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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.

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Response to anonymous referee #2

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 result section was rewritten and the conclusions were transferred to the discussion section.

The data presented here is interesting; however a 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.

Sub-chapters were added in the discussion as the reviewer advised.

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 reviewer is right and the revised MS was rewritten with more precaution and kept simple.

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 reviewer is right- it is clearer to see shift in a figure. Therefore we used Vann diagram in the supplementary figure 1. We have also added a dendrogram of the three depths.

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?

In order to show the different process in the different zones we added profile of functional genes (mcrA, pmoA and dsrA). This addition is made due to the reviewer advice and in order to better understand the processes and microbial diversity in the different depth, despite the small number of the clone libraries.

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.

This table could be added to the supplementary section. However this table is long and it depends on the BG publication space.

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?

Sampling was made every 3 to 4 months (this information was added to the MS) between 2007-2014. However, not all analyses were made at each sampling date, only those of methane and dissolved ferrous iron. All the samples were taken from the same location: station A. Adler et al,. 2011 showed the seasonal variation in the lake porewater and in the sediment. They showed that in the sediment the geochemical variations are small. Schwarz et al 2007 showed that during their study in LK (2 years) the microbial community structure was stable. Therefore the variation of the water col-



umn has probably some effect on the top sediment but much lower effect on the deep sediment.

P9819 L1 – At what temperature were the samples frozen?

The samples were frozen at -20 degrees Celsius. This was added to the MS.

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

We reanalyzed the data using Mothur software and SILVAngs pipeline (Quast et al., 2013). Therefore the paragraph was rewritten.

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.

The reviewer is right; the cut off is low. However using 97% cut off didn't give us a lot of similarity for results of cultured microorganisms. Most of the environmental microorganisms are unculturable. Therefore we have lowered the cut off to 90% to give us some idea for the cultured microorganism's similarity (which gave us less than half similarity results for our sequences). The cut off for the uncultured microorganisms was 97% and was used only for compering the environment that they were found in. All these analyses were made in order to give some sense of the data even though it is only speculation, because none of the microorganisms have been cultured.

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?

The reviewer is right. It would have been best to have all the profiles from the same sampling. However this is not the case, we made only methane profile in the same sampling in order to know where to slice and which zones to sequence. The rest of the profiles are from the closest sampling (like $\delta$13CCH4 and $\delta$56Fe profiles which were

taken 4 month before) or the first time they were made after the sampling.

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?

The reviewer is right, sulfate reduction creates sulfides. Sulfides can precipitate with iron and manganese which are available in the sediment. Therefore we don't see the accumulation of sulfide in the profile.

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

The sentence was corrected.

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

The paragraph was corrected according to the reviewer advice.

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

The reviewer is right, therefore we less emphasize the dominant microorganisms in the sediment and write more about the methane related processes. In addition we added the functional genes to give us more information on the process in the different depths.

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

This explanation was deleted from the material and method part and kept in the result section.

P9824 L11 - Please explain 'functionality'.

As shown by all the diversity indexes, the bacterial diversity is much higher than the archaeal. Therefore it is much harder to try to understand the function (metabolic actions) of each order (not even taking about family or even genus) in the different zones. However this sentence was removed in order not to create confusion.



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

This assumption is based on statistical point of view. When you sample a large microbial diversity with different abundance, it is most probable that you will encounter the more abundant species then the rare ones. Therefore our sequences are representative of the major microbial community.

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

This was modified according to the reviewer advice.

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

The similarity to cultured Halobacteria is low (80%). The data was reanalyzed and therefore slightly different results are shown in the revised MS.

P9826 L13 - 'closely related'

The sentence was corrected.

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

Reference was added accordingly.

Discussion

P9827 L5 - Please change the word 'roughly'.

We changed to broadly.

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.

Our sequences of Deltaproteobacteria were aligned to NCBI database. The 97% similar uncultured sequences were used for estimation of similar environments to our se-

quences. We used also 90% similarity in cultured microorganisms to give us a rough idea about the relative functionality together with the uncultured environments. In the revise MS we added the similarly percentages. In order to know the functionality of microorganism we need it to be cultured. Less than 1% of the environmental microorganisms are cultured. Therefore in microbial ecology we can only speculate what is the microbial functionality based on the similarly of the closest cultivated relatives and the environment of clones from different environments.

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

In the revised MS we added percentages.

P9828 L21-22 - Please expand or explain.

In the water column of LK the conditions for denitrification process are existing. Other studies showed denitrification in the water column. The rapid sedimentation rate ($\sim$4mm a year) allows us to assume that some of the microorganisms that live in the denitrification zones in the water column could sink in to the sediment and could be found there. Those nitrifications microorganisms could survive and prosper when nitrate is available in the top part of the sediment during the mixing period of the lake.

P9830 L19 - A shift in bacterial community diversity?

The environment conditions are changing between the top of the sediment and the 6-9 cm depth. The dissolved organic matter is increasing and the sulfate concentration is depleted. Those changes could lead to change in the microbial populations which govern the top sediment. This was better explained in revised MS.

P9831 L9-11 - Please rewrite this sentence.

Sentence was rewritten according to the reviewer advice.

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 MBGD, MG-III, and TMEG. Please rewrite this paragraph discussing these specific groups.

The reviewer is right. In the revised MS we reanalyzed the data and added similarly percentage. In addition we are discussing higher taxonomic levels (family and genus). Therefore those entire groups are been discussed.

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.

The reviewer is correct, there is no proof. Therefore we took the reviewer advice and toned down the conclusions.

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 discussion on possible functions of the Thaumarchaeota to the clades detected in this study.

In the revised MS the ammonia oxidizing functionality of the Thaumarchaeota are narrowed in the discussion.

P9835 L1 - The discussion needs to be narrowed down to the actual groups of Euryarchaeota detected in this study.

The reviewer advice was taken in to account.

Figures

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

The reviewer is right, however it is also important to see the correlation between the $\delta$13CCH4 to the profile in order to see the effect of methanotrophy in the deep sediment. Adding more profile to the same graph can be some time puzzling. In our case we tried to show the different process that occurs in the sediment by profile measurements of the reactant and products of different respirations. Additionally, the profiles are aligned (the same height and scale) in order to make it easy to compare.

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

The distribution of the archaeal phyla shows the change with depth of the major phyla. It gives the reader the sense of change that occurs with depth. Non the less, in the revised MS we emphasize the higher taxonomic levels.

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