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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 #1

As a general comment to the writing of the paper, there is a substantial amount of discussion in the results section. The results discussion should be kept short and interpretation free. Unfortunately this is not the case here. Please see below specific comments.

We accept this general comment and according to the reviewer's advice, the result section was rewritten and most of the discussion was transferred to the discussion section. Also, in the revised MS we highlighted our conclusion on the link between

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Thaumarchaeota and anaerobic methane oxidation and cut some less significant parts.

A second point which I find surprising is the choice of methodology made by the authors. In a study that focuses on diversity the authors chose to make use of a low throughput sequencing technique, i.e. cloning rather than pyrosequecning or illumine. Additionally the authors present a low number of sequences per sample. The use of cloning would be more understandable had the authors made full use of the method by amplifying full 16S rRNA sequences, to better resolve the taxonomy.

The reviewer is right, next generation sequencing is more suitable for the ecological point of view. However, as the reviewer points, with Sanger clone libraries we can get better resolutions in the taxonomy. Therefore, new and more comprehensive analysis of the data set is presented in the revised version of the MS. In addition, qPCR for functional genes (mcrA, dsrA and pmoA) were added in order to increase the microbial data on the processes occurring in the different zones.

Last, despite the low-throughput, the authors chose to present (analyze) single replicate samples. Overall the author choice of methodology makes their data inappropriate for a full diversity study. Therefore the authors should not over-use their data. The calculation of diversity indexes for comparison between the different samples cannot be meaningful with such a low number of sequences and without any biological or technical replicates. I therefore believe the author should discuss the taxa discovered and their potential biogeochemical role without too much weight on their abundance.

The reviewer advice was very helpful. In the revised MS the diversity indexes and the percentages of the sequences are not mentioned. The comparison of the clone libraries are between the different depths and their similar alignments from the NCBI.

Specific comments (The page numbering refers to the page number in the online pdf version):

One major thing that has not been done throughout the paper is writing taxa in italics.

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This should be applied from the Domain level onwards.

All the taxa were changed to italics.

Title The authors state in the title changes in activity – however activity measurements are not reported. A correct title would include "Changes in microbial community"

The title was changed according to the reviewer advice-thanks!.

Abstract P9814 L 9: There is no need for the abbreviation LK in the abstract since Lake Kinneret appears only once. The abbreviation should be reintroduced in the main body of the manuscript.

The reviewer is right. The abbreviation was reorganized.

P9814 L 12: Erase the word "possible" you examined the changes and not whether they are possible.

Have been corrected.

P9814 L 16: Thaumarchaeota are a group of organisms therefore they do not belong to the family of copper contacting membrane bound monooxygenases. I believe the author refer to the ammonium monooxygenase. Please rewrite the sentence to state that Thaumarchaeota contain such enzymes.

The reviewer is right and the sentence was corrected accordingly.

P9814 L 18: Do the authors mean that they have discovered/showed that Thaumarchaeota in Lake Kinneret are ammonia oxidizers? Or do they refer to the fact that Thaumarchaeota are typically ammonia oxidizers? If the latter is the case, the use of the word "discovered" is inappropriate.

The reviewer is right and the latter is the case. The sentence was corrected.

P9841 L21: I believe that this should be the guiding line throughout the paper: AOM is driven by iron and not by sulfate. Also unless this was the intent of the authors they

should stat that AOM is driven by iron and not sulfate and not as currently written that iron drive AOM is not sulfate driven.

The sentences were corrected according to the reviewer advice.

Materials and methods

P9816 L 26: The references for nitrate and sulfate are too old specifically the one from 1974. If these data are used in the discussion which I believe they are not, the authors should provide newer references or their own data (perhaps if there is a routine monitoring program it could be referenced).

Porewater sulfate profiles are from the recent study of Adler et al (2011). The nitrate concentration is cited form Serruya 1974, however the reviewer is right, and this citation is too old. There is a routine monitoring for total nitrogen in the water column every two weeks (http://kinneret.ocean.org.il/nitur_grp.aspx). The concentrations of total nitrogen in the top 0-15 m in the water column are between 20-60 μM (which is almost the same concentrations as in Serruya 1974). Nishri et al (2000) showed that nitrate is the dominant compound during the mixing time of the lake while in the stratified period ammonia becomes dominant (this reference was added to the MS). In addition, in June 2014 we conducted porewater nitrate profile, by measurements on ion chromatography. No concentrations of nitrate were detected throughout the whole profile. In previous measurement of nitrate profile in the deep part of the sediment (conducted by ion chromatography), nitrate concentrations were below detection limit. Therefore, we kept Serruya's reference, but added also Nishri et al. (2000) and our own results.

