for anoxic pyrite
628 oxidation and stimulation of bacterial sulphate reduction in marine sediments, J. Geol. Soc. London.,
629 157(4), 711–714, doi:10.1144/jgs.157.4.711, 2000.

630 Bray, M. S., Jieying, W., Reed, B. C., Kretz, C. B., Belli, K. M., Simister, R. L., Henny, C., Stewart, F.
631 J., DiChristina, T. J., Brandes, J. A., Fowle, D. A., Crowe, S. A. and Glass, J. B.: Shifting microbial
632 communities sustain multi-year iron reduction and methanogenesis in ferruginous sediment
633 incubations, Geobiology, doi: 10.1101/087783., 2018.

634 Brunner, B., Arnold, G. L., Røy, H., Müller, I. A. and Jørgensen, B. B.: Off Limits : Sulfate below the
635 Sulfate-Methane Transition, , 4(July), 1–16, doi:10.3389/feart.2016.00075, 2016.

636 Buongiorno, J., Turner, S., Webster, G., Asai, M., Shumaker, A. K., Roy, T., Weightman, A.,
637 Schippers, A. and Lloyd, K. G.: Interlaboratory quantification of Bacteria and Archaea in deeply buried
638 sediments of the Baltic Sea (IODP Expedition 347), FEMS Microbiol. Ecol., (93), 1–16,
639 doi:10.1093/femsec/fix007, 2017.

640 Cai, C., Leu, A. O., Jianhua, G. X., Yuexing, G., Zhao, F. J. and Tyson, G. W.: A methanotrophic
641 archaeon couples anaerobic oxidation of methane to Fe (III) reduction, ISME J., 8, 1929-1939,
642 doi:10.1038/s41396-018-0109-x, 2018.

643 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer,
644 N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E.,
645 Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R.,
646 Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R.: QIIME
647 allows analysis of high-throughput com- munity sequencing data., Nat. Methods, (7), 335–336,
648 doi:10.1038/nmeth.f.303, 2010.

649 Chen, W. H., Chen, S. Y., Kumar Khanal, S. and Sung, S.: Kinetic study of biological hydrogen
650 production by anaerobic fermentation, Int. J. Hydrogen Energy, 31(15), 2170–2178,
651 doi:10.4304/jcp.6.4.740-746, 2006.

652 Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters, Limnol.
653 Oceanogr., 454–458, doi:https://doi.org/10.4319/lo.1969.14.3.0454, 1969.

654 Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen concentrations in
655 methanogenic soils and sediments, FEMS Microbiol. Ecol., 28(3), 193–202, 1999.

656 Coolen, M. J. L., Cypionka, H., Sass, A. M., Sass, H. and Overmann, J.: Ongoing modification of
657 Mediterranean pleistocene sapropels mediated by prokaryotes, Science (80-.)., 296(June), 2407–2411,
658 doi:10.1126/science.1071893, 2002.

659 Crowe, S. A., Katsev, S., Leslie, K., Sturm, A., Magen, C., Nomosatryo, S., Pack, M. A., Kessler, J. D.,
660 Reeburgh, W. S., Robert S, J. A., Gonzalez, L., Douglas Haffner, G., Mucci, A., Sundby, B. and Fowle,
661 D. .: The methane cycle in ferruginous Lake Matano, Geobiology, 9, 61–78, doi:10.1111/j.1472-
662 4669.2010.00257.x, 2011.

663 Egger, M., Rasigraf, O., Sapart, C. J., Jilbert, T., Jetten, M. S. M., Röckmann, T., Van Der Veen, C.,
664 Bânda, N., Kartal, B., Ettwig, K. F. and Slomp, C. P.: Iron-mediated anaerobic oxidation of methane in
665 brackish coastal sediments, Environ. Sci. Technol., 49(1), 277–283, doi:10.1021/es503663z, 2014.

666 Egger, M., Kraal, P., Jilbert, T., Sulu-Gambari, F., Sapart, C. J., Röckmann, T. and Slomp, C. P.:
667 Anaerobic oxidation of methane alters diagenetic records of sulfur, iron and phosphorus in Black Sea
668 sediments, Biogeosciences Discuss., (March), 1–39, doi:10.5194/bg-2016-64, 2016.

669 Egger, M., Hagens, M., Sapart, C. J., Dijkstra, N., van Helmond, N. A. G. M., Mogollón, J. M.,
670 Risgaard-Petersen, N., van der Veen, C., Kasten, S., Riedinger, N., Böttcher, M. E., Röckmann, T.,
671 Jørgensen, B. B. and Slomp, C. P.: Iron oxide reduction in methane-rich deep Baltic Sea sediments,
672 Geochim. Cosmochim. Acta, 207, 256–276, doi:10.1016/j.gca.2017.03.019, 2017.

673 Emerson, S., Jahnke, R., Bender, M., Froelich, P. and Klinkhammer, G.: Early diagenesis in sediments
674 from the eastern equatorial pacific, i. pore water nutrient and carbonate results, Earth Planet. Sci. Lett.,
675 49, doi:https://doi.org/10.1016/0012-821X(80)90150-8, 1980.

676 Ettwig, K. F., Zhu, B., Speth, D., Keltjens, J. T., Jetten, M. S. M. and Kartal, B.: Archaea catalyze iron-
677 dependent anaerobic oxidation of methane, Proc. Natl. Acad. Sci., 113(45), 12792–12796,
678 doi:10.1073/pnas.1609534113, 2016a.

679 Ettwig, K. F., Zhu, B., Speth, D., Keltjens, J. T., Jetten, M. S. M. and Kartal, B.: Archaea catalyze iron-
680 dependent anaerobic oxidation of methane, Proc. Natl. Acad. Sci., 113(45), 12792–12796,
681 doi:10.1073/pnas.1609534113, 2016b.

682 Evans, P. N., Parks, D. H., Chadwick, G. L., Robbins, S. J., Orphan, V. J., Golding, S. D. and Tyson,
683 G. W.: Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric

metagenomics, Science, 350(6259), 434–438, doi:10.1126/science.aac7745, 2015.

Feng, X., Wang, Y., Zubin, R. and Wang, F.: Core Metabolic Features and Hot Origin of Bathyarchaeota, Engineering, doi:10.1016/j.eng.2019.01.011, 2019.

