

July 8 2019

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

We would like to thank the editor and the two reviewers for their supportive and constructive comments again.

Response to comments from anonymous reviewer #1 (our response in blue):

We thank the reviewer for the positive review and address below the few technical issues that were left.

L28 and Figure 3: italicize *mcrA*  
Was corrected.

L89, L373: add hyphen between "iron" and "reducing"  
Was added.

L100-L101: there is a grammatical error here that needs to be fixed: "marine's methanic zone"  
Was corrected.

L105: add a subject and verb to sentence: "This \_\_\_\_\_ is \_\_\_\_\_ by...:"  
The sentence was revised to: "The microbial iron reduction is observed by using both geochemical..."

L313: the italics of "uncultured Bathyarchaeota" are different than all the other phyla listed -- format consistently  
Was corrected.

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

The manuscript by Vigderovich represents the revised version of a manuscript that I have reviewed previously. Several of the issues pointed out in my previous review have been addressed by the authors. However, there are still some points and issues that need attention. It is striking that there are still many imprecise statements and numerous typos. I have corrected only a few (see specific comments below). In particular, the English still needs some substantial overhaul and careful polishing and check by a native speaker. Also some of the figures/plots (in particular Fig. 2 and caption) are insufficiently labeled and references cited in the text are missing in the list of references.

We thank the reviewer for the detailed and constructive review again. We are sorry that there were still typos, as the manuscript had been edited twice. We corrected the additional comments, and the ms was edited again by native English colleague. We will be glad to send the revised version to additional editing of the journal if needed.

Besides these formal flaws the main points are: 1) The Discussion chapter still has many statements that are much too general and it is not clear how these relate to your data and to previous work. References are often missing to support the statements made;

We accept this comment and improved the discussion chapter and corrected the statements according to the specific comments below.

2) Please expand and specify and discuss in detail your geochemical evidence showing the contributions of methane from deeper sources (the „gas front“) and that being produced in situ in the shallow sediments.

We accept this comment and expended our discussion on the source of the methane.

To conclude, I find this study and manuscript very interesting and definitely suitable for Biogosciences. However, it needs another intense round of clarification of the scientific discussion and formal polishing before I can recommend publication.

We thank the reviewer again, and improved the manuscript according to the specific comments below.

Specific comments

L. 28: microbially mediated

Corrected

Ls. 33/34: I do not fully understand this last sentence of the abstract. What do you mean with „deeper microbial activity“ and „methanic iron reduction“? I also do not agree with the statement that (what I think you suggest) Fe reduction driven by methane oxidation is observed. Of course you observe Fe reduction within the methanic zone, which leads to liberation of Fe<sup>2+</sup> into the pore water. Please, rephrase and specify accordingly.

By saying "deep microbial activity" we mean the microbial activity deeper than the sediment-water interface. In addition, we did not intend to state that there is iron-coupled AOM here (this possibility is discussed in the discussion chapter). We clarified the sentence to: "We suggest that intensive upward migration of methane in the sedimentary column and its oxidation by sulfate may fuel the microbial activity in the SMTZ. The biomass, created by this microbial activity, can be used further below by the iron reducers at the methanic zone in the sediments of the SE Mediterranean."

Ls. 38, 40: add e.g. in brackets and also cite work by Lovley et al. here who have performed key studies on microbial Fe reduction.

The brackets and the following references were added: Lovley and phillips, 1986; Lovley et al., 1987; Lovley and phillips, 1988; Lovley 1997.

L. 57: add e.g. in brackets; Niewöhner et al. (1998) is missing in the list of references.

Sorry, it was somehow by mistake deleted, and now added.

L. 54: add Hoehler et al. (1994) here

Sorry, it was again somehow by mistake deleted, and now added.

Ls. 68 ff: Sentence is odd. Please rephrase. Furthermore, not all of the studies cited here, have performed modelling. Please, check carefully and correct accordingly.

The sentence was rephrased to: "This process in marine sediments was shown through incubation experiments in marine seeps sediments (Beal et al., 2009; Sivan et al., 2014). It was also suggested to exist mainly through geochemical profiles in deep sea sediments and their modeling (Sivan et al., 2007; März et al., 2008; Riedinger et al., 2014)..."

L. 81: ... „under“ natural conditions ....

Corrected

L. 100: I would suggest to rephrase to: „Despite .... the link between the biogeochemical cycling of iron and methane in the methanic zone of marine sediments ....“

Was rephrased as suggested.

L. 103: ... we report the observation of microbial iron reduction ....

Corrected.

L.105: What are „microbial profiles“? Please specify – profiles of what precisely?

The microbial profiles are the 16S rRNA and qPCR 16S of bacteria and archaea and the functional gene *mcrA*. This is specified in the revised version.

L. 116: is composed of

Corrected.

L. 121: „contents“ inspite of levels

Was changed.

L. 123: I do not agree that TOC contents of more than 1 wt% - as given here – can be described as low or underlying oligotrophic areas. I find this rather high. Please, also specify whether you speak of trophic level of the surface waters (e.g. oligotrophic) or TOC contents of the sediments.

We revised the sentence to clarify that the TOC levels in sediments of the Levantine are lower than in the western basin and the Nile delta. Thus, the term “relatively low” was removed. We also clarified and referred on the oligotrophic nature of the surface water (photic zone) in line 114 (line 119 in the revised MS). It should be noted that the surface water certainly accounts as oligotrophic.

Ls. 125 ff. and 330 ff.: The statement and description of the gas front appear rather imprecise to me. What kind of gas front are you referring to? Why do you put it in brackets? Do you mean free gas? At which sediment depth was this gas front/free gas found. Furthermore, it is not clear to me whether the methane samples you describe as being of biogenic in origin come from this gas front depth or below the gas front or from shallower sediments overlying this gas front. If yes, what is then the link? Does the shallow methane analysed originate from the deeper sediments below the described gas front? Please specify.

We are referring to the gas front that was found and described by Schattner et al. (2012). The gas front is the top of a free gas zone in the sediment, which is limited to the shelf in area of about 72 km<sup>2</sup> from few to tens meters below the sea floor. The origin of the gas was speculated in Schattner et al. (2012), however it has not been sampled for isotopic values. The methane that was sampled by us was from the sediments overlying this gas front (~1-5 m depth). This shallow sediment methane is probably biogenic based on its low  $\delta^{13}\text{C}_{\text{CH}_4}$  values and the high C1/C2 ratio (Sela-Adler et al., 2015). The microbial and geochemical pore-water profiles indicate also that at least part of it is produced *insitu*. However, we do not know how much of the methane in the shallow sediments is originated from the gas front and how much of it is produced *insitu*. This was clarified and added to the text.

L. 130: delete „seafloor“

Was deleted.

L. 135: What do you mean with „methanogenesis characteristics“?

The sentence was rephrased to: "... and the possibility for methanogenesis to occur..."

Ls. 136 ff. and 171 ff.: How was porosity determined? This is needed to calculate methane concentrations in pore-water.

Porosity was determined by drying at 60°C wet sediment samples from different depths, until there was no weight loss (~48 hr). The porosity was calculated as the weight loss from the initial weight of the samples. The porosity was indeed considered in the methane concentration in the pore-water calculations. This was added to the text.

L. 137: „anoxic“ instead of anaerobic

Corrected.

L. 153: apparant „from (instead of in)

Changed as suggested.

L- 156: ... to reach „a“ 1:1 .....

Corrected.

L. 176: with „a“ detection limit

Corrected.

Ls. 179/180: ... the measured total sulfur concentrations in „pore water“ were ....

The phrase "pore-water" was added.

L. 182: delete „Several“

Was deleted

Ls. 189 ff.: How was the sediment dried? Was it freeze-dried. Please specify.

Dried in the oven at 60°C. We added this to the text.

Ls. 195 ff.: Where/in which figures are these data - i.e. the results of sequential extraction – plotted. At least I could not find them in Fig. 2.

It is the first graph on the right-hand side. We revised the figure to clarify it (graphs (f) and (l) in the revised version).

L. 196: The profile of pyrite was taken from Wurgaft et al. (2019).

The citation was there, but the sentence was changed as suggested.

Ls. 222 ff. This sentence needs to be rephrased because the syntax is odd. How can you investigate the „source“ of a process? This makes no sense.

The sentence was rephrased to: "... in order to characterize the iron reduction process in the methanic zone of the SE Mediterranean continental shelf and to identify its potential sources."

Ls. 224: The next sentence also makes no sense. How can several profiles show complete depletion of total sulfur at one station?! (You say: The pore-water profiles ....)

The sentence was rephrased to: "The pore-water profiles at Station SG-1 (Fig. 2) show complete depletion of total sulfur at approximately 150 cm depth in all extracted cores."

L. 231 and throughout the manuscript: I do not like the term „traditional“ iron reduction zone. This is imprecise and makes no sense. Better speak of the „upper Fe reduction zone“ or „upper iron-rich zone“.

The term was changed as suggested.

L. 234: „sediments“ instead of sediment cores.

Changed.

L. 238 ff.: Are these methane concentrations in pore water? i.e. have the measured values been corrected for porosity? Please specify!

Yes, these are methane concentrations in the pore-water after correction to the porosity (this was added to the methods section in the revised MS).

L. 249: resemble

Corrected.

L. 256: I would not speak of „iron mineral profiles“ but of „operationally defined iron mineral fractions“

Changed as suggested.

L. 266: I thought the pyrite profile was from Wurgaft et al. (2019)?! If yes, please cite this study as the source of these data here.

Was cited.

L. 267 and throughout the manuscript: use „uppermost“ instead of „first“ etc.

Changed as suggested where the uppermost part was the correct term.

Ls. 320 ff. and Fig. 2: The plots and profiles referred to here and depicted in Fig. 2 are insufficiently labeled. It is not clear to me at all what is shown in Fig. 2. Label the individual plots with a,b,c .... And refer to it in the figure caption and label the sequentially extracted Fe fraction as done in related publications.

Labeled as suggested.

L. 317 ff: This contradicts your statement in lines 223 ff. where you are mentioning „sources“. Please precisely present the objective of your study.

The statement in line 223 was rephrased, please see above comment.

L. 321, last sentence, and Ls. 152 ff.: This belongs to the Materials and Methods chapter. Moreover, I would suggest to give a table in which the experimental set-up of the different slurry incubation experiments/vials is listed – otherwise it is very hard to follow.

The sentence was deleted. In addition, a table with the experimental set up was added to the text.

Ls. 334 ff. and 495ff.: I have no idea which of your data show that and to what extent the „shallow sediment processes“ studied in this contribution are linked to the deeper gas reservoir. How do you know and show that part of the methane you have analysed in the shallow sediments originates from the gas front (whatever that is) and from methanogenesis in situ? Please expand and specify and discuss in detail your geochemical evidence showing the contribution of methane from deeper sources (the „gas front“) and that being produced in situ in the shallow sediments. I find it hard to believe that methanogenesis is really possible in these low-TOC (oligotrophic) deposits. If the sediments were really so low in reactive TOC as suggested by the title, I find it hard to believe that methanogenesis can happen in situ at all. I find it more plausible that the methane you have detected in the shallower sediments originates from deeper sources.

Please see the response to the comment on L. 125 regarding the gas front. The TOC levels were ~0.8% in Station SG-1 and ~1% in PC-3. These levels can support *insitu* methanogenesis (e.g. Sivan et al., 2007) in addition to the methane immigration from the gas front. We clarified this point in the revised version.

