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Interactive comment on “Methane related changes in prokaryotic activity along geochemical profiles in sediments of Lake Kinneret (Israel)” by I. Bar Or et al.

Anonymous Referee #2

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Bar or et al. present a study on prokaryotic community diversity in freshwater sediments, in association with geochemical measurements. The authors conclude on possible new prokaryotic drivers of iron-associated AOM in deeper sediment depths of Lake Kinneret.

General comments

Overall both the Results and Discussion parts are too long and contain data/paragraphs that are repeated throughout the main text. The manuscript needs substantial rewriting in this regard. Combining Results and Discussion would be an interesting way to circumvent this. The data presented here is interesting; however a

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lot of information is buried in descriptive paragraphs and it is not clear how some paragraphs are linked to each other. It would be a considerable help for the readers to have sub-chapters with clear titles added to the Discussion.

As only 3 samples were analyzed it is surprising that an average of 50 sequences was reported for each clone library. As such the conclusions based on the number of sequences affiliated with each prokaryotic groups in each sample should be dealt with more precaution and kept simple. The same comment goes to the analysis of prokaryotic species richness.

The authors state in the introduction that the main goal of this study is to examine a possible shift in microbial communities. In order to visualize this shift it would help the readers to have a cluster analysis carried out resulting in a dendrogram figure that would clearly show this/these shift(s) and where it/they may occur in regards to sediment depth. The authors also state that they aim to study the AOM related prokaryotic diversity in the deeper sediments. If so why did they not analyze the *mcrA* genes to further discuss methanogenic/methanotrophic diversity?

It would also be helpful if the authors could add, as supplementary material, a table listing each clone, their closest matches in the NCBI database along with the % of identity and where they were retrieved.

Specific comments

Material and Methods

P9817 L24 - The authors state that only slight seasonal changes were measured. How often were the bio-geochemical parameters measured and over how many years? Were the analyzed sediment pore-water samples taken at the same location than the samples for the molecular work? Also could the authors provide a statistical analysis supporting the fact that variation overtime of the parameters used in this study is not significant?

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P9819 L1 – At what temperature were the samples frozen?

P9820 L9-19 – This paragraph is unclear. Please rewrite it.

P9820 L25 - Why use an identity cutoff of only 96% for uncultured matches and 90% for cultured matches? I would expect 97% for cultured and 90% for uncultured matches.

Results

P9821 L13 - Why were those profiles specifically chosen? Would it be more relevant to show geochemical profiles from samples retrieved the same month and year as the samples for the molecular work?

P9821 L25 - If bacterial sulfate reduction is occurring then why are the sulfide concentrations decreasing and not increasing? In other words why is sulfide not being produced as sulfate is being consumed?

P9822 L3 - The figure shows a value of ca. 1.25 mM. Please correct.

P9822 L6 - Please put this conclusion at the end of the paragraph after discussing the isotopic data.

P9823 L22 - Coverage for the bacterial clone libraries are extremely low.

P9823 L19-22 - This was already explained in the material and methods part.

P9824 L11 - Please explain 'functionality'.

P9824 L14-15 - What is this assumption based on?

P9826 L4 - Group C3 is actually a subgroup of the MCG, please modify accordingly.

P9826 L8 - How close are the sequences affiliated to the Halobacteria to cultured halophilic organisms?

P9826 L13 - 'closely related'

P9826 L18-19 - Please add a reference to support this statement.

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P9827 L5 - Please change the word 'roughly'.

P9828 L2 - What was aligned? What is the percentage of identity? If lower than 97% than nothing can be concluded as to the function of the organisms these sequences belong to.

P9828 L9 - Again, please specify the percentage of identity.

P9828 L21-22 - Please expand or explain.

P9830 L19 - A shift in bacterial community diversity?

P9831 L9-11 - Please rewrite this sentence.

P9831 L14 - How similar (percentage of identity) are the sequences from this study to sequences from the clade of methanogens belonging to the Thermoplasmata? Are the authors referring to the Methanoplasmatales order of methanogens belonging to the Rice Cluster III clade? If so, based on 16S rRNA phylogeny, the MBG-D and RC-III Archaea are distinct organisms belonging to the Thermoplasmata class. Also Fig.5b shows that the Thermoplasma clones detected in this study are affiliated with the MBG-D, MG-III, and TMEG. Please rewrite this paragraph discussing these specific groups.

P9831 L16-20 - Both these conclusions should be toned down and rewritten as no proof exists that MBG-D are indeed methanogens, yet alone methanotrophs. Also as only a very small number of sequences were analyzed and coverage is low it is possible that other organisms perform methanogenesis and/or AOM and were not detected.

P9834 L4-17 - This paragraph discusses functions for an entire phylum even though it has been stated previously that the only Thaumarchaeota detected in the present study are MCG. No Marine Group I Thaumarchaeota, the only proven ammonia oxidizers within the Thaumarchaeota, have been detected hence any discussion on functions related to ammonia oxidizing seem irrelevant to this study. Please narrow the discus-

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sion on possible functions of the Thaumarchaeota to the clades detected in this study.
P9835 L1 - The discussion needs to be narrowed down to the actual groups of Eur-
yarchaeota detected in this study.

Figures

Fig.2 - It would be interesting to have methane, sulfate and sulfide all in one graph.

Fig.4A - Distribution of the archaeal phyla gives no useful information for the discussion
so this should be put in the supplemental material.

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