

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.

Anonymous Referee #1

Received and published: 23 September 2020

The paper addresses a topic of importance to readers of this 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

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missing citations:

1) 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.

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, $\leq 22\%$ aromatic gaps and aromatic amino acids at 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/Python-script>) 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>.

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 BioProject does not contain any genomes with accessions to cite, so it is important for the FASTA

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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 PR-JNA637457 (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.

4) Consider citing papers by Kelly Wrighton's group on the importance of *Candidatus Methanotherix 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?

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.

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.

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

L252: correct the misspelling of Methanosarcinales

L287: ORFs per what? Per metagenome?

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L301: capitalize "P" in PilA when referring to protein; italicize and lowercase when referring to gene. No such thing as "pilA" non-italicized.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-329>, 2020.

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