Froelich, P. N., Klinkhammer, G. P., Bender, M. L., Luedtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B. and Maynard, V.: Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: Suboxic diagenesis, Geochim. Cosmochim. Acta, 43, 1075–1090, doi:https://doi.org/10.1016/0016-7037(79)90095-4, 1979.

He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., Sievert, S. M. and Wang, F.: Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments, Nat. Microbiol., (April), 1–9, doi:10.1038/nmicrobiol.2016.35, 2016.

Herut, B., Almogi-Labin, A., Jannink, N. and Gertman, I.: The seasonal dynamics of nutrient and chlorophyll a concentrations on the SE Mediterranean shelf-slope, Oceanol. Acta, 23(7), 771–782, doi:10.1016/S0399-1784(00)01118-X, 2000.

Hinrichs, K., Hayes, J. M. and Sylva, S. P.: Methane-consuming archaebacteria in marine sediments, Lett. to Nat., 398(April), 802–805, 1999.

Holmkvist, L., Ferdelman, T. G. and Jørgensen, B. B.: A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark), Geochim. Cosmochim. Acta, 75(12), 3581–3599, doi:10.1016/j.gca.2011.03.033, 2011.

Iversen, N. and Jorgensen, B. B.: Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark), Limnol. Oceanogr., 1983(5), 944–955, 1985.

Jorgensen, B. B., Bottcher, M. E., Luschen, H., Neretin, L. N. and Volkov, I. I.: Anaerobic methane oxidation and a deep H_2S sink generate isotopically heavy sulfides in Black Sea sediments, Geochim. Cosmochim. Acta, 68(9), 2095–2118, doi:10.1016/j.gca.2003.07.017, 2004.

Kawaichi, S., Ito, N., Kamikawa, R., Sugawara, T., Yoshida, T. and Sako, Y.: Ardenticatena maritima gen. nov., sp. nov., a ferric iron- and nitrate-reducing bacterium of the phylum 'Chloroflexi' isolated from an iron-rich coastal hydrothermal field, and description of Ardenticatenia classis nov., Int. J. Syst. Evol. Microbiol., (63), 2992–3002, doi:10.1099/ijs.0.046532-0, 2013.

Knittel, K. and Boetius, A.: Anaerobic Oxidation of Methane : Progress with an Unknown Process, Annu. Rev. Microbiol., 63, 311-334, doi:10.1146/annurev.micro.61.080706.093130, 2009.

Kulichevskaya, I. S., Suzina, N. E., Rijpstra, W. I. C., Dedysh, S. N. and Damste, J. S. S.: a facultative anaerobe capable of dissimilatory iron reduction from subdivision 3 of the Acidobacteria, Int. J. Syst. Evol. Microbiol., (64), 2857–2864, doi:10.1099/ijs.0.066175-0, 2014.

Li, Y., Yu, S., Strong, J. and Wang, H.: Are the biogeochemical cycles of carbon , nitrogen , sulfur , and phosphorus driven by the " Fe III – Fe II redox wheel " in dynamic redox environments ?, J. soils

719 sediments, 12(5), 683–693, doi:10.1007/s11368-012-0507-z, 2012.

720 Lilley, M. D., Baross, J. A. and Gordon, L. I.: Dissolved hydrogen and methane in Saanich Inlet,
721 British Columbia, Deep Sea Res. Part A, Oceanogr. Res. Pap., 29(12), 1471–1484, doi:10.1016/0198-
722 0149(82)90037-1, 1982.

723 Liu, D., Dong Hailiang, H., Bishop, M. E., Wang, H., Agrawal, A., Tritschler, S., Eberl, D. D. and Xie,
724 S.: Reduction of structural Fe(III) in nontronite by methanogen Methanosarcina barkeri, Geochim.
725 Cosmochim. Acta, 75(4), 1057–1071, doi:10.1016/j.gca.2010.11.009, 2011.

726 Lloyd, K. G., Schreiber, L., Petersen, D. G., Kjeldsen, K. U., Lever, M. A., Steen, A. D., Stepanauskas,
727 R., Richter, M., Kleindienst, S., Lenk, S., Schramm, A. and Jørgensen, B. B.: Predominant archaea in
728 marine sediments degrade detrital proteins, Nature, 496(7444), 215–218, doi:10.1038/nature12033,
729 2013.

730 Lovley, Derek R. and Goodwin, S.: Hydrogen concentrations as an indicator of the predominant
731 terminal electron-accepting reactions in aquatic sediments, Geochim. Cosmochim. Acta, 52, 2993–
732 3003, doi:10.1016/0016-7037(88)90163-9, 1988.

733 Lovley, D. R.: Dissimilatory Fe(III) and Mn(IV) reduction., Microbiol. Rev., 55(2), 259–287, 1991.

734 Lovley, D. R. and Phillips, E. J.: Novel mode of microbial energy metabolism: organic carbon
735 oxidation coupled to dissimilatory reduction of iron or manganese., Appl. Environ. Microbiol., 54(6),
736 1472–1480, doi:10.1103/PhysRevLett.50.1998, 1988.

737 Lovley, D. R. and Phillips, E. J. P.: Organic matter mineralization with reduction of ferric iron in
738 anaerobic sediments., Appl. Environ. Microbiol., 51(4), 683–689, doi:10.1080/01490458709385975,
739 1986.

740 Lovley, D. R., Coates, J. D., BluntHarris, E. L., Phillips, E. J. P. and Woodward, J. C.: Humic
741 substances as electron acceptors for microbial respiration, Nature, 382(6590), 445–448,
742 doi:10.1038/382445a0, 1996.

743 März, C., Hoffmann, J., Bleil, U., Lange, G. J. De and Kasten, S.: Diagenetic changes of magnetic and
744 geochemical signals by anaerobic methane oxidation in sediments of the Zambezi deep-sea fan (SW
745 Indian Ocean), Mar. Geol., 255(3–4), 118–130, doi:10.1016/j.margeo.2008.05.013, 2008.

746 März, C., Riedinger, N., Sena, C. and Kasten, S.: Phosphorus dynamics around the sulphate-methane
747 transition in continental margin sediments : Authigenic apatite and Fe (II) phosphates, Mar. Geol.,
748 404(July), 84–96, doi:10.1016/j.margeo.2018.07.010, 2018.

