Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-329-AC1, 2020

Biogeosciences Discussions
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EGU

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.

Michal Elul et al.

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Received and published: 31 October 2020

Dear anonymous referee #1, We would like to thank you for taking the time to evaluate our paper and for your constructive review and suggestions which helped increase the overall quality of the manuscript. We have carefully considered all your notes and suggestions 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 #1: The paper addresses a topic of importance to readers of this

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journal: the microbial ecology of ferruginous sediments. The title is descriptive and therefore does not as clearly summarize the paper's major finding as a declarative title would, but it does accurately describe the paper's topic. The abstract provides a concise and complete summary. The paper is overall well-structured and clearly written, with fluent and precise language, and of appropriate length. The figures are of high quality. The findings largely confirm a previous study (Vuillemin et al 2018), and thus the findings overall are more confirmatory than novel, but important nonetheless. I have several suggestions for strengthening the methods and results as well as some missing citations: The paper includes metagenomic data on sediments incubated with various substrates for 470-days but never mentions specifics about the activities of these sediments for methane oxidation, iron reduction, methanogenesis, etc. Please summarize those geochemical data from the Bar-Or et al 2017 study at the start of the results section to set the stage for the metagenomics findings.

response: We thank the referee for this observation and agree that a summary presenting the geochemical data on Bar-Or et al 2017 slurries is needed. In the revised version, as recommended, we devoted a section (3.1) at the beginning of the results for this purpose. In this section, we describe the concentrations of relevant elements (methane, dissolved iron, manganese, nitrate, and sulfate) in the investigated sedimentary zone as well as the geochemical data on methane oxidation, iron reduction, and methanogenesis processes in Bar-Or et al 2017 slurries. We have attached to this response form the above mentioned new section (3.1).

2) My second main concern is regarding the methods and results for the PilA proteins, which were identified through a simple KEGG annotation without a detailed analysis necessary to confirm that the aromatic abundance and spacing was sufficient for predicted electroactivity. The authors should add that analysis, as in this paper (https://doi.org/10.1111/1758-2229.12809) to check that the PilA sequences contain the requisite cutoffs for predicted electroactivity ($\geq$9.8% aromatic amino acids, $\leq$22âAËŸ Raa aromatic gaps, and aromatic amino acids

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at ËĞ residues 1, 24, 27, 50 and/or 51, and 32 and/or 57) because there are many other type IV-a pilin genes that can easily be mistaken as electroactive PilA. A script is available for calculation of mature pilin length, percent aromatic amino acids and aromatic free gaps (https://github.com/GlassLabGT/Pythonscript) as described in this paper: https://doi.org/10.1111/1758-2229.12809. Also for the multiheme cytochromes, there are scripts available from a published study: 'cytochrome_stats.py' described in https://doi.org/10.3389/fmicb.2016.00913 and available at https://github.com/bondlab/scripts. Also, note that electroactive PilA are present in lineages outside of Deltaproteobacteria: see https://doi.org/10.1111/1758-2229.12809 https://doi.org/10.1038/ismej.2017.141 and https://doi.org/10.1128/mBio.00579-19

response: As suggested, we confirmed that the aromatic abundance and spacing was sufficient for predicted electroactivity in the metagenome pilA sequences using the recommended script. We corrected Figure 4d, which now shows only the PilA open reading frames that correspond to the stringent parameters (the amended figure 4d in attached to the response form). Accordingly, we adjusted the text in this paragraph to: " The overall abundance of the MHC (secreted and trans-membranal), PilA and OmcS ORFs was 364-493, 35-45, 5-9 and 4-9 counts per million reads mapped, respectively. Our findings confirm that the phylogenetic diversity of microbes are capable of nanowire-mediated DIET extends beyond deltaproteobacterial lineages (Bray et al. 2020), as strict searches attributed pilA-like sequences not only to Desulfobacterota (Deltaproteobacteria), but also to Thermodesulfovibrionales, Burkholderiales, Gemmatimonadales, Aminicenantales, as well as WOR-3 and Firmicutes (Fig, 4d)." We thank the reviewer for pointing out the 'cytochrome_stats.py' script, this will streamline our future analyses.

3)As supplemental data, the authors should include FASTA files with the hits for each of the major genes discussed, so that readers can easily use the sequences, unless the metagenomes have been deposited in annotated form into NCBI. The NCBI Bio-

Project does not contain any genomes with accessions to cite, so it is important for the FASTA files to be provided with the publication, or else there is no way for readers to locate the new sequences without reprocessing the raw metagenomes in the BioProject PRJNA637457 (indeed, there are no genomes listed on the BioProject page, so the data are hidden in SRAs, and not easily accessible for BLAST searches). Even better would be to include annotated metagenomes on NCBI and include the assigned NCBI accession numbers in the paper, but currently that is not simple except for metagenome assembled bins.

Response: As suggested by the reviewer, we submitted the metagenome to NCBI within the PRJNA637457 project. The metagenome is currently being processed and will be released ASAP. We supplemented the manuscript with amino acid sequences of the enzymes discussed (those involved in methanogenesis and extracellular electron transfer, as well as heterodisulfide reductase âĂŐsubunits) in FASTA files, referred to as Supplementary Database 6, 7, and 8. The above-mentioned FASTA files can be found in the following links: (which are also listed as S.DB.6,7 and 8 in the revised version of the Supplementary Information).