Ls. 344 ff.: See previous comment!

Please see above.

L. 349: Do you mean pore-water Fe here? If yes, please say so.

Yes. Was specified in the revised version.

Ls. 348 – 351: These statements are much too general and it is not clear how this relates to your data.

We described the data, referred to the figures and removed the general statements regarding the hydrogen and the iron (see the response to L 425).

Ls. 356 ff: Also this part is much too general and not at all supported by references. As has been shown by numerous studies, pore-water Fe is often below detection limit at the SMT due to pyrite formation (e.g. Riedinger et al., 2005, GCA, and 2017)

We agree that this is often the case at the SMT, however, in these lines we are discussing our specific results from the shelf of the SE Mediterranean Sea, where in a few profiles we see low concentrations of dissolved Fe(II) in the pore-water, for example, in the profile from June 2015 at SG-1 station.

Ls. 358 ff.: The only study cited here is the one by Whiticar (1999). Please, give more recent ones as well. There is an enormous amount of studies and literature on this issues published in recent years.

The references Holler et al. (2009) and Conrad (2005) were added.

Ls. 370/371: Please also add Oni et al. (2015) here.

Was added.

Ls. 375/376: This part of the sentence is odd. How can organic matter be formed from upward migrating methane at the SMTZ?

We accept the reviewer's comment and rephrased to: "... where it is produced by the microorganisms that live there and benefits from the upward migrating methane."

L. 380: As already pointed out in a previous comment. I do not find TOC contents of more than 1 wt% particularly low.

We removed the sentence.

L- 381: Again, how do you know how much of the methane is produced in situ and how much is coming from deeper sources? Please discuss.

Please see discussion to comment in L. 125. We do not have a way to quantify the exact relative contribution of each source and we clarify it in the text.

Ls. 384 and 401: What kind of „biomass formation“ at the SMTZ are you referring to here? Again there are no references at all.

We are referring to the microbial community that live at the SMTZ, including ANMEs and sulfate reducing bacteria. The reference of Boetius et al. (2000) was added.

L. 386: To which „deep microbial community“ are you referring to here? Do you mean those at the depth of the „gas front“ or that in your shallower sediments? This is not clear here.

The "deep microbial community" is referred to the community at zone 2 and 3 (i.e. SMTZ and methanic zone). We agree that the sentence is not clear, and it was revised to: "The importance of the methane flux as a carbon source that supports the microbial community at zone 2 and 3 in the sediments of the SE Mediterranean..."

Ls. 399 ff.: Please also add Fischer et al. (2013), Nature Geoscience here.

Was added

Ls. 409 and 412: Please also cite Riedinger et al. (2005) and (2014) here. They were the first to highlight these environmental/depositional prerequisites.

Were added.

Ls. 425 ff.: Discuss why the H<sub>2</sub> levels are that particularly high at these study sites.

Hydrogen levels are dictated from its sink (i.e. sulfate reduction, methanogenesis, iron reduction) and sources processes (mostly fermentation). It is usually found in low steady state concentration in the deep sediments. High concentrations can be explained by fermentation being the dominant process (regarding the hydrogen levels). At station PC-3 the H<sub>2</sub> levels do not increase in the methanic zone, which means that there is H<sub>2</sub> consumption. At station SG-1 the concentrations are lower than PC-3, meaning that in general the H<sub>2</sub> consuming processes are more intensive than in PC-3. At station SG-1 there is a maximum peak in the methanic zone, meaning that the production of H<sub>2</sub> is higher at that depth compared to the SMT zone, however it doesn't necessarily mean that H<sub>2</sub> is not being consumed. This was added shortly to the text instead of the general statement.

Ls. 470: Which evidence makes you „believe“ that – i.e. makes you make this assumption. Please discuss.

We assume that the microbial characterization in this station is representative, based on preliminary low resolution (not published) measurements in other stations using old classifications. However, we agree that the word "believe" here does not fit and we removed the sentence.

L. 625: has to be: Jørgensen, B. B., Böttcher, M. E., Lüschen, H.,  
Check and correct the spelling of Jørgensen throughout the manuscript and the references.

Corrected.



# Evidence for microbial iron reduction in the methanic sediments of the oligotrophic SE Mediterranean continental shelf

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**Abstract.** Dissimilatory iron reduction is probably one of the oldest types of metabolisms that still participates in important biological 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 to the shallow part of the sedimentary column in most aquatic systems. However, iron reduction has also been observed in the methanic zone of many marine and freshwater sediments, well below its expected zone, occasionally accompanied by decreases in methane, suggesting a link between the iron and the methane cycles. ~~Yet~~Nevertheless, 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 methanic zone of the oligotrophic Southern Eastern (SE) Mediterranean continental shelf. Geochemical pore-water profiles indicate iron reduction in two zones, ~~the traditional one, in the upper part~~the uppermost part of the sediment, and the deeper zone, located in the enhanced methane concentration layer. Results from a slurry incubation experiment indicate that the deep methanic iron reduction is microbially mediated. The sedimentary profiles of microbial abundance and qPCR of the *mcrA* gene, together with Spearman correlation between the microbial data and Fe(II) concentrations in the pore-water, suggest types of potential microorganisms that may be involved in the iron reduction via several potential pathways: H<sub>2</sub> 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-the microbial activity in the sulfate methane transition zone (SMTZ). ~~The biomass, created by this microbial activity, can be used below by the iron reducers that allows methanic iron reduction~~in the methanic zone ~~in of the~~ sediments of the SE Mediterranean.

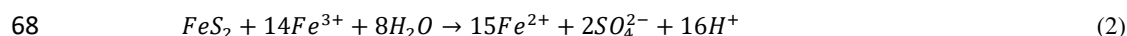
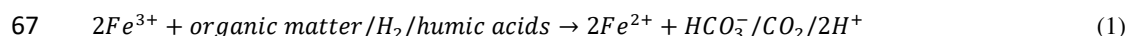
## 1 Introduction

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 (e.g. Roden, 2006). Dissimilatory

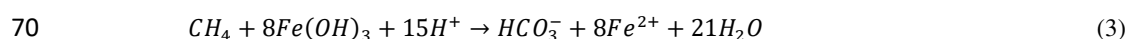
41 microbial iron reduction may be one of the first evolutionary metabolisms, and plays a key role in the  
42 reductive dissolution of Fe(III) minerals in the natural environment ~~(Lovley and Phillips, 1986; Lovley~~  
43 ~~et al., 1987; Lovley and Phillips, 1988; Lovley, 1997;~~ Weber et al., 2006) and in the mineralization of  
44 organic matter in freshwater sediments (Roden and Wetzel, 2002). It also serves as a redox wheel that  
45 drives the biogeochemical cycles of carbon, nitrogen, sulfur and phosphorous (Li et al., 2012 ; Slomp et  
46 al., 2013; Sivan et al., 2014; Egger et al., 2016; Ettwig et al., 2016; Riedinger et al., 2017; März et al.,  
47 2018).

48 Dissimilatory iron reduction is part of the anaerobic respiration cascade, in which different organic  
49 substrates are used for energy by microorganisms and oxidized to dissolved inorganic carbon (DIC). This  
50 is accomplished by reduction of electron acceptors, other than oxygen, according to their availability and  
51 energy yield. Denitrification is the first respiratory process in anoxic sediments, followed by manganese  
52 reduction, iron reduction and then sulfate reduction. Methane (CH<sub>4</sub>) production (methanogenesis) by  
53 archaeal methanogens is traditionally considered to be the terminal process of microbial organic matter  
54 mineralization in anoxic environments, after the other electron acceptors have been exhausted (Froelich  
55 et al., 1979). When the produced methane diffuses away from the methanic layer and meets an electron  
56 acceptor it can be consumed by microbial oxidation (methanotrophy). In marine sediments anaerobic  
57 oxidation of methane (AOM) coupled to sulfate reduction has been shown to occur (Iversen and  
58 Jørgensen, 1985; ~~Hoehler et al., 1994;~~ Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001;  
59 Knittel and Boetius, 2009), and was found to consume up to 90 % of the methane that diffuses upwards  
60 to the sulfate methane transition zone (SMTZ) (e.g. ~~Neiwöhner et al., 1998;~~ Valentine, 2002).

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



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



71 This process in marine sediments was ~~evident-shown~~ through incubation experiments of-with marine  
72 seeps sediments (Beal et al., 2009; Sivan et al., 2014). It was also suggested to exist mainly through  
73 geochemical profiles in deep sea sediments the-and their modeling of-geochemical-profiles-in-deep-sea  
74 sediments (Sivan et al., 2007; März et al., 2008; Riedinger et al., 2014), and in brackish coastal sediments  
75 (Slomp et al., 2013; Segarra et al., 2013; Egger et al., 2014; Egger et al., 2016; Rooze et al., 2016; Egger  
76 et al., 2017). In freshwater environments, it was suggested to occur in lakes (Crowe et al. 2011; Sivan et  
77 al., 2011; Nor~~di~~ et al., 2013), and in denitrifying cultures from sewage, where it was performed by

78 methanogens (Ettwig et al., 2016). Iron-coupled AOM in natural lake sediments was indicated using  
79 isotope pore-water depth profiles (Sivan et al., 2011), rate modeling based on these profiles (Adler et al.,  
80 2011), microbial profiles (Bar-Or et al., 2015), and directly from a set of sediment slurry incubation  
81 experiments (Bar-Or et al. 2017). The few microbial studies on iron-coupled AOM (mainly in cultures)  
82 showed either the involvement of methanogenic/methanotrophic archaea (Scheller et al., 2016; Ettwig et  
83 al., 2016; Rotaru and Thamdrup, 2016; Cai et al., 2018; Yan et al., 2018) or a cooperation between  
84 methanotrophs and methanogens (Bar-Or et al., 2017).

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

104 Despite the above studies, the nature of the link between the biogeochemical cycling of iron and the  
105 methane cycles in the marine methanic zone of marine sediments, which creates suitable conditions for  
106 iron reduction, has not yet been determined. Furthermore, this microbial iron reduction in ~~the~~ methanic  
107 zones has not been shown in the sediments of oligotrophic shallow marine environments. In this study  
108 we report the observations of microbial iron reduction in the methanic depth of marine sediments from  
109 the oligotrophic SE Mediterranean continental shelf. ~~This~~ The microbial iron reduction is observed by  
110 using ~~both~~ geochemical pore-water profiles, qPCR profile of archaea, bacteria and the *mcrA* functional  
111 gene and microbial-16S rRNA gene sequencing profile at three different stations combined with a simple  
112 slurry incubation experiment from the methanic zone. The slurries were amended with hematite and  
113 magnetite, ~~as, given~~ Given their low reactivity, these are the expected Fe(III) minerals to survive the  
114 sulfide zone (Canfield, 1989; Poulton et al., 2004). Furthermore, these minerals were found to be active  
115 in iron-coupled AOM in lake sediments (Bar-Or et al., 2017). The profiles, ~~and~~ the incubation experiment

116 ~~as well as; including~~ the related microorganisms, are discussed in terms of the possible links between  
117 the cycles of iron and methane.