749 McGlynn, S. E., Chadwick, G. L., Kempes, C. P. and Orphan, V. J.: Single cell activity reveals direct
750 electron transfer in methanotrophic consortia, Nature, 526(7574), 531–535, doi:10.1038/nature15512,
751 2015.

752 Meng, J., Xu, J., Qin, D., He, Y., Xiao, X. and Wang, F.: Genetic and functional properties of
753 uncultivated MCG archaea assessed by metagenome and gene expression analyses, ISME J., 8(3), 650–

754 9, doi:10.1038/ismej.2013.174, 2014.

755 Milkov, A. V: Global estimates of hydrate-bound gas in marine sediments : how much is really out
756 there ?, Earth-Science Rev., 66, 183–197, doi:10.1016/j.earscirev.2003.11.002, 2004.

757 Milkov, A. V and Sassen, R.: Economic geology of offshore gas hydrate accumulations and provinces,
758 Mar. Pet. Geol., 19, 1–11, doi:doi.org/10.1016/S0264-8172(01)00047-2, 2002.

759 Mountfort, D. O. and Asher, R. A.: Role of Sulfate Reduction Versus Methanogenesis in Terminal
760 Carbon Flow in Polluted Intertidal Sediment of Waimea Inlet, Nelson, New Zealand, Appl. Environ.
761 Microbiol., 42(2), 252–258, doi: 0099-2240/81/080252-07$02.00/0, 1981.

762 Mountfort, D. O., Asher, R. a, Mays, E. L. and Tiedje, J. M.: Carbon and electron flow in mud and
763 sandflat intertidal sediments at delaware inlet, nelson, new zealand., Appl. Environ. Microbiol., 39(4),
764 686–94 doi: 0099-2240/80/04-0686/09$02.00/0, 1980.

765 Moutin, T. and Raimbault, P.: Primary production , carbon export and nutrients availability in western
766 and eastern Mediterranean Sea in early summer 1996 (MINOS cruise), J. Mar. Syst., 34, 273–288,
767 doi: doi.org/10.1016/S0924-7963(02)00062-3, 2002.

768 Nir, Y.: Recent sediments of the Israel Mediterranean continental shelf and slope, University of
769 Gothenburg. http://hdl.handle.net/2077/13267, 1984.

770 Niu, M., Fan, X., Zhuang, G., Liang, Q. and Wang, F.: Methane-metabolizing microbial communities
771 in sediments of the Haima cold seep area , northwest slope of the South China Sea, FEMS Microbiol.
772 Ecol., (March), 1–13, doi:10.1093/femsec/fix101, 2017.

773 Nordi, K. Á., Thamdrup, B. and Schubert, C. J.: Anaerobic oxidation of methane in an iron-rich Danish
774 freshwater lake sediment, Limnol. Oceanogr., 58(2), 546–554, doi:10.4319/lo.2013.58.2.0546, 2013.

775 Novelli, P. C., Scranton, M. I. and Michener, R. H.: Hydrogen Distributions in Marine Sediments
776 Hydrogen distributions, , 32(3), 565–576, doi:10.4319/lo.1987.32.3.0565, 1987.

777 Nusslein, B., Eckert, W. and Conrad, R.: Stable isotope biogeochemistry of methane formation in
778 profundal sediments of Lake Kinneret (Israel), , 48(4), 1439–1446, doi:10.4319/lo.2003.48.4.1439,
779 2003.

780 Oni, O., Miyatake, T., Kasten, S., Richter-Heitmann, T., Fischer, D., Wagenknecht, Laura Kulkarni, A.,
781 Blumers, M., Shylin, S. I., Ksenofontov, Vadim Costa, B. F. O., Klingelhöfer, G. and Friedrich, M. W.:
782 Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic
783 sediments of the Helgoland mud area , North Sea, Front. Microbiol., 6(May), 1–15,
784 doi:10.3389/fmicb.2015.00365, 2015.

785 Orphan, V. J., House, C. H. and Hinrichs, K.: Methane-Consuming Archaea Revealed by Directly
786 Coupled Isotopic and Phylogenetic Analysis, Science (80-.)., 293(July), 484–488,
787 doi:10.1126/science.1061338, 2001.

788 Paull, C. K., Normark, W. R., Ussler, W., Caress, D. W. and Keaten, R.: Association among active
789 seafloor deformation , mound formation , and gas hydrate growth and accumulation within the seafloor
790 of the Santa Monica Basin , offshore California, Mar. Geol., 250, 258–275,
791 doi:10.1016/j.margeo.2008.01.011, 2008.

792 Poulton, S. W. and Canfield, D. E.: Development of a sequential extraction procedure for iron:
793 Implications for iron partitioning in continentally derived particulates, Chem. Geol., 214(3–4), 209–
794 221, doi:10.1016/j.chemgeo.2004.09.003, 2005.

795 Riedinger, N., Formolo, M. J., Lyons, T. W., Henkel, S., Beck, A. and Kasten, S.: An inorganic
796 geochemical argument for coupled anaerobic oxidation of methane and iron reduction in marine
797 sediments, Geobiology, 12(2), 172–181, doi:10.1111/gbi.12077, 2014.

798 Riedinger, N., Brunner, B., Krastel, S., Arnold, G. L., Wehrmann, L. M., Formolo, M. J., Beck, A.,
799 Bates, S. M., Henkel, S., Kasten, S. and Lyons, T. W.: Sulfur Cycling in an Iron Oxide-Dominated ,
800 Dynamic Marine Depositional System : The Argentine Continental Margin, Front. earth Sci., 5(May),
801 doi:10.3389/feart.2017.00033, 2017.

802 Roden, E. E.: Fe(III) Oxide Reactivity Toward Biological versus Chemical Reduction, Environ. Sci.
803 Technol., 37(7), 1319–1324, doi:10.1021/es026038o, 2003.

804 Roden, E. E.: Geochemical and microbiological controls on dissimilatory iron reduction, Comptes
805 Rendus - Geosci., 338(6–7), 456–467, doi:10.1016/j.crte.2006.04.009, 2006.

