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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 #2 We thank you for the thorough and thoughtful comments on our submitted article. We went through the comments and suggestions and the paper has been revised accordingly. We present below, point-by-point, answers to the issues raised (after each comment you will find a response paragraph). We hope that you will find the revised version of our manuscript suitable for publication in Biogeosciences.

Sincerely yours, Michal Elul, on behalf of all co-authors

Anonymous Referee #2: This study "Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret sediments" use metagenomics to investigated microbial communities associated with iron reduction and methane cycling from both natural Lake Kinneret sediments and iron amended slurry incubations. The data and interpretation is generally good. While I find the topic of this study certainly interesting for Biogeosciences, there are several aspects which should be addressed before publication. Lack of accompanying geochemical analysis, enzyme assay or transcripts analysis make the study descriptive, mostly putative or based on prediction from reference database in results and discussion.

Response: We agree with the reviewer that a geochemical background and analyses of both the sedimentary zone and slurry incubations examined here is needed to be added (also noticed by reviewers 1 and 3). In the revised version, we added a full section (3.1) that address the geochemical aspect of the manuscript. This new section is attached to this response form. Since our study was based on metagenomics, it can only raise hypotheses regarding the functionality of the studied communities. We agree that further experiments, such as enzyme assay or metatranscriptomics are needed to base our assumptions. We strongly believe, however, that this study provides a valuable basis for further investigation of Lake Kinneret communities and iron and methane metabolisms.

Moreover, metagenomic analysis of four treatments shows not much different between them or at least the authors didn't present much difference, which question the experiment design or validity of method due to poor coverage of metagenomic method, especially when targeting a minor group in a complex sample.

Response: Albeit the overall similarities, we find some differences between the treatment, yet lack the statistical power to show them and often can only speculate regarding their nature. For example, BES additions appear to reduce the relative abundance of Methanosarcinales, but not Methanomicrobiales, as observed in the 16S rRNA amplicon read results. We agree that the small changes following the addition of BES are

curious, yet at this point, we prefer not to overinterpret these changes. Iron mineral amendments may have little effect on the community structure, as iron are not limiting in these sediments. The overall similarity of the communities allowed us to increase the coverage and co-assemble the reads from the different libraries, being in our favor in this case. We believe that although the coverage was insufficient to cover the rare taxa in the way that high-quality bins could be assembled, metagenome-wide functional predictions and taxonomic assignments still provided important insights into this system.

Metagenomics analysis only covers the ferruginous part of sediment core, so the title, abstract and descriptions throughout the text should be specific, rather than use "whole" lake sediment.

Response: We agree - in the revised version we emphasize in both the title, abstract and descriptions throughout the text that our analyses address only the deep iron-rich methanic part of the sediment in Lake Kinneret. The title has been changed to "Metagenomic insights into the metabolism of microbial communities that mediate iron and methane cycling in Lake Kinneret iron-rich methanic sediments".

The names of microbes and genes should be in italic, first letter of proteins should be in Capital, please check and correct throughout the whole text

Response: Thank you for these observations, we made amendments throughout the text accordingly.

Specific comments: Line 35 "on average" and "up to" are redundant and not logical here, delete one.

Response: Corrected as suggested.

Line 40 "largely unknown" is not precise here, actually there have many studies in recent years, in ferruginous sediments will be more specific.

Response: This sentence now reads: "However, the diversity and metabolic poten-

tial of the microbial communities in natural anoxic ferruginous sediments are not fully understood."

Line 46 change depleting to depleted

Response: Corrected as suggested.

Line 71 Diversity of what?

Response: We refer to the diversity of bacteria and archaea. For clarity, this line now reads: "In all the treatments, the diversity of bacteria and archaea was similar to that of the natural sediments"

Line 208-211 Did the author measured concentrations of H_2 and SO_4 in this study? Otherwise, they need to explain how they get these numbers.

Response: Our group measured these species. H_2 concentrations were measured by Michal Adler and shown in her doctoral dissertation, and SO_4 concentrations were measured in Adler et al. 2011;Sivan et al.2011 and Bar-Or et al., 2015. The references were added as suggested.

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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 $^{13}CH_4$ + 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.