## 118 2 Methods

### 119 2.1 Study site

120 The surface water in the Levantine Basin of the SE Mediterranean Sea, including Israel's continental  
121 shelf, is an oligotrophic nutrient-poor marine system (Herut et al., 2000; Kress and Herut, 2001). The  
122 continental shelf narrows from south to north and ~~comprises is composed~~ of Pliocene-Quaternary  
123 Nile-derived sediments. The sedimentation rate decreases with increasing distance from the Nile Delta  
124 and from the shoreline (Nir, 1984; Sandler and Herut, 2000). Off the shore of ~~Israe~~Israel, the  
125 sedimentation rate is ~~a~~relatively high at  $\sim 0.1 \text{ cm y}^{-1}$  (Bareket et al., 2016). The bottom seawater along  
126 the continental shelf is well oxygenated and sulfate concentrations at the water-sediment interface are  
127  $\sim 30 \text{ mmol L}^{-1}$  (Sela-Adler et al., 2015). The central and eastern regions of the Levantine Basin have  
128 relatively low total organic carbon (TOC) levels ( $\sim 0.1 - 1.4\%$ ; Almogi-Labin et al., 2009; Sela-  
129 Adler et al., 2015; Astrahan et al., 2017) ~~as compared to~~ While the highest levels of total organic carbon  
130 (TOC) ( $1 - 2\%$ ) in sediments were found in the Western Mediterranean Basin and offshore the Nile River  
131 delta ( $1 - 2\%$ ), ~~the central and eastern regions of the Levantine Basin have relatively low TOC levels~~  
132 ( $\sim 0.1 - 1.4\%$ ; Almogi-Labin et al., 2009; Sela-Adler et al., 2015; Astrahan et al., 2017). Along the  
133 Egyptian coast, the TOC in surface sediments on the shelf reaches maximum values of 1.5% (Aly Salem  
134 et al., 2013). The finding of a free gas zone, which is located near the ~~from few to tens meters below the~~  
135 seafloor down as far as tens of meters deep 'gas front' (i.e. gas front), ~~from close to the seafloor down~~  
136 to tens meters deep, in seismic profiles ~~within the sediments of the continental shelf of Israel (Schattner~~  
137 ~~et al., 2012)~~, led to the discovery of biogenic methane formation at some locations in the shallow  
138 sediments (Sela-Adler et al., 2015).

### 139 2.2 Sampling

140 Seven sediment cores ( $\sim 5 - 6 \text{ m}$  long) were collected using a Benthos 2175 piston corer, from the  
141 undisturbed ~~seafloor~~ sediments of the SE Mediterranean continental shelf of Israel at water depths of 81  
142  $- 89 \text{ m}$  from three stations; SG-1, PC-3 and PC-5 (Fig. 1). The cores were sampled during cruises of R.V.  
143 *Shikmona* between 2013 to 2017, and by the R.V. *Bat-Galim* on January 2017 (Table 1). The sediment  
144 cores were sliced on board every 25  $- 35 \text{ cm}$  within minutes upon retrieval from the seafloor. This area  
145 was previously investigated for other purposes, such as the sulfate reduction in the SMTZ (Antler et al.,  
146 2015; Wurgaft et al., 2019.), and the possibility of methanogenesis ~~characteristics~~ (Sela-Adler et al.,  
147 2015).

148 From each interval, a 2.5 mL of ~~total~~ sediment sample was collected and inserted immediately into an  
149 ~~anaerobic-anoxic~~ 10 mL glass bottle filled with 5 mL NaOH 1.5 N for headspace measurements of  
150 methane concentration (after Nüsslein et al, 2003). Approximately 3 mL of sediment was sampled every  
151 50 cm for porosity. In addition, another 2.5 mL sediment sample was taken from each segment of the  
152 cores and transferred into a 20 mL glass bottle filled with NaCl saturated solution for  $\text{H}_2$  concentrations

153 measurements. Sediment samples from each segment of the cores were centrifuged on board if possible  
154 or in the lab within a day by Sorval centrifuge at 9500 RPM under 4 °C and Ar atmosphere in order to  
155 extract pore-water for chemical analysis. The supernatant was filtered (0.22 µm) and analyzed for Fe(II),  
156 sulfate, sulfide, DIC and the stable carbon isotope composition of the DIC ( $\delta^{13}\text{C}_{\text{DIC}}$ ). After the pore-water  
157 extraction, the sediment was analyzed for the content of the different reactive iron minerals (Table 2). In  
158 addition, sediment sub-sample from each segment of the January 2017 core from [Station SG-1](#) ~~station~~  
159 was kept at -20 °C for molecular analysis. Due to high water content and movement in the uppermost  
160 part of the sediments, two ~30 cm sediment cores were also sub-sampled separately, using a 0.0625 m<sup>2</sup>  
161 box corer (Ocean Instruments BX 700 AI) and Perspex tubes during the September 2015 and January  
162 2017 cruises. The short cores were stored at 4 °C, cut in the lab within 24 hours after their collection and  
163 their results are presented for the top sediment (Fig. [2a – d](#)).

### 164 **2.3 Slurry incubation experiment**

165 The experimental set-up ([Table 2](#)) consisted of 11 bottles with sediment from the methanic zone (265-  
166 285 cm depth) from Station SG-1, where iron reduction was apparent ~~in~~-[from](#) the pore-water profiles  
167 (Fig. [2d](#)). Prior to the beginning of the experiment, sediment from the designated depth had been  
168 homogenized in an [anaerobic-anoxic](#) bag under N<sub>2</sub> atmosphere. It was then transferred under anoxic  
169 conditions to a 250 mL glass bottle with the addition of synthetic sea water without sulfate to reach [a](#) 1:1  
170 sediment:—water slurry ratio for 3 months incubation period. After the incubation period the slurry was  
171 sub-divided anoxically to the 11 experiment bottles (60 mL each), and synthetic sea water was added for  
172 final sediment:—water ratio of 1:3. The bottles were sealed with a crimped cap and were flushed with  
173 N<sub>2</sub> for 5 minutes, shaken vigorously and flushed again, (repeated 3 times). Three experimental bottles  
174 were autoclaved twice to serve as "killed" control for the experiment. The experimental bottles were  
175 amended with 1.6 g L<sup>-1</sup> of hematite (Fe<sub>2</sub>O<sub>3</sub>) or 2.3 g L<sup>-1</sup> of magnetite (Fe<sub>3</sub>O<sub>4</sub>) to reach Fe(III) final  
176 concentration of 10 mmol L<sup>-1</sup>. The three killed bottles were amended with the iron oxides after they  
177 cooled down to room temperature. H<sub>2</sub> was added to some treatments to test its potential as an electron  
178 donor. One mL of H<sub>2</sub> was injected by gas tight syringe to the three killed bottles, to two bottles with the  
179 addition of hematite and to two bottles with the addition of magnetite (to reach final concentration of  
180 ~4% of the head space volume). The experimental bottles were sampled several times for dissolved Fe(II)  
181 concentrations during the 14 day experiment period.

### 182 **2.4 Analytical methods**

#### 183 **2.4.1 Pore-water analyses**

184 Methane concentrations [in the pore-water](#) were analyzed by Focus Gas Chromatograph (GC; Thermo)  
185 equipped with FID detector with [a](#) detection limit of 50 µmol L<sup>-1</sup>. [To calculate the methane concentrations](#)  
186 [the sediment porosity was considered. Porosity was determined by drying wet sediment samples at 60](#)  
187 [°C until there was no weight loss \(~48 h\). It was calculated as the weight loss from the initial weight of](#)  
188 [the samples.](#) H<sub>2</sub> concentrations were analyzed in a Reducing Compound Photometer Gas Chromatograph  
189 (RCP-GC; Peak Laboratories). Dissolved Fe(II) concentrations were measured using the ferrozine  
190 method (Stookey, 1970) by a spectrophotometer at 562 nm wavelength with detection limit of 1 µmol L<sup>-1</sup>.  
191 Sulfide was measured using the Cline (1969) method by a spectrophotometer at 665 nm wavelength

192 with detection limit of  $1 \mu\text{mol L}^{-1}$ . Total sulfur concentrations were measured in an inductively coupled  
193 plasma atomic emission spectrometer (ICP-AES), Perkin Elmer Optima 3300, with an analytical error of  
194  $\pm 1\%$  (average deviations from repeated measurements of a seawater standard). Since sulfide was not  
195 detected in any of the sediment cores, the ~~measured~~-total sulfur concentrations in each pore-water sample  
196 ~~were was~~ assumed to be the sulfate concentration of that sample. The  $\delta^{13}\text{C}_{\text{DIC}}$  values were measured on  
197 a DeltaV Advantage Thermo© isotope-ratio mass-spectrometer (IRMS) at a precision of  $\pm 0.1 \text{‰}$ . Results  
198 are reported versus VPDB standard. ~~Several p~~Pore-water profiles of dissolved total sulfur,  $\text{CH}_4$ ,  $\delta^{13}\text{C}_{\text{DIC}}$ ,  
199  $\text{Fe(II)}$  and  $\text{H}_2$  were produced during the study, and all of them are presented (Fig. 2). For each profile  
200 where duplicate samples were taken, the error bar is that of the average deviation of the mean of the  
201 duplicates, in cases where they only single samples were taken, ~~otherwise~~ it is ~~that of~~ the analytical error  
202 (if larger than the symbol).

#### 203 **2.4.2 Sediment analysis**

204 Reactive Fe(III) in the sediments was measured according to Poulton and Canfield (2005) definition and  
205 sequential extraction procedure. The different reactive iron minerals were separated to (1) carbonate-  
206 associated Fe ( $\text{Fe}_{\text{carb}}$  (i.e. siderite and ankerite); (2) easily reducible oxides ( $\text{Fe}_{\text{ox1}}$ ) (i.e. ferrihydrite and  
207 lepidocrocite); (3) reducible oxides ( $\text{Fe}_{\text{ox2}}$ ) (i.e. hematite, goethite and akageneite) and (4) magnetite  
208 ( $\text{Fe}_{\text{mag}}$ ). ~~Sediment samples were dried at  $60^\circ\text{C}$ . About then,~~ approximately 0.6 g dry sediment was inserted  
209 to a centrifuge tube with 10 ml of a specific extractant at every stage under ~~oxie~~-atmospheric conditions  
210 and constant agitation (Table 23). The fluids were separated from the sediment by centrifugation and  
211 removed from the tube with Pasteur pipette after every extraction stage. At the end of each extraction  
212 stage, the extractant was transferred to a 15 mL falcon tube with 0.1 mL ascorbic acid and 0.1 mL  
213 ferrozine solution to reduce all the Fe(III) to Fe(II) and fix it, then it was measured  
214 spectrophotometrically. The results presented as "total reactive Fe(III)" are the sum of  $\text{Fe}_{\text{ox1}}$ ~~the easily~~  
215 ~~reducible oxides,~~  $\text{Fe}_{\text{ox2}}$ ~~reducible oxides~~ and magnetite $\text{Fe}_{\text{mag}}$ . The profile of pPyrite ( $\text{Fe}_{\text{py}}$ ) ~~profile was~~  
216 ~~produced by taken from~~ Wurgaft et al., (2019).