806 Roden, E. E. and Wetzel, R. G.: Organic carbon oxidation and suppression of methane production by
807 microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments, Limnol.
808 Oceanogr., 41(8), 1733–1748, doi:10.4319/lo.1996.41.8.1733, 1996.

809 Roden, E. E. and Wetzel, R. G.: Kinetics of microbial Fe (III) oxide reduction in freshwater wetland
810 sediments, Limnol. Ocean., 47(1), 198–211, doi:10.4319/lo.2002.47.1.0198, 2002.

811 Rooze, J., Egger, M., Tsandev, I. and Slomp, C. P.: Iron-dependent anaerobic oxidation of methane in
812 coastal surface sediments: potential controls and impact, Limnol. Oceanogr., (1),
813 doi:10.1002/lno.10275, 2016.

814 Salem, D. M. S. A., Khaled, A. and Nemr, A. El: Assessment of pesticides and polychlorinated
815 biphenyls (PCBs) in sediments of the Egyptian Mediterranean Coast, Egypt. J. Aquat. Res., 39(3),
816 141–152, doi:10.1016/j.ejar.2013.11.001, 2013.

817 Sandler, A. and Herut, B.: Composition of clays along the continental shelf off Israel: Contribution of
818 the Nile versus local sources, Mar. Geol., 167(3–4), 339–354, doi:10.1016/S0025-3227(00)00021-9,
819 2000.

820 Schattner, U., Lazar, M., Harari, D. and Waldmann, N.: Active gas migration systems offshore northern
821 Israel, first evidence from seafloor and subsurface data, Cont. Shelf Res., 48, 167–172,
822 doi:10.1016/j.csr.2012.08.003, 2012.

Scheller, S., Yu, H., Chadwick, G. L., McGlynn, S. E. and Orphan, V. J.: Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction, Science, 351(6274), 1754–1756, doi:10.1126/science.aad7154, 2016.

Segarra, K. E. a, Comerford, C., Slaughter, J. and Joye, S. B.: Impact of electron acceptor availability on the anaerobic oxidation of methane in coastal freshwater and brackish wetland sediments, Geochim. Cosmochim. Acta, 115, 15–30, doi:10.1016/j.gca.2013.03.029, 2013.

Sela-Adler, M., Herut, B., Bar-Or, I., Antler, G., Eliani-Russak, E., Levy, E., Makovsky, Y. and Sivan, O.: Geochemical evidence for biogenic methane production and consumption in the shallow sediments of the SE Mediterranean shelf (Israel), Cont. Shelf Res., 101, 117–124, doi:10.1016/j.csr.2015.04.001, 2015.

Sivan, O., Schrag, D. P. and Murray, R. W.: Rates of methanogenesis and methanotrophy in deep-sea sediments, Geobiology, 5(2), 141–151, doi:10.1111/j.1472-4669.2007.00098.x, 2007.

Sivan, O., Adler, M., Pearson, A., Gelman, F., Bar-Or, I., John, S. G. and Eckert, W.: Geochemical evidence for iron-mediated anaerobic oxidation of methane, Limnol. Oceanogr., 56(4), 1536–1544, doi:10.4319/lo.2011.56.4.1536, 2011.

Sivan, O., Antler, G., Turchyn, A. V., Marlow, J. J. and Orphan, V. J.: Iron oxides stimulate sulfate-driven anaerobic methane oxidation in seeps, Proc. Natl. Acad. Sci., 111, E4139–E4147, doi:10.1073/pnas.1412269111, 2014.

Sivan, O., Shusta, S. and Valentine, D. L.: Methanogens rapidly transition from methane production to iron reduction, Geobiology, 190–203, doi:10.1111/gbi.12172, 2016.

Slomp, C. P., Mort, H. P., Jilbert, T., Reed, D. C., Gustafsson, B. G. and Wolthers, M.: Coupled Dynamics of Iron and Phosphorus in Sediments of an Oligotrophic Coastal Basin and the Impact of Anaerobic Oxidation of Methane, PLoS One, 8(4), doi:10.1371/journal.pone.0062386, 2013.

Song, Z., Wang, F., Zhi, X., Chen, J., Zhou, E., Liang, F., Xiao, X., Tang, S., Jiang, H., Zhang, C. L., Dong, H. and Li, W.: Bacterial and archaeal diversities in Yunnan and Tibetan hot springs , China, , 15, 1160–1175, doi:10.1111/1462-2920.12025, 2013.

Stookey, L. L.: Ferrozine-a new spectrophotometric reagent for iron, Anal. Chem., 42(7), 779–781, doi:10.1021/ac60289a016, 1970.

Tan, S., Liu, J., Fang, Y., Hedlund, B. P., Lian, Z., Li, L. H. J., Li, L. H. W., Dong, H. J. H. and Shu, W.: Insights into ecological role of a new deltaproteobacterial order Candidatus Acidulodesulfobacterales by metagenomics and metatranscriptomics, ISME J., doi:10.1038/s41396-019-0415-y, 2019.

Treude, T., Krause, S., Maltby, J., Dale, A. W., Coffin, R. and Hamdan, L. J.: Sulfate reduction and methane oxidation activity below the sulfate-methane transition zone in Alaskan Beaufort Sea continental margin sediments: Implications for deep sulfur cycling, Geochim. Cosmochim. Acta, 144,

217–237, doi:10.1016/j.gca.2014.08.018, 2014.

Walters, W., Hyde, E. R., Berg-lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J. A. and Jansson, J. K.: Transcribed Spacer Marker Gene Primers for Microbial Community Surveys, Am. Soc. Microbiol., 1(1), 1–10, doi:10.1128/mSystems.00009-15, 2015.

Wang, Z., Guo, F., Liu, L. and Zhang, T.: Evidence of Carbon Fixation Pathway in a Bacterium from Candidate Phylum SBR1093 Revealed with Genomic Analysis, PLoS One, 9(10), doi:10.1371/journal.pone.0109571, 2014.

Weber, K. A., Urrutia, M. M., Churchill, P. F., Kukkadapu, R. K. and Roden, E. E.: Anaerobic redox cycling of iron by freshwater sediment microorganisms, Environ. Microbiol., 8(1), 100–113, doi:10.1111/j.1462-2920.2005.00873.x, 2006.