P9817 L 8-12: The units here are mixed. Total iron is given in 3% - not stated of what dry/wet weight? Manganese is given in _g g-1. Organic carbon is given again in percent. Please be consistent.

As the reviewer asked, the units were adjusted to percentage from dry weight. It has been corrected in the MS.

P9817 L 11: Can the authors be certain that the Mn concentration measured 43 years ago is still valid??? Don't you provide actual values from your profiles which in fact are much lower?

We have measured the concentrations of dissolved manganese in pore water. The profile of dissolved manganese shows lower concentrations than iron and different pattern. Those implies of less activity of manganese in the deep sediment than iron. However we still don't deny the involvement of manganese in anaerobic methane oxidation.

P9817 L 12: A similar remark as above the concentration and trend in the sediment is from 1978. Surely there has been sedimentation at the lake bottom since then. This is not a valid reference.

We agree, and the concentration of total carbon from a newer study was added to the MS (Eckert, 2000).

P9817 L22: Can you provide the sampling frequency in these 4 years (yearly, monthly weekly).

Sampling was made every 3 to 4 months (this information was added to the MS). However, not all analyses were made at each sampling date, only those of methane and dissolved ferrous iron.

P9818 L7: Please add the model of the GC.

The model of the SHIMADZU GC is 8IF. The model was added to the MS.

P9819 L13: Are the primers 87-907R designed by Ben-Dov as suggested here. I believe that they are older. Unless they were modified in the cited paper, please cite the original reference.

The reviewer is right. The 8F primer was modified by Ben-Dove et al. (2006). The 907R was taken from Lane et al. (1985). Those corrections in the citations were made in the revised MS.

P9820: L1-5. This paragraph needs some rewriting. Something like "inserts were amplified from white colonies using the M13F and M14R primers."

The Paragraph was rewritten accordingly to the reviewer advice.

P9820: L 10: The second check for chimeras is not clear. To what did the authors refer: when the two halves did not align? Do you refer if they didn't align to the same reference sequence? Do you mean aligned or do you mean their final location in the ARB guide tree? I am not certain this is a good measure for Chimera as a 450 nt sequence from different parts of the 16S molecule may easily end up aligned to a slightly different sequences. Was there a cutoff in the decision to throw out sequences?

The reviewer is right. This method is not bullet proof, and just can help finding the more suspicious sequences. However, the data set was reanalyzed to find chimera using Mothur software. The Mothur chimera analyses were made by uchime, bellerophon and ccode algorithms. In addition SILVAngs pipeline (Quast et al., 2013) was used to find unclassified sequences in the clone libraries. The suspected sequences from uchime, bellerophon, ccode and SILVA were aligned single handedly to NCBI database to identify if they are truly chimera. The new analyses were added to the MS.

P9820: L14: The authors probably refer to the placement in the ARB guide tree rather than alignment.

The reviewer is right, however the dataset is reanalyzed and ARB wasn't used.

Results

P9821 L10: Over a dozen can be 13 or 50. Please be specific.

The reviewer is right. About 30 profiles were conducted, and we changed to this number.

P9821 L11: please explain seasonally. This was missing from the method section as well.

We sampled the lake every 3-4 month for 48 months. We added the time interval of the sampling to the MS in the methods section which hopefully explain the seasonality.

P9821 L13: profiles of: : : The word of seems to be forgotten from a previous sentence.

The sentence was corrected according to the suggestion.

P9821 L19-22: the authors provide here data from old references. The result section should present only results obtained during the course of this study. Interpretation or references to previous studies should be left to the discussion.

The paragraph was changed according to the suggestion.

P9822 L1: The methane profile is in Fig 2B rather than A. The panels are inverted also in the figure caption. Additionally the figures are, at least in my version, of low quality and cannot be read properly.

In our version the figures have the right caption and are in good quality. We will notify the editor about this.

P9822: This entire section is mixed with results and their interpretation. Any sentence that uses "suggests", "probably", "support": : : belongs to the discussion and should be removed from the results section. This entire section can be much shorter and "cleaner".

The reviewer is right. The results section was changed and now there are no "suggestions" in the MS.

P9823 L10-13: The decision which samples to sequence belongs to the methods and can be mentioned once more in the discussion.

We accept this advice, and the decision paragraph was moved to the methods.

