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Biogeosciences Discussions

Interactive comment on "Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret sediments" *by* Michal Elul et al.

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Dear anonymous referee #3 Thank you for taking the time to assess our manuscript. We appreciate your valuable comments, suggestion and corrections. We have carefully addressed each concern raised and revised the manuscript accordingly. We hereby present point-by-point answers to the issues raised (after each comment you will find a response paragraph). We hope that the manuscript will now be suitable for publication in Biogeosciences. Sincerely yours, Michal Elul, on behalf of all co-authors.

Anonymous Referee #3 This manuscript on "Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret sed-

iments" is very well written and organized. The title accurately describes the subject of the manuscript, though it is a bit dry and lacks any insight into what was concluded in the study. The abstract is clean and concise and effectively summarizes the key findings of the manuscript, which are largely descriptive.

Response: We thank the reviewer for the positive feedback.

The introduction is also well constructed and (mostly) properly referenced, though the statement at line 40 of "largely unknown isn't exactly true.

Response: Following the recommendation of all three reviewers this line was changed to "However, the diversity and metabolic potential of the microbial communities in natural anoxic ferruginous sediments are not fully understood"

However, my main concern with this paper is that there is no geochemical data from the incubations to confirm/support the metagenomic interpretations. The authors state at line 374 : "our geochemical experiments suggest: : :." however, no geochemical data is provided. As such, while the authors engage in thorough, well referenced discussion of inferred function based on homology searches, implying that there is experimental geochemical evidence to support their conclusions is misleading unless that data is presented. If it is available it needs to be presented, even if only in the supplement and not the focus of the main text.

Response: We thank the reviewer for this helpful comment. In the revised manuscript, we added a new section (3.1) that briefly addresses the geochemistry of the sampled sediments and the geochemical analyses of the slurry incubations. We supplement this discussion with figure S1 in the Supplement, which shows the change in δ13C of the DIC of the slurry incubations, after Bar-Or et al.2017. Both the new section (3.1) and figure S1 are attach to this response form.

I find similarity between the in situ sediment samples and all of the incubations for which metagenomes are available to also be curious, especially in the presence of

inhibitors. Perhaps some geochemical data could shed some light on this?

Response: We have observed some dissimilarities between the treatments, however, our analyses lack the statistical power to clearly define these differences. We can speculate that iron amendments had little effect on the composition of microbial communities, as iron is not a limiting factor in these sediments. Similarly, as we suspect that sulfate plays only a minor role in these sediments due to the low concentrations, the addition of molybdate may have only a negligible effect on the community structure. Bar Or et al. 2017 geochemical data (now presented as Supplementary Figure S1) shows that the addition of BES completely halted methanotrophy and methanogenesis. We observed that read abundance of some lineages, such as Methanosarcinales, declined in BES amendments (Supplementary Figure S2, S1 in the previous version). It is still unclear how other methanogens persist in BES-amended treatments, transcriptomics may elucidate this interesting phenomenon. It is important to note that the results here describe only the relative abundance. It is feasible that the cell numbers declined following the BES addition. In this study, the fact that the communities are similar among the treatments is, in fact, helpful for our analyses, allowing co-assembly and thus better genomic coverage.

At line 71-72 the authors state that " slurry incubations: : :: : :produced substantial amounts of 13C-labelled DIC". How much is "substantial amounts"?

Response: We clarify this in the text and refer to the new Supplementary Figure 1: "These incubations, including a) 13CH4, b) 13CH4 + Hematite, or c) 13CH4 + amorphous iron + molybdate (A.Fe(III)+MoO4) produced substantial amounts of 13C-labelled dissolved inorganic carbon over 470 days (80-450‰ Fig. S1 in the Supplement)". As stated above, we added section 3.1 to introduce the geochemical data.

Was there iron reduction? H2 production? Or did the slurry just sit there static and are just a reflection of the initial sediment slurry sitting there for over a year, as it sort of looks like from the non-departure from the t0 microbial community (Figure S2).

Response: Iron reduction occurred in the slurry incubations. We address this subject in the newly added section 3.1-L154 " Ferrous iron concentrations increased by âĹij20−50 μM following iron oxide amendments (with and without molybdate addition), indicating that iron was reduced." Unfortunately, H2 was not measured in the slurry incubations.

There seems to be some presentation of in situ geochemical data (lines 208-209) though it's unclear if this was measured or a previously reported value.