Whiticar, M. J.: Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane, Chem. Geol., 161(1–3), 291–314, doi:10.1016/S0009-2541(99)00092-3, 1999.

Wurgaft, E., Findlay, A. J., Vigderovich, H., Herut, B. and Sivan, O.: Sulfate reduction rates in the sediments of the Mediterranean continental shelf inferred from combined dissolved inorganic carbon and total alkalinity profiles, Mar. Chem., (February), 1–11, doi:10.1016/j.marchem.2019.03.004, 2019.

Yan, Z., Joshi, P., Gorski, C. A. and Ferry, J. G.: A biochemical framework for anaerobic oxidation of methane driven by Fe(III)-dependent respiration, Nat. Commun., (Iii), 1–9, doi:10.1038/s41467-018-04097-9, 2018.

Yu, T., Wu, W., Liang, W., Alexander, M. and Hinrichs, K.: Growth of sedimentary Bathyarchaeota on lignin as an energy source, Proc. Natl. Acad. Sci., 115(23), 6022–6027, doi:10.1073/pnas.1718854115, 2018.

Zehnder, a. J. B. and Brock, T. D.: Anaerobic methane oxidation: Occurrence and ecology, Appl. Environ. Microbiol., 39(1), 194–204 http://aem.asm.org/content/39/1/194, 1980.

Zhang, C. L. and Lanoil, B.: Geomicrobiology and biogeochemistry of gas hydrates and cold seeps, Chemocal Geol., 205, 187–194, doi:10.1016/j.chemgeo.2004.01.001, 2004.

Zhang, J., Dong, H., Liu, D., Fischer, T. B., Wang, S. and Huang, L.: Microbial reduction of Fe(III) in illite–smectite minerals by methanogen Methanosarcina mazei, Chem. Geol., 292–293, 35–44, doi:10.1016/j.chemgeo.2011.11.003, 2012.

Zhang, J., Dong, H., Liu, D. and Agrawal, A.: Microbial reduction of Fe(III) in smectite minerals by thermophilic methanogen Methanothermobacter thermautotrophicus, Geochim. Cosmochim. Acta, 106, 203–215, doi:10.1016/j.gca.2012.12.031, 2013.

Zhou, Z., Pan, J., Wang, F., Gu, J. and Li, M.: Bathyarchaeota : globally distributed metabolic generalists in anoxic environments, FEMS Microbiol. Rev., (May), 639–655, doi:10.1093/femsre/fuy023, 2018.

892

893

Table 1: Sampling details: dates, water depths and locations of cores.

Date	station	water depth (m)	Latitude	Longtitude
August 14, 2013	PC-5	87	32°55.47'	34°54.01'
	PC-3	81	32°55.29'	34°54.14'
February 6, 2014	PC-3	82	32°55.30'	34°54.14'
January, 2015	PC-3	82	32°55.30'	34°54.14'
June 9, 2015	SG-1	89	32°57.87'	34°55.30'
September 17, 2015	SG-1	84	32°57.91'	34°55.27'
January 24, 2017	SG-1	85	32°57.51'	34°55.15'

Table 2: Summary of reactive iron extraction procedure (after Poulton and Canfield, 2005)~~s~~.

Extractant	Target compounds	Analyzed species	Formula	Shaking time (h)
Magnesium chloride	Ion-exchangeble Fe(II)	Adsorbed ferrous iron	Fe^{2+}	2
Sodium acetate	Iron carbonates	Siderite Ankerite	$FeCO_3$ $Ca(Fe^{+2},Mg^{+2},Mn^{+2})(CO_3)_2$	24
Hydroxylamine hydrochloride	"Easily reducible" Iron(hydr)oxides	Ferrihydrite, Lepidicrocite	$Fe^{3+}{}_2O_3*0.5(H_2O)$ γ-FeOOH	48
Sodium dithionite	"Reducible" oxides	Goethite, Hematite, Akageneite	α-FeOOH Fe_2O_3 β-FeOOH	2
Ammonium oxalate	Poorly crystalline	Magnetite	Fe_3O_4	6

Figures captions:

Figure 1: A map of the study area in the SE Mediterranean with the location of the three stations that were sampled: SG-1, PC-3 and PC-5 (after Wurgaft et al., 2019)

Figure ~~1~~2: Geochemical pore-water profiles of sediment cores collected from Station SG-1 (top~~),~~) and Station PC-3 ~~(middle) and PC-5~~ (bottom) in the SE Mediterranean. The profiles are divided roughly to three zones according to the dominant processes: upper microbial iron and sulfate reduction, sulfate-methane transition zone (SMTZ), and the methan~~ogen~~ic zone at the deep part. The dashed line in the CH_4 graph at SG-1 station represents the CH_4 saturation value in the pore-water. The following iron minerals profiles of stations SG-1 and PC-3 are from the September 2015 and January 2015 cruise (respectively): siderite, ankerite ( ), ferrihydrite, lepidocrocite ( ) goethite, hematite, akaganeite ( ), magnetite ( ), pyrite ( ) and total reactive iron ( ). The error bars for CH_4 are presented where duplicate sediment samples were collected. The error bars for Fe(II), $\delta^{13}C_{DIC}$ and H_2 are presented where measurement repetition of each sample was taken (at least twice). The analytical errors were smaller than the symbols.

Figure ~~2~~3: Sedimentary depth profiles of bacterial and archaeal 16S rRNA and mcrA functional genes of S~~S~~tation SG-1 from January 2017, divided to three zones (as described in figure 2).~~.~~ Triplicates were ~~made for~~produced from each sample with error bars smaller than the symbols.

Figure ~~3~~4: Phyla level classification of bacterial (a) and archaeal (b) diversity in the sediments of Station SG-1 from January 2017.

Figure ~~4~~5: Dissolved Fe(II) results of the sediment slurry incubation experiment ~~from SG-1 core~~. The sediment was collected from Station SG-1 on January 2017 from sediment depth of ~~260~~ 265-285 cm. The error bars were smaller than the symbol.

Figure ~~5~~6: The relationship between dissolved Fe(II) concentrations and methane concentrations in zone 3 ~~sediments at~~of (a) Station SG-1 and (b) Station PC-3. An inverse association is observed between the two species, suggesting a relationship of competition or ~~Fe(III)~~iron-coupled ~~anaerobic methane oxidation~~AOM.