#### 217 **2.4.3 Quantitative PCR and 16S rRNA gene V4 amplicon pyrosequencing**

218 DNA was extracted from the sediment core of Station SG-1 from January 2017 using Power Soil DNA  
219 Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's instructions. Copy  
220 numbers of selected genes were estimated with quantitative PCR (qPCR) as described previously (Niu  
221 et al., 2017) using specific primers: Uni519f/Arc908R and bac341f/519r for archaeal and bacterial 16S  
222 rRNA genes, respectively, and mlas/mcrA-rev for the *mcrA* gene, which encodes the  $\alpha$ -subunit of methyl-  
223 coenzyme M reductase. The amplification efficiency was 94.5%, 106.3% and 92.4% for the archaeal 16S  
224 rRNA, bacterial 16S rRNA and the *mcrA* gene, respectively (the respective  $R^2$  of the standard curve was  
225 0.998, 0.998 and 0.995).

226 The V4 regions of bacterial and archaeal 16S rRNA genes were amplified using barcoded 515FB/806RB  
227 primers (Walters et al., 2015) and Arch519/Arch806 primers (Song et al., 2013), respectively. PCR  
228 mixture contained 6 – 10 ng total DNA, 5  $\mu\text{L}$  10 $\times$  Ex Taq buffer, 4  $\mu\text{L}$  2.5 mmol  $\text{L}^{-1}$  dNTP mix, 1  $\mu\text{L}$  of  
229 each primer, 0.25  $\mu\text{L}$  Ex Taq polymerase (Ex-Taq; TaKaRa, Dalian, China) and 5  $\mu\text{L}$  bovine serum

230 albumin (25 mg mL<sup>-1</sup>) in a total volume of 50 µL. DNA was sequenced as 2x150 bp reads using Illumina  
231 MiSeq platform (Illumina, USA). Sequence quality assessments, chimera detection and down-stream  
232 phylogenetic analyses were conducted in QIIME (Caporaso et al., 2010). Taxonomical assignments for  
233 each OTU were performed in QIIME using the BLAST method and the SILVA128 reference database.  
234 24056 to 132042 high quality sequences were obtained per sample, with the proportion of high-quality  
235 sequence versus total sequence between 81.97 – 99.89%. Spearman correlation was performed using the  
236 online calculator (<http://www.sthda.com/english/rsthd/correlation.php>) to test the relevance of  
237 microbial abundance and communities with Fe(II) concentration along the depth of the sediment core  
238 from 185 cm to the bottom 575 cm, which is the methanic zone of the sediment core according to the  
239 geochemical profile (see the results below).

## 240 3 Results

### 241 3.1 Geochemical profiles

242 Geochemical pore-water profiles of several sediment cores from the three stations (SG-1, PC-3 and PC-  
243 5 (Fig. 1, Table 1)) were produced in order to ~~investigate~~ characterize the iron reduction process in the  
244 methanic zone of the SE Mediterranean continental shelf and to identify its potential sources. The pore-  
245 water profiles ~~from~~ at Station SG-1 (Fig. 2a) show complete depletion of total sulfur at approximately  
246 150 cm depth in ~~all the station cores~~ all cores. Sulfide concentrations were below the detection limit in all  
247 cores, indicating that the total sulfur is mostly sulfate. ~~The m~~ Methane concentrations in the pore-water  
248 (Fig. 2b) show an increase with depth immediately after the consumption of sulfate. The maximum  
249 methane concentration was approximately 10 mmol L<sup>-1</sup> at ~140 cm depth in June 2015. The other  
250 methane depth profiles show an increase in the concentrations to approximately 2 mmol L<sup>-1</sup> and then  
251 leveling off throughout the bottom of the cores (~600 cm). Detected dissolved Fe(II) concentrations (Fig.  
252 2d) were found in the traditional upper iron reduction zone ~~in the upper part of the sediment~~ (between 30  
253 – 90 cm depth), and a second peak was found in the deeper part of the sediment, at the methanic zone  
254 (below 180 cm depth). Maximum dissolved Fe(II) concentrations reached 84 µmol L<sup>-1</sup> in the ~~traditional~~  
255 upper iron reduction zone of the ~~sediments~~ cores and 65 µmol L<sup>-1</sup> in the methanic zone. The δ<sup>13</sup>C<sub>DIC</sub>  
256 values (Fig. 2c) were the lowest (-35 ‰) as expected at the SMTZ depth, and the highest in the methanic  
257 zone. H<sub>2</sub> concentrations (Fig. 2e) decreased to a minimum of 0.017 µmol L<sup>-1</sup> at 155 cm depth, and then  
258 increased to a maximum of 0.147 µmol L<sup>-1</sup> at 485 cm depth.

259 Pore-water profiles from Station PC-3 (Fig. 2g–i) show similar patterns to Station SG-1 on all three  
260 sampling dates, but with lower methane concentrations. Total sulfur (Fig. 2g) was completely depleted  
261 within the upper 300 cm depth. Sulfide concentrations were below the detection limit at this station as  
262 well. Methane profiles show an increase in methane concentration immediately after the consumption of  
263 sulfate. The maximum methane concentration (Fig. 2h) reached 0.8 mmol L<sup>-1</sup> at 450 cm depth in the  
264 Aug-13 core. The dissolved Fe(II) profiles (Fig. 2i) show two peaks ~~also here at this station as well~~, one  
265 in the upper part of the sediment with maximum value of 32 µmol L<sup>-1</sup> at 177 cm depth, and another one  
266 with maximum value of 64 µmol L<sup>-1</sup> at 390 cm depth at the methanic depth. The δ<sup>13</sup>C<sub>DIC</sub> values (Fig. 2i)  
267 decreased from approximately -10 ‰ at the water-sediment interface to -20 ‰ at the SMTZ. Below that

268 zone there was an increase in  $\delta^{13}\text{C}_{\text{DIC}}$  values to about -5 ‰ due to methanogenesis.  $\text{H}_2$  concentrations  
269 (Fig. 2k) remained around  $2 \mu\text{mol L}^{-1}$  along the core. The three deviating points that do not fit the clear  
270 pattern are attributed to an analytical or sampling error.

271 Pore-water profiles from the core collected at Station PC-5 (Fig. S1) resembles the profiles of Station  
272 PC-3. Total sulfur was depleted at approximately 300 cm, and methane concentrations increased below  
273 that depth to  $0.3 \text{ mmol L}^{-1}$ . The Fe(II) profile shows two peaks in this core as well, one in the upper  
274 sediment of  $20 \mu\text{M}$  at 150 cm depth and the second of  $30 \mu\text{mol L}^{-1}$  in the methanic zone. The  $\delta^{13}\text{C}_{\text{DIC}}$   
275 value decreased from -5 ‰ at the water-sediment interface to -25 ‰ at the SMTZ, and below that depth  
276 increased to -17 ‰ at the methanic zone.

277 In addition to the dissolved constituents' profiles, reactive iron minerals were extracted from the  
278 sediment collected on September 2015, and operationally defined iron minerals fractions profiles from  
279 Stations SG-1 and PC-3 were produced (Fig. 2f and 1). In Station SG-1 there appears to be a slight  
280 variability in the content of the minerals (Fig. 2f). The Fe-carbonate minerals<sub>carb</sub> (i.e. siderite and ankerite)  
281 content in the upper part of the sediment was 0.22 dry wt%, increased to ~0.45 dry wt % at 103 cm depth  
282 and then remained constant. The iron (hydr)oxides<sub>Fe<sub>ox1</sub></sub> (i.e. ferrihydrite and lepidocrocite) content was  
283 0.49 dry wt % in the upper part of the sediment, peaked at 203 cm depth to 0.64 dry wt % and then  
284 decreased to 0.50 dry wt % at the bottom of the core. The reducible oxides<sub>Fe<sub>ox2</sub></sub> (i.e. hematite, goethite  
285 and akageneite) content was 2.15 dry wt % in the upper part of the sediment, decreased to 1.03 dry wt %  
286 at 312 cm depth, and then it increased to 1.55 dry wt % at 427 cm depth. Magnetite-Fe<sub>mag</sub> content was  
287 0.34 dry wt % in the upper part of the sediment, decreased to 0.32 dry wt % at 153 cm depth, increased  
288 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  
289 % at the bottom. A pyrite content profile from Station SG-1 was also produced (Wurgaft et al., 2019)  
290 from the September 2015 cruise data and shows two peaks; the first uppermost of 1.10 wt % at 153 cm  
291 depth, and the second lower one of 1.80 wt % at 312 cm depth. The total reactive Fe(III) oxides profile  
292 showed a general decrease from 3.00 dry wt % at 13 cm depth to 2.27 dry wt % at 507 cm depth, with  
293 two minimum peaks of 2.42 dry wt % at 103 cm and of 1.88 dry wt % at 312 cm.

294 In Station PC-3 there appeared to be smaller changes in the different reactive oxides iron mineral fractions  
295 with depth (Fig. 2j). The Fe-carbonate<sub>carb</sub> minerals content in the upper part of the sediment was 0.50 dry  
296 wt % and reached 0.69 dry wt % in the deep sediment. The iron (hydr)oxides<sub>Fe<sub>ox1</sub></sub> concentrations content  
297 were was approximately 1.00 dry wt % throughout the sediment column. The reducible oxides<sub>Fe<sub>ox2</sub></sub>  
298 concentrations content were was 0.78 dry wt % in the upper part of the sediment, increased to 0.89 dry  
299 wt % at 167 cm depth and then decreased to 0.76 dry wt % at 495 cm depth. Magnetite-Fe<sub>mag</sub>  
300 concentration content was 0.83 dry wt % in the upper part of the sediment, increased to 0.89 dry wt % at  
301 167 cm, and then decreased again to 0.75 dry wt % at 495 cm depth. The total reactive Fe(III) oxides  
302 content varied between 2.10 dry wt % (at 167 cm depth) and 1.76 dry wt % (at 137 cm depth).

### 303 3.2 Abundance and diversity of bacteria and archaea

304 The qPCR of bacterial and archaeal 16S rRNA genes from the SG-1 core (collected on January 2017)  
305 revealed an abundance of bacterial genes between  $1.46 - 9.45 \times 10^6$  copies per g wet sediment, while that



306 of archaea was between  $8.15 \times 10^5$  –  $2.25 \times 10^7$  copies per g wet sediment (Fig. 3). The abundance of  
307 bacteria and archaea decreased gradually in the top 95 cm, increased sharply at 125 cm depth within the  
308 SMTZ, remained relatively stable with high abundance at 185 – 245 cm (the top layer of the methanic  
309 zone), and then decreased. Notably, the abundance of both bacteria and archaea peaked within the  
310 methanic zone at 245 cm in correspondence with a Fe(II) concentration peak. However, it is not feasible  
311 to compare the abundance of archaea and bacteria by this method due to bias caused by the PCR primers  
312 used (Buongiorno et al., 2017). The abundance of the *mcrA* gene (Fig. 3) increased sharply from the  
313 surface layer to the SMTZ, peaked at 155 cm and remained stable at 155 – 245 cm, indicative of active  
314 anaerobic methane metabolism in the SMTZ and an active methanic zone (Fig. 2). Spearman correlation  
315 test (Table S2) shows that the abundance of the bacteria and archaea 16S rRNA genes and *mcrA* genes  
316 correlated with Fe(II) concentration in the methanic zone, where *mcrA* gene correlated the most  
317 significantly ( $r = 0.5429$ ,  $p$  value = 0.04789).