Response: The values mentioned here are previously reported values. To clarify this issue, the respective references we added: The hydrogen concentration in the Fe-AOM horizon is $\sim$20 μM gr-1 sediment (Adler 2015). Given that sulfate is below the detection limit there (<10μM, Adler et al., 2011, Sivan et al., 2011), hydrogen scavenging may also be coupled to metal reduction, most likely by Deltaproteobacterial lineages, some of which may be syntrophic (e.g. Syntrophobacterales). "

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3.1 Geochemical evidence for iron coupled AOM in Lake Kinneret iron-rich methanic sediments

We explore here slurries amended with Lake Kinneret sediments from the deep methanic zone (26-41 cm). In this potentially ferruginous zone, sedimentary profiles show that the concentration of methane decreases from its maximum values of above 2mM at around 10 cm depth to 500 μM at 40 cm depth, and that of dissolved ferrous iron increases (from 1-6μM at the first 10 cm depth to ~60-100μM, depending on sampling season). This, combined with an increase of $\delta^{13}C$ of methane (from -65‰ at 7 cm depth to -53.5‰ at 24 cm depth) and a decrease of $\delta^{13}C$ of total lipid compounds (from 27‰ at 23 cm depth to -31‰ at 27 cm depth), suggests AOM in the deep sediment coupled to iron reduction (Adler et al. 2011; Sivan et al. 2011). This was supported by rate modeling and by microbial profiles (Adler et al. 2011; Sivan et al. 2011; Bar-Or et al. 2015, 2017). Alternative electron acceptors are scarce: dissolved manganese oxides concentrations are ~ 0.04% and nitrate and sulfate are below the detection limit (Sivan et al. 2011).

The slurries investigated microbially here were amended with isotopically labeled $^{13}CH_4$, $^{13}CH_4$ + hematite and $^{12}CH_4$ + amorphous iron + molybdate for 470 days. In these incubations, we observed a marked enrichment of labeled carbon after ten months of incubation (up to 250‰ enrichment in the treatment with hematite addition, up to 80‰ enrichment in the natural treatment and up to 450‰ in the treatment with amorphous iron + molybdate Fig. S1 in the Supplement). Ferrous iron concentrations increased by ~20−50 μM following iron oxide amendments (with and without molybdate addition), indicating that iron was reduced. The BES amendments resulted in the highest increase in ferrous iron concentrations (~50-110 μM), most likely due to the abiotic reaction of BES with iron minerals. The evidence for iron reduction, together with the fact that $\delta^{13}C_{DIC}$ values increased by 250-450‰ in the different iron amended treatments, but not in methane-only additions (only up to 80‰, Fig. S1 in the Supplement), indicate iron coupled AOM. Sulfate did not play a role in the AOM, as the addition of molybdate, sulfate reduction and disproportionation antagonist, did not inhibit methane turnover (Fig. S1 in the Supplement). The addition BES to specific slurries inhibited the production of $\delta^{13}C_{DIC}$, indicating the essential role of methanogens in the AOM activity (Fig. S1 in the Supplement).

Fig. 1.

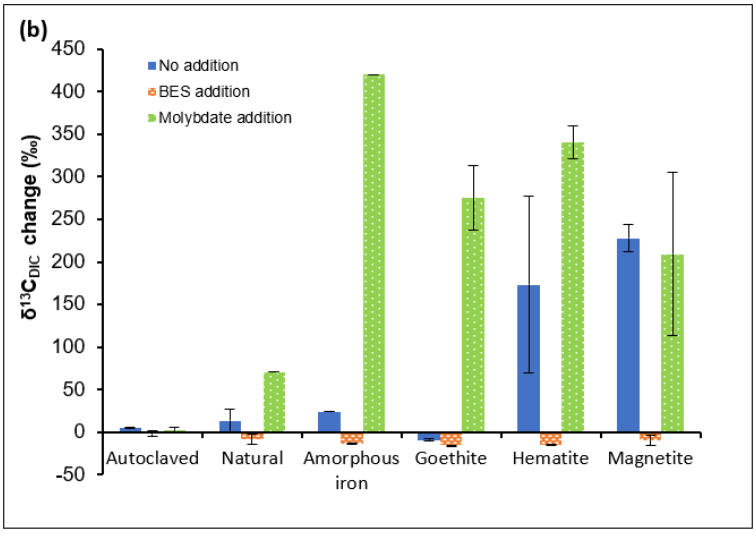

Figure S1: Net change in $\delta^{13}C_{DIC}$ values in Bar-or et al.2017 slurry incubations after 470 days. Solid blue: without inhibitor addition; Fence red: inhibition of methanogenesis by BES addition; Dot green: inhibition of sulfate reduction by molybdate addition. Analysis of DNA 16S rRNA genes was performed for all of these incubations and the untreated sediments. The following treatments: Natural (without additions), Amorphous iron with the addition of molybdate and the hematite (without additions) treatment were sequenced for metagenome analysis. After Bar-Or et al. 2017

Fig. 2.