Figures:

Figure 1

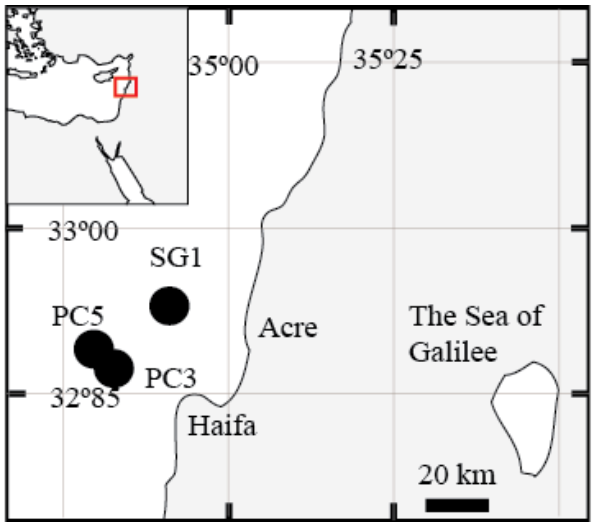
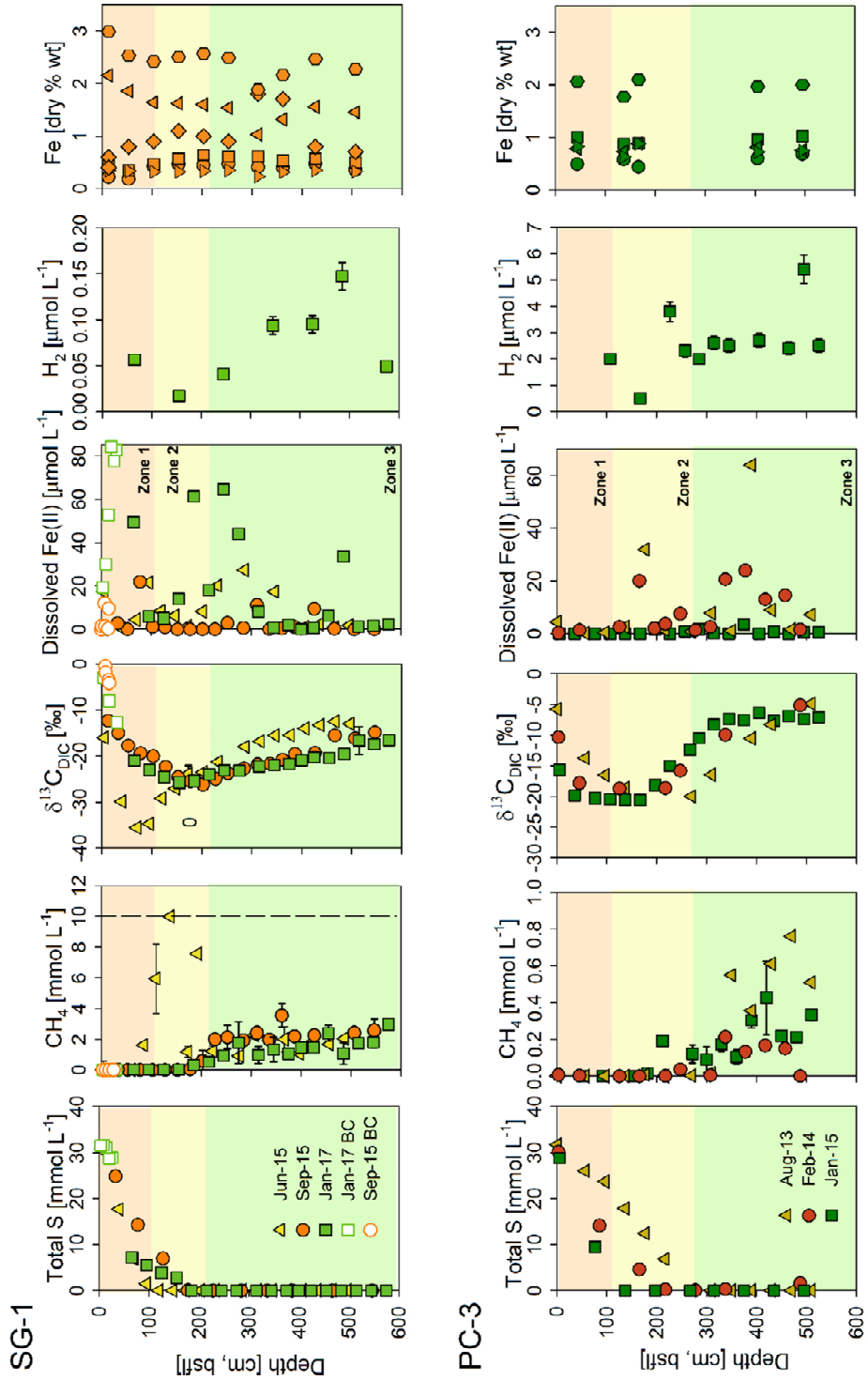


Figure 2


SG-1
PC-3
Total S [mmol L-1]
CH4 [mmol L-1]
δ13CDIC [‰]
Dissolved Fe(II) [µmol L-1]
H2 [µmol L-1]
Fe [dry % wt]
Depth [cm, bsf]
Zone 1
Zone 2
Zone 3
Jun-15
Sep-15
Jan-17
Jan-17 BC
Sep-15 BC
Aug-13
Feb-14
Jan-15