318 Illumina-sequencing of the 16S rRNA gene revealed diverse bacterial and archaeal communities  
319 throughout the SG-1 core (Fig. 4). Although no clear plateau was observed on species rarefaction curve  
320 for the current sequencing depth (Fig. S2), Shannon diversity indices reached stable values, indicating  
321 that those sequences well covered the diversity of bacterial and archaeal populations in the samples (Fig.  
322 S3). Shannon index, based on 16S rRNA gene sequences, shows higher diversity in the top layers of the  
323 sediment along with similar values through the core using the bacterial primers, while for sequences  
324 using archaeal primers, the values varied in different layers (Table S1). The bacterial sequences were  
325 affiliated with the following phyla: Planctomycetes (25.7%), Chloroflexi (23.2 %), Proteobacteria  
326 (12.9%), Deinococcus-Thermus (9.9 %), Acidobacteria (3.5%), Aminicenantes (3.3 %), Spirochaetes  
327 (2.3%), Deferribacteres (1.7%), Elusimicrobia (1.6%), Aerophobetes (1.6%), Nitrospirae (1.4%),  
328 Firmicutes (1.4 %), Actinobacteria (1.4 %), TM6 (Dependentiae) (1.2%), Marinimicrobia (SAR406 clade)  
329 (1.0%), and other taxa with less than 1% of the bacterial communities (Fig. 4a). Bathyarchaeota were the  
330 predominant archaea in all the sediment layers, based on the high relative abundance of their 16S rRNA  
331 gene sequences (91.0%). The remaining archaeal phyla comprised Euryarchaeota (3.2%),  
332 Thaumarchaeota (2.4%), Lokiarchaeota (1.0%), and other phyla with less than 1% of the archaeal  
333 communities (Fig. 4b). Spearman correlation analysis (Table S2) revealed that uncultured SBR1093 ( $r =$   
334  $0.6176$ ,  $p$  value = 0.01859) from bacterial Candidate Phylum SBR1093, subgroup 26 of Acidobacteria ( $r =$   
335  $0.5841$ ,  $p$  value = 0.02828), the uncultured bacterium from TK10 Class of Chloroflexi phylum ( $r =$   
336  $0.5297$ ,  $p$  value = 0.0544) and uncultured Bathyarchaeota sp. (archaea) ( $r = 0.5516$ ,  $p$  value = 0.04388)  
337 correlated significantly with Fe(II) concentration.

### 338 3.3 Incubation experiment

339 Sediment from the observed deep iron reduction zone of Station SG-1 from January 2017 core was used  
340 for a simple short-term (couple of weeks) slurry incubation experiment in order to characterize the iron  
341 reduction process in the methanic zone. Hematite and magnetite, which were expected to survive the  
342 sulfate zone, and were shown to be a source for AOM in lake sediments, were added to the slurries.  
343 Indeed, the operationally defined iron oxide-mineral fractions profiles (Fig. 2f) confirm that hematite and

344 magnetite were abundant in the methanic zone in this core. ~~Hydrogen was added as well to some of the~~  
345 ~~bottles.~~

346 The results of the experiment are shown in figure 5. Dissolved Fe(II) concentrations show significant  
347 increase from 11  $\mu\text{mol L}^{-1}$  to approximately 90  $\mu\text{mol L}^{-1}$  during the first three days in all the experimental  
348 bottles, except for the killed bottles, implying that the reduction is microbially mediated. Another  
349 observation was that the microorganisms were able to reduce both hematite and magnetite to the same  
350 extent. In addition, no difference in the Fe(II) concentrations between bottles with and without the  
351 addition of  $\text{H}_2$  was observed.

## 352 4 Discussion

### 353 4.1 General

354 This study was performed in the SE Mediterranean (Fig. 1) ~~in above~~ the area of ~~the a~~ recently discovered  
355 'gas front' (Schattner et al., 2012). The investigated ~~biogenic~~ methane was found in ~~the~~ shallow sediments  
356 ~~(~1-5 m deep) and seems biogenic based on its low  $\delta^{13}\text{C}_{\text{CH}_4}$  values and high C1/C2 ratio with low TOC~~  
357 ~~content~~ (Sela-Adler et al., 2015). Station SG-1 is located at the center of ~~this the~~ gas front area, while  
358 Stations PC-3 and PC-5 ~~stations are located~~ at the edges, and indeed methane related processes were  
359 more intensive at ~~station~~ Station SG-1, ~~linking the shallow sediment processes to this reservoir. The~~  
360 ~~source of this gas front is not certain, but it was speculated to be terrestrial organic matter (Schattner et~~  
361 ~~al., 2012).~~ Our results suggest that there are two sources for methane in the sediment: the first is from  
362 migration of methane from this gas front area (Wurgaft et al., 2019), and the second is from *in-situ*  
363 methane formation. ~~The relative contribution of each source is currently unknown.~~ *In-situ*  
364 methanogenesis in the shallow shelf sediments is evident by the geochemical profiles of  $\delta^{13}\text{C}_{\text{DIC}}$  and  
365  $\delta^{13}\text{C}_{\text{CH}_4}$  (Sela-Adler et al., 2015), ~~and~~ by the microbial ~~population abundance profile profiles of~~  
366 ~~population~~ and ~~by the~~ functional *mcrA* gene ~~profile~~ (Figs. 3 and 4, further discussed below). ~~The TOC~~  
367 ~~content in the methanic zone are ~0.8% at Station SG-1 (Sela-Adler et al., 2015; Wurgaft et al., 2019)~~  
368 ~~and ~1% at Station PC-3 (Sela-Adler et al., 2015), and these levels are known to be able to support in-~~  
369 ~~situ methanogenesis (Sivan et al., 2007).~~

370 The comparison between the sites shows that methane reaches the highest concentrations at Station SG-  
371 1 (up to the saturation level (Sela-Adler et al., 2015)), specifically in the June 2015 profile (Fig. 2b). This  
372 leads to intensive AOM by sulfate at the SMTZ, causing it to occur at shallower depth and to produce  
373 lower  $\delta^{13}\text{C}_{\text{DIC}}$  values than the other two stations. The relation between the upward fluxes of methane, the  
374 SMTZ depth and the  $\delta^{13}\text{C}_{\text{DIC}}$  values fits previous studies (e.g. Sivan et al., 2007). The higher methane  
375 concentrations in the June 2015 profile is presumably due to intensive migration of methane from the  
376 deeper sediments and/or more intensive methane production at the exact location of the core collected at  
377 that time. The  $\text{H}_2$  concentrations at Station SG-1 (Fig. 2e) were lower by two orders of magnitude than  
378 the concentrations at Station PC-3 (Fig. 2k), ~~perhaps.~~ ~~This is possibly~~ due to ~~the~~ more intensive hydrogen  
379 consuming processes at Station SG-1 (i.e. sulfate reduction, methanogenesis, iron reduction (Conrad et  
380 al., 1986; Lovley, 1991)). Dissolved Fe(II) pore-water profiles (Fig. 2d and j) show some variability  
381 between the cores within the same station, ~~probably as a result of.~~ ~~This is reasonable as iron reduction is~~

382 sensitive to environmental changes such as shifts in local pH, the different types of electron shuttles, and  
383 organic compounds that are present in the surroundings variations.

384 Despite the pore-water profiles variability between the stations, they show a resemblance in their trends.  
385 All geochemical pore-water and reactive Fe(II) iron mineral fraction profiles suggest that the sediments  
386 in this area of the SE Mediterranean shelf can be classified into three general depth-zones (Fig. 2): **zone**  
387 **1** is the upper part of the sediment, where the traditional classical iron reduction occurs, probably coupled  
388 to organic matter oxidation, with sulfate reduction below it; **zone 2** is the SMT depth, where methane  
389 starts to increase, sulfate is completely depleted, and Fe(II) (Fig. 2d and j) is either present in low  
390 concentrations or absent (probably due to the precipitation of iron-sulfide minerals). In addition, the  
391  $\delta^{13}\text{C}_{\text{DIC}}$  values are the lowest in this zone, as expected from the intensive AOM process there, which uses  
392 the isotopically light carbon of the methane as a carbon source with small fractionation (Whiticar, 1999);  
393 Holler et al., 2009); **zone 3** is the methanic zone, where methane concentrations increased to the highest  
394 values in all stations, as did the  $\delta^{13}\text{C}_{\text{DIC}}$  since the carbon source for the methane comes mainly from  $\text{CO}_2$ ,  
395 leaving the residual DIC heavier by about 60 ‰ (Whiticar, 1999; Conrad, 2005). At this zone, local  
396 maxima of Fe(II) concentrations in the pore-water were found in all cores, indicating reduction of iron  
397 oxides. The slurry experiment results show only a slight increase in Fe(II) concentrations in the killed  
398 bottles compared to their significant increase in the non-killed bottles, inferring that the iron reduction in  
399 zone 3 is microbial (Fig. 5).

#### 400 **4.2 Potential methanic iron reduction pathways**

401 This observed intensive iron reduction in the methanic sediments is the first discovered in the SE  
402 Mediterranean shelf. The phenomenon of iron reduction in the methanic depth has been observed before  
403 in other marine provinces ( Jørgensen et al., 2004; März et al., 2008; Slomp et al., 2013; Riedinger et  
404 al., 2014; Treude et al., 2014; Oni et al., 2015; Egger et al., 2016). Yet, the type of link to the methane  
405 cycle is not well understood. Usually, iron reduction is coupled to oxidation of organic matter (Lovley  
406 and Phillips, 1988) and is performed by iron-iron-reducing bacteria, which is probably the case in zone  
407 1. It is however questionable if this also stands for zone 3 and if not, what process is responsible for the  
408 iron reduction at this depth and its relation to methane. The iron reduction in zone 3 can occur potentially  
409 via four pathways: 1) oxidation of organic matter arriving from the SMTZ, where it is produced by the  
410 microorganisms that live there and benefit from the upward migrating methane, 2) oxidation of the  
411 methane itself, 3)  $\text{H}_2$  oxidation or 4) oxidation of sulfur species through a cryptic cycle.

412 The oligotrophic nature of the water column in the studied area would suggest that intensive bacterial  
413 iron reduction coupled simply to with the oxidation of organic matter in zone 3 is less likely. The low  
414 nutrient and low chlorophyll concentrations in the water column results in low TOC amounts in the  
415 sediments (<1%) (Sela-Adler et al., 2015). Nevertheless, we observe high methane concentrations in  
416 zone 3 in all three stations, where part of it is from upward migration. This indicates that regardless of  
417 the surface water area's present oligotrophic nature, the TOC substrate may be enough to sustain all the  
418 microbial activity and to take part in the iron reduction process in the methanic zone. This is possibly  
419 due to just from biomass production in the SMTZ (i.e. the microbial community including ANMEs and

420 [sulfate reducing bacteria \(Boetius et al., 2000\)](#) and its ~~fast-rapid~~ use ~~below in the methanic zone~~ (so the  
421 TOC content ~~still~~ seems ~~still~~-low).