S.DB.6| Metagenomic hits (amino acid sequences) for methanogenesis related enzymes -FwdC/FmdC, Ftr, Mch , MtrA, Mer, Mtd, mcrA. https://doi.org/10.6084/m9.figshare.13091126.v2

S.DB.7| Metagenomic hits (amino acid sequences) for extracellular electron transferâĂŐ related enzymes - MHC, omcS and pilA. https://doi.org/10.6084/m9.figshare.13092821.v1

S.DB.8| Metagenomic hits (amino acid sequences) for heterodisulfide reductase âĂŐsubunits A, D and E. https://doi.org/10.6084/m9.figshare.13092842.v1

4) Consider citing papers by Kelly Wrighton's group on the importance of Candidatus Methanothrix paradoxum for methanogenesis in terrestrial sediments with oxygen exposure. For example: https://doi.org/10.1038/s41467-017-01753-4. Could also help

explain the occurrence of genes encoding oxygen-dependent methane monooxygenases if there is occasional oxygen exposure in these sediments. Are they bioturbated?

Response: We thank the reviewer for this information. We assume that Lake Kineret sediments are not bioturbated in the depths that we examined (26-41cm). We now cite a paper from Kelly Wrighton's group (https://doi.org/10.1038/s41467-017-01753-4): "Other notable archaeal lineages included the acetoclastic Methanothrix (1-3% read abundance), which are often found en masse in anoxic lake sediments (Smith and Ingram-Smith 2007; Schwarz et al. 2007; Carr et al. 2018) as well as in oxygenated soil, as recently discovered for Methanothrix paradoxum (Angle et al. 2017)."

Specific comments: Line 40-41: There has been quite a great deal of research on the diversity and metabolic potential of microbial communities in natural anoxic sediments over the past 40 years. I would not characterize this topic as "largely unknown". Please correct language here to focus on a more specific question, perhaps on ferruginous sediments.

Response: We agree with the reviewer that this line needed to be more focused on a specific topic. The text now reads "However, the diversity and metabolic potential of the microbial communities in natural anoxic ferruginous sediments are not fully understood"

L163-164: It is notable that Bathyarchaeia remained one of the dominant lineages even after sediment incubation. It is typical that Bathys quickly "die out" when sealed in bottles for a few weeks-months (for example, https://doi.org/10.1111/gbi.12239) and these were sealed for 470 days! The authors may want to attempt to culture Bathys out of these bottles, since they seem to be persisting, and perhaps even growing.

Response: We are thrilled to try it!

L205: change "anaerobic conditions" to "anoxic conditions" (metabolisms are anaerobic/aerobic; environments are oxic/anoxic).

Response: Corrected as suggested.

L252: correct the misspelling of Methanosarcinales.

Response:Corrected as suggested.

L287: ORFs per what? Per metagenome?

Response: Indeed, per metagenome, we added this clarification in the text.

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-329/bg-2020-329-AC1-supplement.pdf

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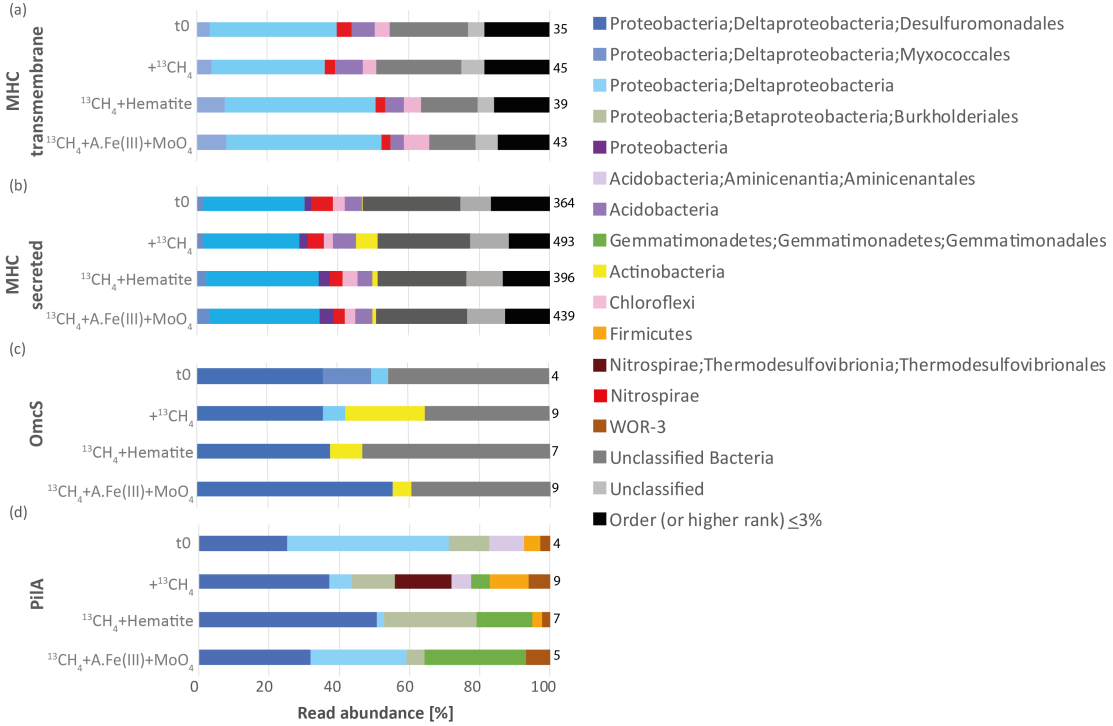

Fig. 1.

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