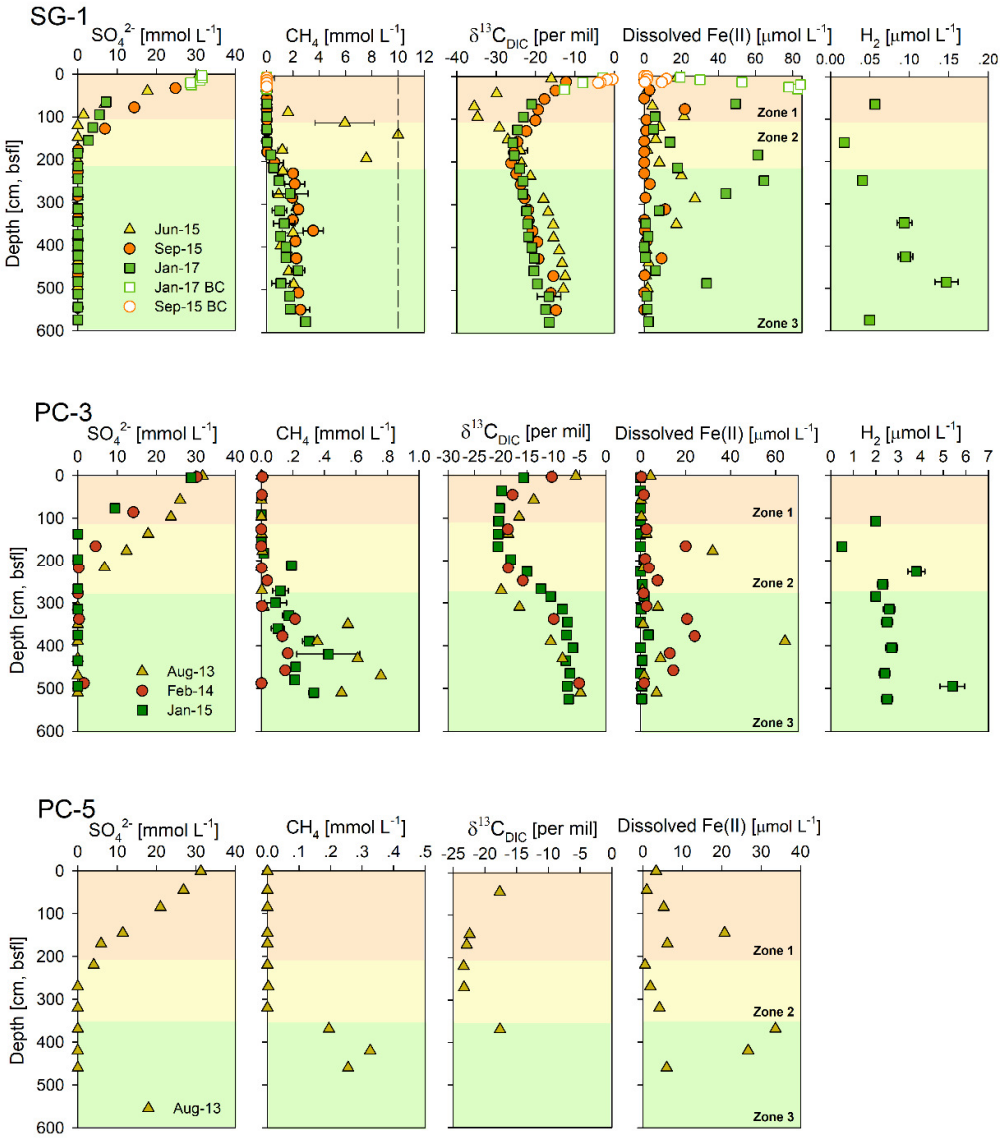
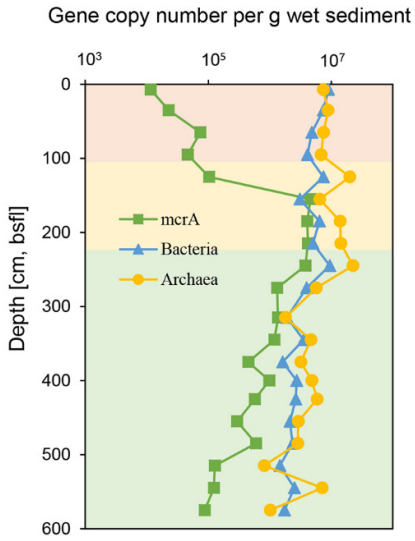
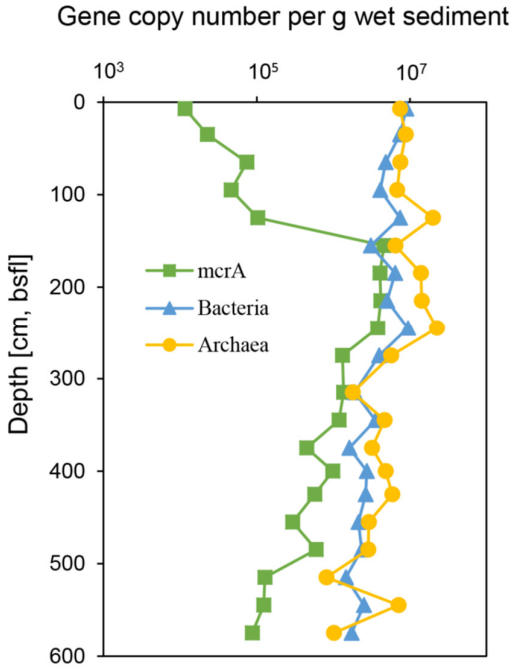

SG-1
SO4 2- [mmol L-1]
CH4 [mmol L-1]
δ13CDIC [per mil]
Dissolved Fe(II) [µmol L-1]
H2 [µmol L-1]
Depth [cm, bsfl]
Jun-15
Sep-15
Jan-17
Jan-17 BC
Sep-15 BC
Zone 1
Zone 2
Zone 3
PC-3
Aug-13
Feb-14
Jan-15
PC-5

Figure 3

938


Gene copy number per g wet sediment
10³
10⁵
10⁷
0
100
200
300
400
500
600
Depth [cm, bsfl]
mcrA
Bacteria
Archaea