422 The importance of the methane flux as a carbon source that supports the deep microbial community [at](#)  
423 [zone 2 and 3](#) in the sediments of the SE Mediterranean can be illustrated by comparing the organic carbon  
424 flux from the photic zone, with the flux of organic carbon that is oxidized by sulfate in the pore-water.  
425 Using traps, Moutin and Raimbault (2002) estimated an export flux of  $7.4 \pm 6.3 \text{ mgC m}^{-2} \text{ d}^{-1}$ , which leaves  
426 the photic zone. However, Wurgaft et al. (2019) estimated that the flux of DIC toward the SMTZ from  
427 sulfate reduction is equivalent to  $8 \pm 3 \text{ mgC m}^{-2} \text{ d}^{-1}$ . Whereas the difference between the two fluxes is  
428 statistically insignificant, it should be noted that the flux of organic material that survives aerobic  
429 oxidation in the water column and the upper part of the sediment column, as well as anaerobic oxidation  
430 by other electron acceptors with higher energy yield (Froelich et al., 1979; Emerson et al., 1980), is likely  
431 to be substantially smaller than the flux measured by Moutin and Raimbault (2002). Therefore, it is  
432 unlikely that export flux from the photic zone constitutes the sole source of carbon to the SMTZ. Wurgaft  
433 et al. (2019) suggested that “external” methane, originates in deeper portions of the sediments, provides  
434 [an](#) important source of carbon to the SMTZ in Station SG-1. Such fluxes of “external” methane are  
435 common along continental margins sediments (e.g. Milkov and Sassen, 2002; Milkov, 2004; Zhang and  
436 Lanoil, 2004; Paull et al., 2008; ~~;) Fischer et al., 2013~~). Here, we suggest that this supply of methane, leads  
437 to intensive sulfate-mediated AOM in the SMTZ, and that this intensive process and biomass may serve  
438 as an additional substrate that “fuels” ~~the deeper zone~~ [zone 3](#), activating the iron-oxides.

439 The recently discovered iron-coupled AOM process (Eq. 3) is the second potential process that can  
440 involve iron-oxide reduction in the deep methanic zone (Sivan et al., 2011; Segarra et al., 2013; Slomp  
441 et al., 2013; Riedinger et al., 2014; Egger et al., 2015; Rooze et al., 2016; Egger et al., 2017; Bar-Or et  
442 al., 2017). Fe(III) as an electron acceptor for AOM provides a greater free energy yield than sulfate  
443 (Zehnder and Brock, 1980), and its global importance was emphasized (Sivan et al., 2011; Segarra et al.,  
444 2013; Sivan et al., 2014). Two of the main environmental conditions for iron-coupled AOM to occur are  
445 high dissolved methane concentrations and abundant reducible iron oxides (~~(Riedinger et al., 2005); (~~  
446 [Riedinger et al., 2014](#)); Egger et al., 2017). Thus, from our profiles it seems that AOM could be a valid  
447 option, considering the high methane concentrations and the high sedimentation rates ( $0.1 \text{ cm y}^{-1}$  (Bareket  
448 et al., 2016)), which allow the iron oxides to survive the sulfidic zone and reach the methanic zone  
449 (~~(Riedinger et al., 2005); Riedinger et al., 2014~~; Egger et al., 2017). This can also be inferred from figure  
450 6, where some association was observed between the dissolved Fe(II) concentrations and the methane  
451 concentrations in zone 3. It seems that at high concentrations of Fe(II), methane concentrations are low  
452 and vice versa. This could be a result of iron-coupled AOM that uses methane to reduce Fe(III)-oxides,  
453 releasing dissolved Fe(II) to the pore-water. It can also suggest a type of competitive relationship between  
454 methanogenesis and microbial iron reduction, or microbial population switching from methanogenesis  
455 to iron reduction metabolism (e.g. Sivan et al., 2016). It should be noted that our experiment was not  
456 designed to test AOM due to its short time scale of a few weeks, hence another long experiment with the  
457 addition of the  $^{13}\text{C}$ -labeled methane will enable us to shed more light on this association.

458 The third potential process that can be coupled to iron reduction in the methanic zone is H<sub>2</sub> oxidation. H<sub>2</sub>  
459 is an important intermediate in anoxic aquatic sediments. In this type of environment, it is produced  
460 mainly by fermentation of organic matter (Chen et al., 2006), and can be involved in different microbial  
461 processes where each process would need a certain amount of H<sub>2</sub> in order to occur (Lovley and Goodwin,  
462 1988). The H<sub>2</sub> levels at Stations SG-1 and PC-3 stations (Fig. 2e and k) are relatively high in comparison  
463 to other marine environments (Lilley et al., 1982; Novelli et al., 1987), suggesting that there is enough  
464 H<sub>2</sub> to sustain the iron reduction process. The relatively high H<sub>2</sub> concentrations at these stations could be  
465 explained by the dominance of H<sub>2</sub> production processes (i.e. fermentation (Chen et al., 2006)) compared  
466 to H<sub>2</sub> consuming processes (i.e. sulfate reduction, methanogenesis, iron reduction (Conrad et al., 1986;  
467 Lovley, 1991)). At Station PC-3, the H<sub>2</sub> concentrations (Fig. 2k) are constant in zone 3, this suggest that  
468 in addition to being produced, H<sub>2</sub> is consumed as well. At Station SG-1 (Fig. 2e) there is a maximum  
469 peak at zone 3, indicating that there is either more H<sub>2</sub> production or less H<sub>2</sub> consumption at this zone  
470 compared to zone 2. This is reasonable considering the intensive microbial activity in zone 2. The  
471 decrease in the H<sub>2</sub> concentrations below the peak suggests that H<sub>2</sub> consuming processes are  
472 activeintensive in this zone. The increase in H<sub>2</sub> concentration profile at the methanic zone in SG-1 station  
473 could be explained by the occurrence of fermentation processes, which enables H<sub>2</sub> to accumulate (Chen  
474 et al., 2006). The H<sub>2</sub> involvement was tested by injecting 1 mL of this gas to the experimental bottles in  
475 the methanic iron reduction process (Fig. 5). We observed that the increase of Fe(II) concentration was  
476 similar in the bottles with H<sub>2</sub> addition compared to the bottles without H<sub>2</sub>. This could mean that either  
477 there is enough H<sub>2</sub> in the sediments as it is, as implied by the H<sub>2</sub> pore-water profiles, or that at the  
478 methanic depth H<sub>2</sub> is not involved in the iron reduction process.

479 The fourth potential way to reduce iron in zone 3 is by an active sulfur cycle. The pyrite profile supports  
480 this possibility by showing two peaks, ~~one~~ uppermost in zone 2 of ~1 wt% and the ~~second~~ other in zone  
481 3 of ~2 wt% at about 300 cm depth (Fig. 2f). The peak at 300 cm depth indicates possible active sulfur  
482 cycle, even though sulfate is already undetected at 200 cm. Thus, a possible scenario is that Fe(III) is  
483 reduced by pyrite oxidation (Eq. 3) (Bottrell et al., 2000), which triggers the 'cryptic' sulfur cycle, as  
484 observed in other marine sediments (Holmkvist et al., 2011; Brunner et al., 2016; Egger et al., 2016). In  
485 this cycle, elemental sulfur, and eventually by disproportionation also sulfide and sulfate, are produced.  
486 The sulfide reacts with iron-oxide and precipitates as FeS or as pyrite (FeS<sub>2</sub>) (Holmkvist et al., 2011).  
487 The sulfate can inhibit methanogenesis (Mountfort et al., 1980; Mountfort and Asher, 1981), which can  
488 result in the enhancement of the iron reduction process due to competition for substrate with the  
489 methanogenesis process. Another indication for an active sulfur cryptic cycle comes from the 16S rRNA  
490 sequencing analysis (Fig. 4), which shows that Proteobacteria, a potential sulfur related bacteria phylum,  
491 is one of the most abundant phyla in the sediments. Moreover, the increase in the abundance of Sva0485  
492 order of the deltaproteobacteria class, a known sulfate reducer (Tan et al., 2019), with depth, supports an  
493 active sulfur cycle in zone 3 as well.

#### 494 **4.3 Potential microbial players**

495 Our data profiles and incubations indicate that the observed iron reduction in the methanic zone of the  
496 SE Mediterranean shelf is performed by microbial activity. The microbial results show first that the

497 abundances of the bacteria and archaea (Fig. 4) are typical to oligotrophic marine sediments (e.g. South  
498 China Sea that contains ~0.5 – 1 % TOC (Yu et al., 2018)). Second, even though potential bacterial iron  
499 reducers, such as *Alicyclobacillus*, *Sulfobacillus*, *Desulfotomaculum* genera (Firmicutes), *Acidiphilium*  
500 (Alphaproteobacteria), *Desulfobulbus*, *Desulfuromonas*, *Geobacter*, *Geothermobacter*,  
501 *Anaeromyxobacter* (Deltaproteobacteria) and *Shewanella* (Gammaproteobacteria) (Weber et al., 2006)  
502 comprise less than 0.1% of bacteria detected in the methanic zone (from 185 cm and below), it appears  
503 that both the microbial abundance and the Fe(II) concentration peaked at this zone. Cultivation efforts  
504 indicated that archaeal methanogens may also play a role in iron reduction within sediments (Sivan et al.,  
505 2016). Moreover, the relative abundance of methane-metabolizing archaea was shown to correlate with  
506 Fe(II) concentrations in Helgoland muds from the North Sea, where microbial abundance and the Fe(II)  
507 concentrations peaked at the methanic zone (Oni et al., 2015), similarly to the [results found in the SE](#)  
508 Mediterranean sediments. It is possible that methane-metabolizing archaea were involved in the iron  
509 reduction in the [SE](#) Mediterranean sediments, as the highest *mcrA* gene copies per gram wet sediment  
510 were detected in the SMTZ and in the top of the methanic zone ([Fig. 3](#)) where the Fe(II) concentrations  
511 are high ([Fig. 2d](#)). Methanotrophs, such as ANMEs, were found to be involved in ~~iron-iron~~-coupled AOM  
512 in marine and freshwater cultures (Scheller et al., 2016; McGlynn et al., 2015; Ettwig et al., 2016; Cai et  
513 al., 2018). ANMEs were found here with relatively low frequencies (ANME1, below 1% in most  
514 samples, circa 5% in the 185 cm layer), and their role in iron reduction within the [SE](#) Mediterranean  
515 sediments remains to be tested.

516 ~~It should be noted that even though the microbial population was tested only on one sediment core that~~  
517 ~~was extracted on January 2017 at Station SG-1, we believe that it represents the general microbial~~  
518 ~~population abundance in the SE Mediterranean continental shelf.~~ In our study, Spearman correlation  
519 analysis [at Station SG-1](#) (Table S2) revealed that bacterial phyla SBR1093 (candidate Phylum),  
520 Acidobacteria and Chloroflexi, as well as archaeal Phylum Bathyarchaeota showed significant positive  
521 correlation with a Fe(II) concentration in the methanic zone. The Candidate Phylum SBR1093 was firstly  
522 identified in phosphate-removing activated sludge from a sequencing batch reactor (Bond et al., 1995),  
523 and continuously detected in a short-chain fatty acid rich environment such as wastewater treatment, and  
524 marine sediments (Wang et al., 2014). It was thought to be capable of growing autotrophically, but the  
525 metabolic capabilities related to iron reduction remain unclear. Strains of Acidobacteria and Chloroflexi  
526 phylum were found to be capable of iron reduction (Kawaichi et al., 2013; Kulichevskaya et al., 2014).  
527 In addition, members of Acidobacteria were found in iron-coupled AOM enrichment (Beal et al., 2009).  
528 The metabolic properties of Subgroup 26 from Acidobacteria and TK10 Class of Chloroflexi are still not  
529 known. Bathyarchaeota are globally distributed and account for a considerable fraction of the archaeal  
530 communities in the marine sediments, particularly, in the Mediterranean Pleistocene sapropels (Coolen  
531 et al., 2002; Zhou et al., 2018). While Bathyarchaeota have diverse metabolic capabilities (Lloyd et al.,  
532 2013; Meng et al., 2014; Evans et al., 2015; He et al., 2016; Yu et al., 2018; Feng et al., 2019), their role  
533 in iron reduction warrants further studies, as suggested from their high abundance here. Therefore, iron  
534 reduction and methane cycling within the deep methanic zone may be facilitated by an interplay among  
535 bacterial and archaeal groups, whose physiology and functions needs further investigation.

## 536 **5 Conclusions**

537 Our study used combined geochemical and microbial profiles together with [a](#) slurry incubation  
538 experiment to show microbial iron reduction in methanic sediments, and the potential microbial  
539 population performing this reduction. The Spearman analysis points out several potential microbial  
540 players (both bacterial and archaeal) that correlate to the dissolved Fe(II) profiles (e.g. Bathyarchaeota,  
541 Acidobacteria and Chloroflexi). Moreover, our study emphasizes that this [methanic](#)-iron reduction [in the](#)  
542 [methanic zone](#) can occur even in sediments of oligotrophic seas such as the SE Mediterranean. We  
543 suggest that the availability of iron minerals for reduction is linked to intensive upward fluxes of methane  
544 and high sulfate-AOM rates that may produce available biomass or/and hydrogen, which fuel deeper  
545 microbial processes. The deep iron reduction may also be linked to a cryptic sulfur cycle and iron-coupled  
546 AOM.

## 547 **5 Author contribution**

548 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  
549 research and analyzed the data; H.V, O.S, B.H, F.W, M.RB and L.L synthesized the data and wrote the  
550 paper.

551 The authors declare that they have no conflict of interest.

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898

899

900 **Table 1:** Cores sampling details: dates, water depths and locations.

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

901

902 **Table 2:** Experimental set-up of the slurry incubation experiment.

<u>Treatment</u>	<u>Number of bottles</u>
<u>Hematite</u>	<u>2</u>
<u>Magnetite</u>	<u>2</u>
<u>Hematite + H<sub>2</sub></u>	<u>2</u>
<u>Magnetite + H<sub>2</sub></u>	<u>3</u>
<u>Killed + hematite + H<sub>2</sub></u>	<u>2</u>
<u>Killed + magnetite + H<sub>2</sub></u>	<u>1</u>

903

904 **Table 23:** Summary of reactive iron extraction procedure (after Poulton and Canfield, 2005).

Extractant	Target compounds	Analyzed species	Formula	Shaking time (h)
<b>Magnesium chloride</b>	Ion-exchangeable Fe(II)	Adsorbed ferrous iron	Fe <sup>2+</sup>	2
<b>Sodium acetate</b>	Iron carbonates	Siderite Ankerite	FeCO <sub>3</sub> Ca(Fe <sup>+2</sup> ,Mg <sup>+2</sup> ,Mn <sup>+2</sup> )(CO <sub>3</sub> ) <sub>2</sub>	24
<b>Hydroxylamine hydrochloride</b>	"Easily reducible" Iron(hydr)oxides	Ferrihydrite, Lepidocrocite	Fe <sup>3+</sup> <sub>2</sub> O <sub>3</sub> *0.5(H <sub>2</sub> O) γ-FeOOH	48
<b>Sodium dithionite</b>	"Reducible" oxides	Goethite, Hematite, Akageneite	α-FeOOH Fe <sub>2</sub> O <sub>3</sub> β-FeOOH	2
<b>Ammonium oxalate</b>	Poorly crystalline	Magnetite	Fe <sub>3</sub> O <sub>4</sub>	6

905

906 **Figures captions:**

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

907 **Figure 2:** Geochemical pore-water profiles of: total S, CH<sub>4</sub>, δ<sup>13</sup>C<sub>DIC</sub>, dissolved Fe(II), H<sub>2</sub> and extractable Fe fractions from sediment cores collected from at the two stations: SG-1 (top) and PC-3 (bottom) in the Eastern SE Mediterranean. The profiles are divided roughly into three zones according to the dominant processes: upper microbial iron and sulfate reduction, sulfate-methane transition zone (SMTZ), and the methanic zone at the deep part. The dashed line in the CH<sub>4</sub> graph at SG-1 station represents the CH<sub>4</sub> saturation value in the pore-water (Sela-Adler et al., 2015). The following iron-extractable Fe minerals fraction profiles of stations SG-1 (f) and PC-3 (l) are from the September 2015 and January 2015 cruise (respectively): siderite, ankerite Fe<sub>carb</sub> ( ), ferrihydrite, lepidocrocite Fe<sub>ox1</sub> ( ), goethite, hematite, akaganeite Fe<sub>ox2</sub> ( ), magnetite Fe<sub>mag</sub> ( ), pyrite Fe<sub>py</sub> ( ) (Wurgaft et al., 2019) and total reactive iron ( ). The error bars for CH<sub>4</sub> are presented where duplicate sediment samples were collected. The error bars for Fe(II), δ<sup>13</sup>C<sub>DIC</sub> and H<sub>2</sub> are presented where measurements from the same sample were repeated at least twice (at least twice). The analytical errors were smaller than the symbols too small to be displayed.

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

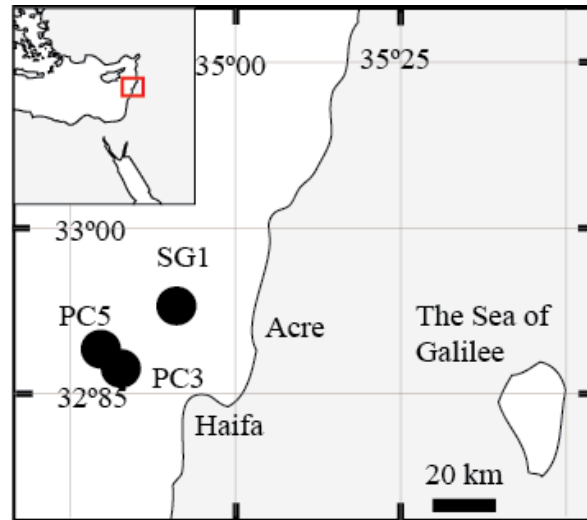
**Figure 4:** Phyla level classification of bacterial (a) and archaeal (b) diversity in the sediments of Station SG-1 from January 2017.

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

**Figure 6:** The relationship between dissolved Fe(II) concentrations and methane concentrations in zone 3 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 iron-coupled AOM.

908 **Figures:**

909 **Figure 1**



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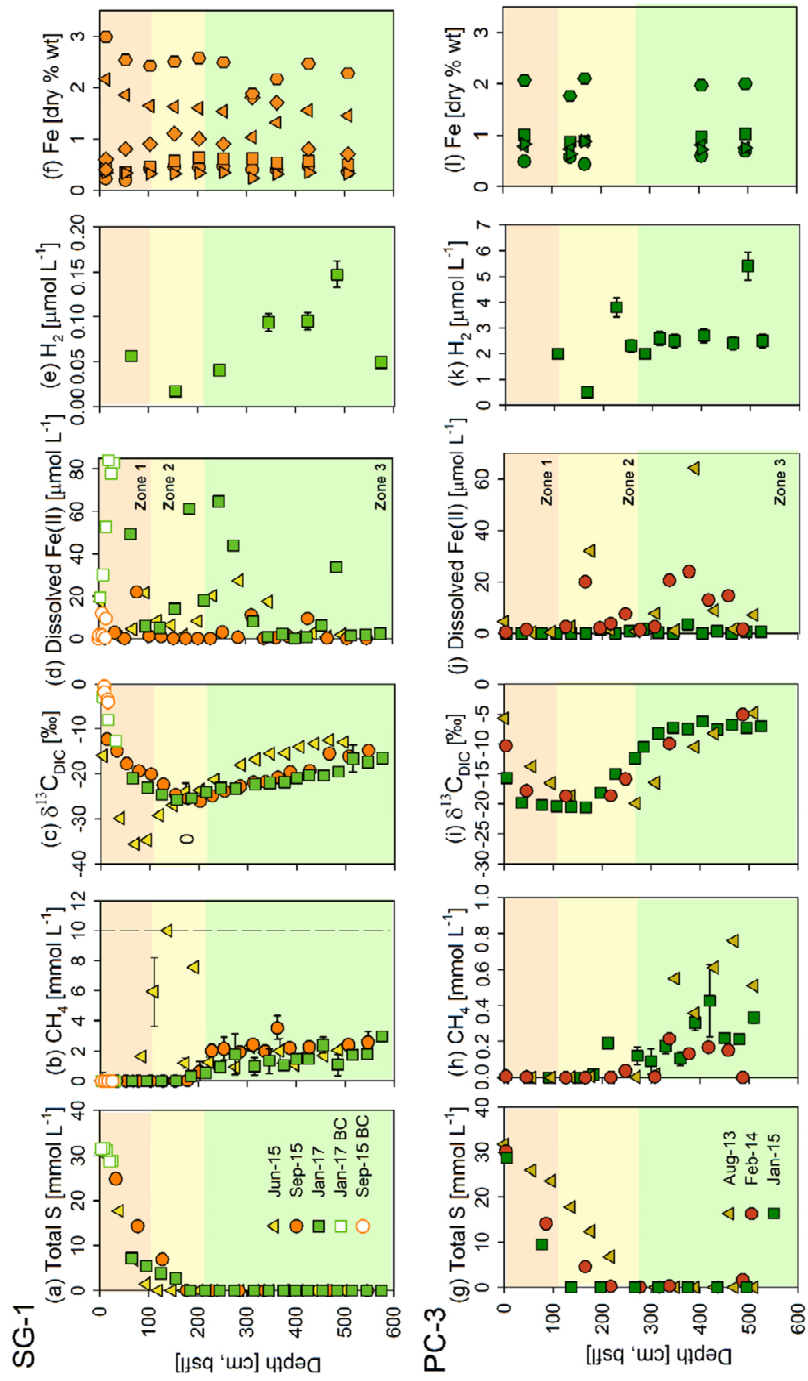
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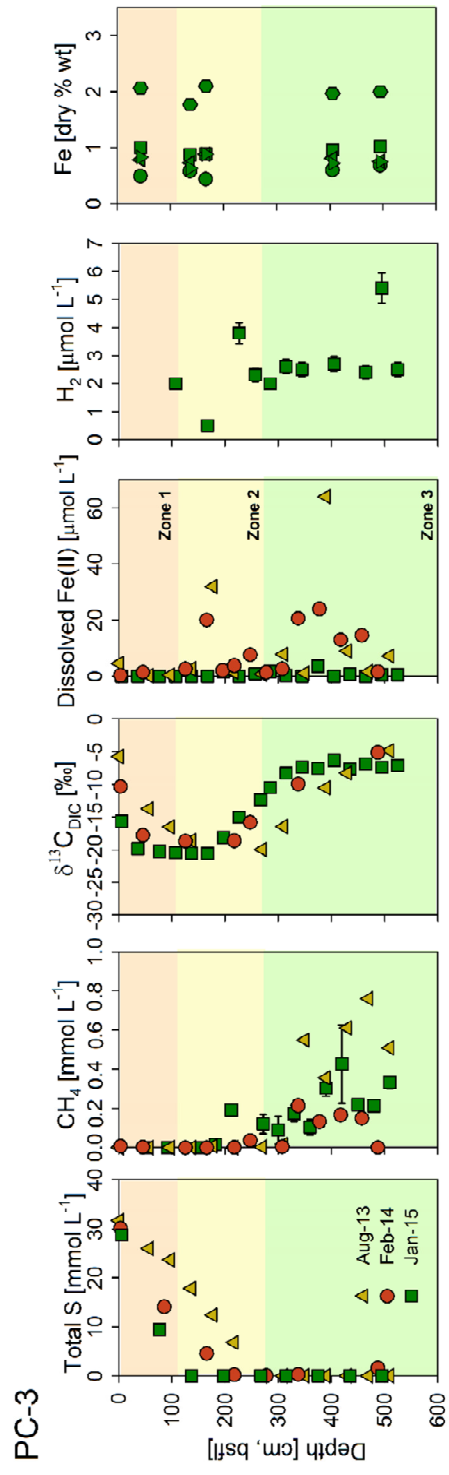
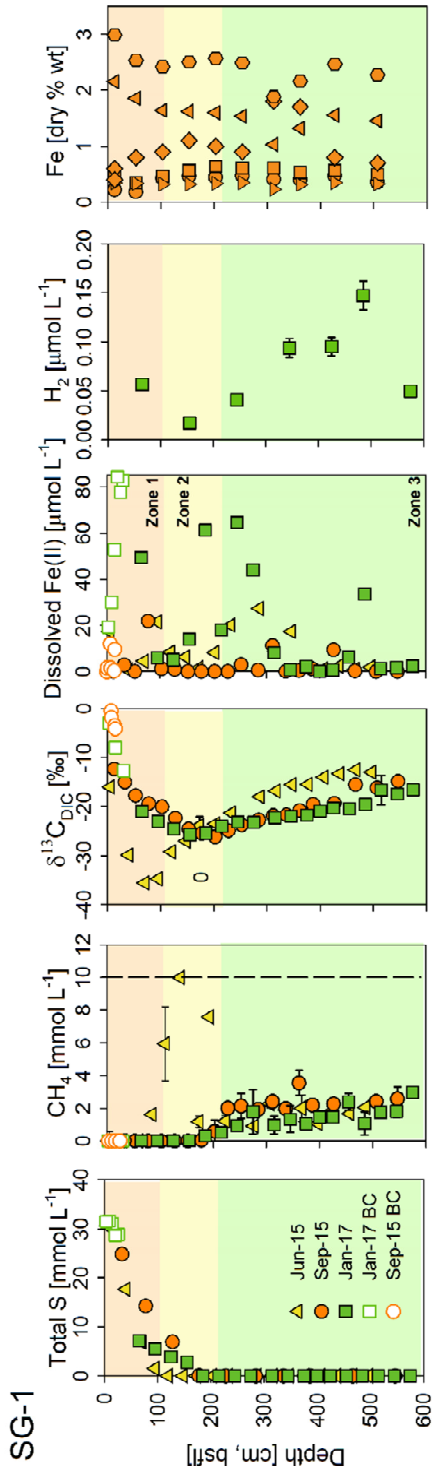
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940 **Figure 2**