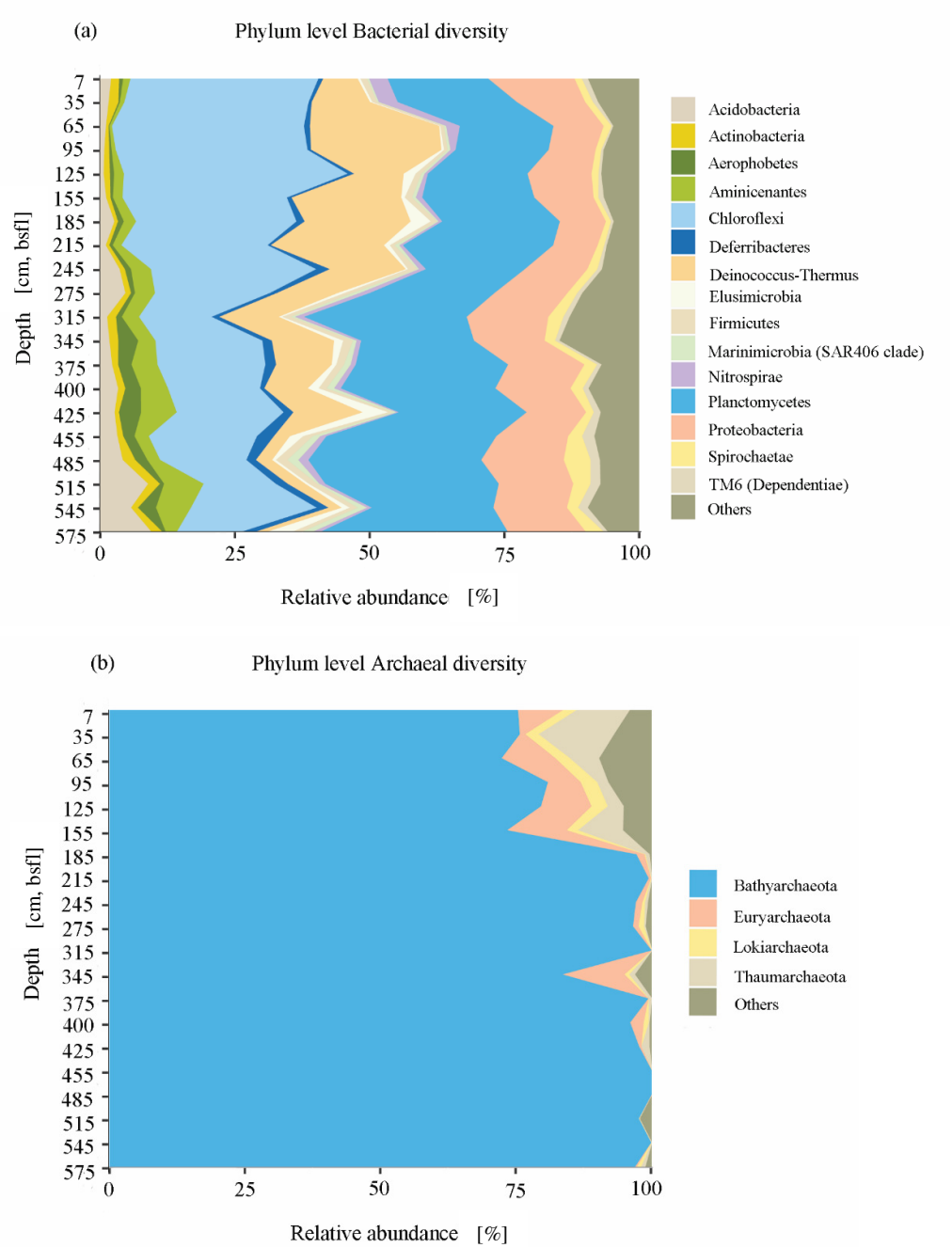

Gene copy number per g wet sediment
10³
10⁵
10⁷
0
100
200
300
400
500
600
Depth [cm, bsfl]
mcrA
Bacteria
Archaea

939

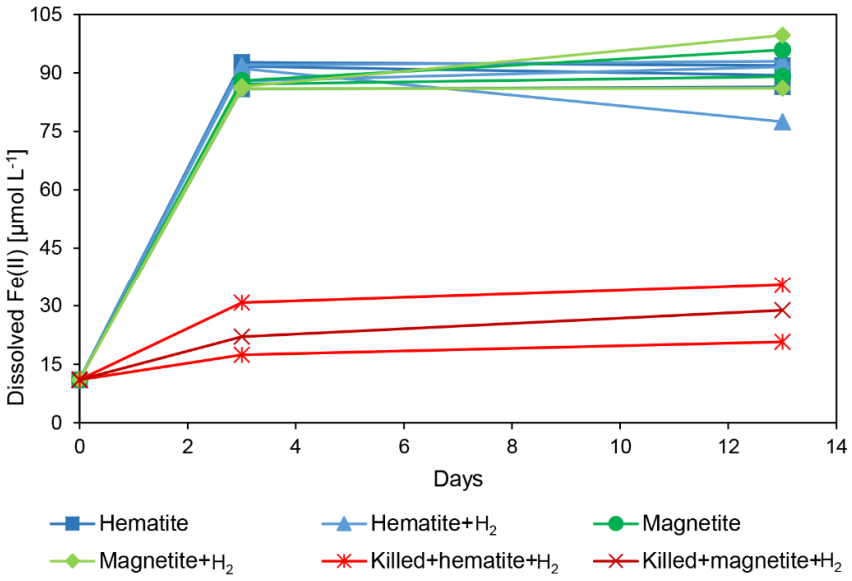
940

941 **Figure 4**

942



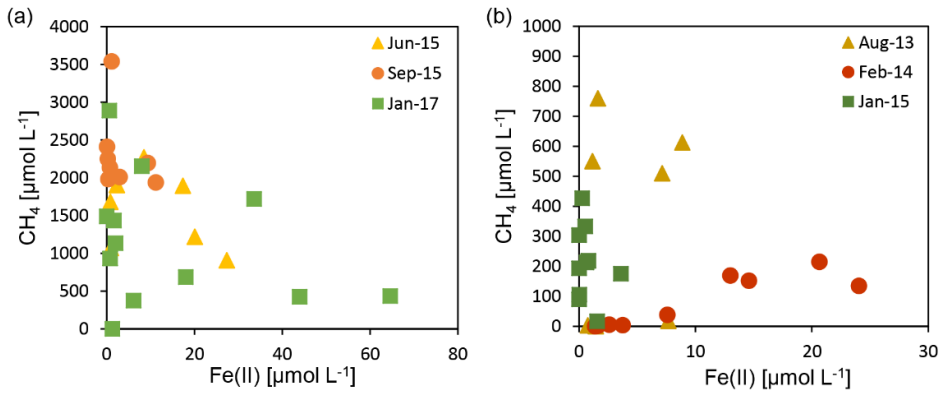
943 **Figure 5**



944

945

946 **Figure 6**



947

948

949

950

951

952

953

954