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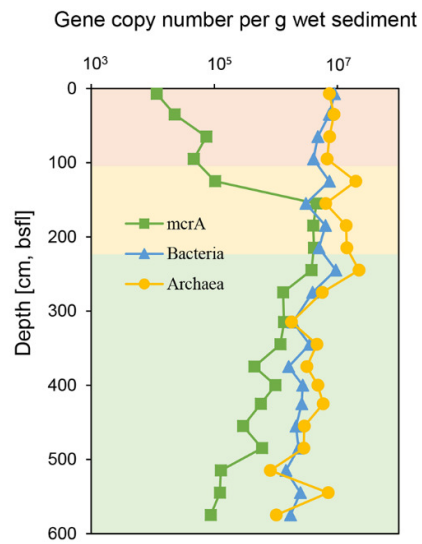
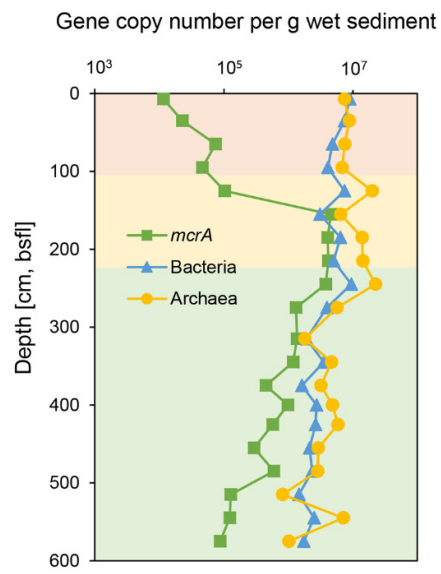
948 **Figure 3**

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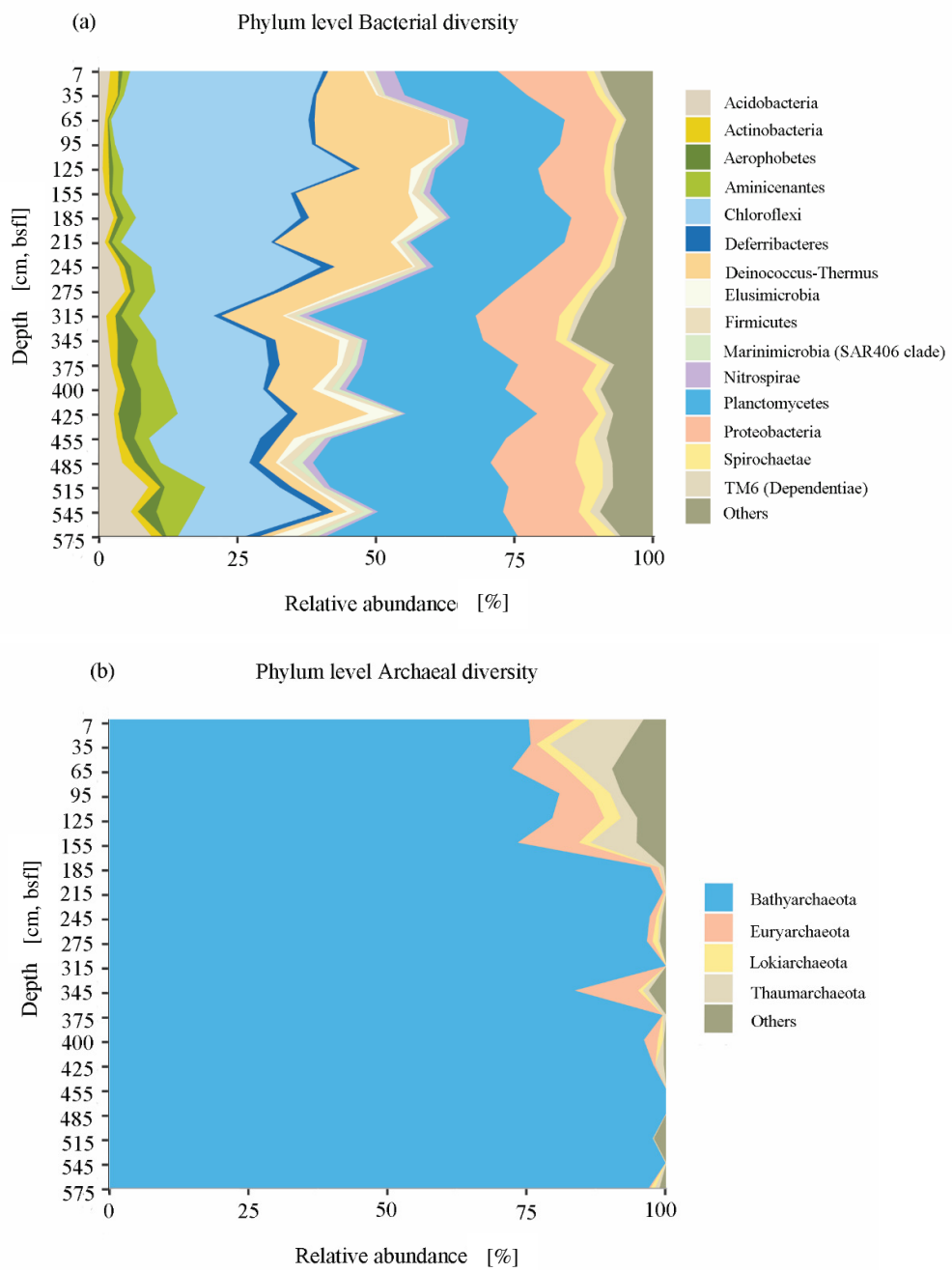


953 **Figure 4**

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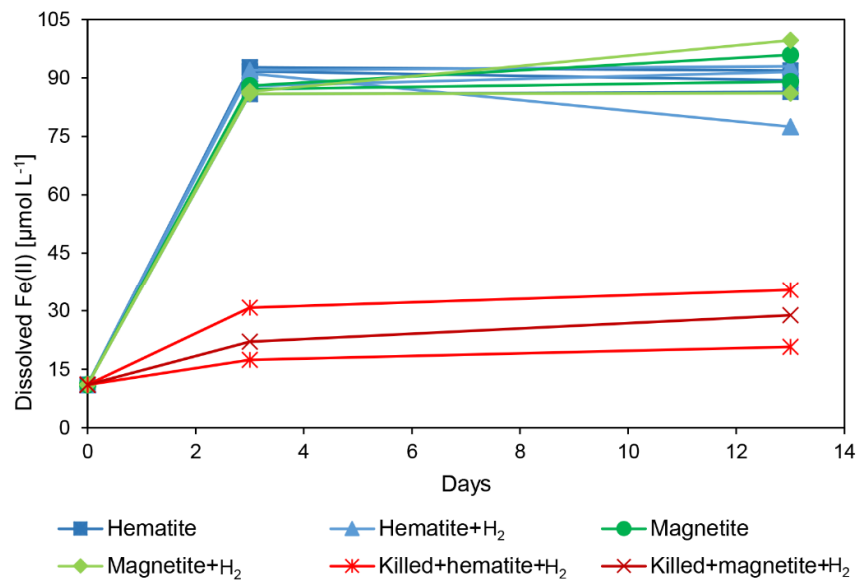
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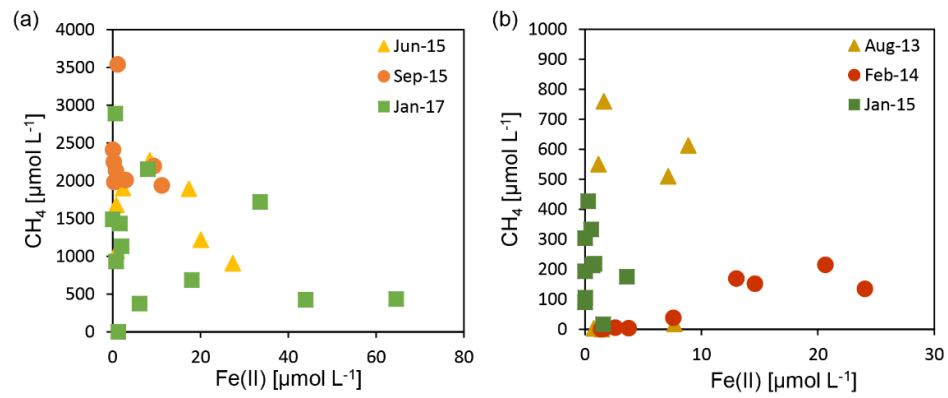
957 **Figure 5**



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960 **Figure 6**



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