P9823 L22-25: This is valid to all sequencing methods. As long as direct counts are not available (via FISH), PCR based data should be used cautiously.

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The reviewer is right, we wrote this sentence in order to show our understanding of the PCR bias and that we took it under consideration.

P9823 L26: High degree of richness as compared to what?

The comparison was between richness of the different depths. However, the paragraph was deleted.

P9824 L4-5: highly diverse community – this has to be used comparatively to other environments. And belongs to the discussion.

The reviewer is right, therefore the paragraph was deleted and part of the statement was added to the discussion.

P9824 L16-23: The use of percentage is not valid in my opinion. Over 10% means 4 sequences. This is meaningless. An increase in Nitrospira to a relatively high percentage (11%) – One replicate, 4 sequences (11% of 38). You can say they are found in the deeper samples and not in the shallow one but I would refrain from using any percentages.

We agree with the reviewer. The percentage is not relevant (we tried to show the cut off more abundant clones in the clone libraries). The sentence was changed. P9825 L1-3: The authors jump from phyla (Nitrospirae) to family (Nitrospiraceae) to genera (Nitrospira). If it was done intentionally, make use of the prefix family or genera.

The reviewer is right. The SILVA and ARB classification showed the class Nitrospira and the family Nitrospiraceae. The sentence was fixed. P9825 L6: Please specify which families do: "Our Deltaproteobacteria" refer to. The classification was better specified and changed slightly with the new analyses.

P9825 L26: Rephrase. About 17% : : : could not be classified using SINA and were classified using ARB instead.

The sentence was rephrased according to the reviewer advice.

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P9826 L2: To the 13%-40% refer to % out of the total community or % out of the Thaumarchaeota - specify?

The percentage meant from the total community. This was added to the MS.

P9826 L 13: closely instead of close related.

The word was changed.

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P9827 L10: ferrous – the word iron is missing.

The word was added.

P9827 L14: and its resemblance (not resemble)

The sentence was corrected according to the reviewer advice.

P9827 L17-21: I would avoid making use of the diversity indexes given the limitation of the methods used and samples sizes and numbers.

The reviewer is right and the text was changed.

P9827 L22: Therefore (not Therfor)

The word was corrected.

P9827 L26: Proteobacteria are the most described phyla of bacteria (especially from environmental samples) therefore it is not a big surprise that it is among the most abundant phyla. The discussion should be held at the family level or higher taxonomic resolution.

The reviewer is right especially using our long sequences that allow higher taxonomy. Therefore, the data was reanalyzed and higher taxonomy is discussed also in the new MS.

P9828 L2: It is more common and correct to say that the sequences were related

to or clustered with sequences of: : : rather than aligned to. Specifically since the sequences aligned to other sequences and not to organisms.

The reviewer is correct. The sentence was changed.

P9828 L3: Some sulfate reducers are also iron reducers. This may be relevant to the iron based AOM discussion. Please specify families found.

The reviewer is right, sulfate reducers can use iron and be relevant to AOM. Therefore the new analyses of the data show higher taxonomy and the families that can be relevant.

P9828 L5: upper part of LK. Does this refer to sediment of water column?

It refers to the upper 10 cm of the sediment.

P9828 L9: Chloroflexi are usually rather small. You have stated that sulfate reduction was the main process in the upper part of the sediment. How does this fit with your thoughts regarding the role of Chloroflexi.

Chloroflexi was one of the most dominant phyla in contaminated soil environment, which had a lot of polycyclic aromatic hydrocarbons (Winderl et al., 2008). In natural environments they may be involved in biodegradation of aromatic organic compounds (Zhao et al., 2012), as may be in LK.

P9828 L15-23 This entire sections discusses organic matter usage by different groups. Though interesting it deviates from the AOM topic of the paper. Furthermore the discussion does not follow a single line but rather states that all the groups found may be organic matter consumers. I am curious how does the activity attributed to these organisms fit with the relatively deep O2 penetration of 4 mm which was mentionedearlier. As well as the denitrifies which should be anaerobes. My guess is that the 4 mm O2 penetration is seasonal and was not the case during some of the periods discussed here. But all of this should not be left for the reader to assume or guess but rather be clearly stated.

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The MS is focused on the methane cycle, however there are some dominant phyla that are not involved in the methane cycle and still need to be addressed. This paragraph tries to explain the role of the Bacteroidetes found in the upper part of the sediment regardless to the methane cycle. The reviewer is right about the seasonality of the oxygen (Monomictic Lake as mentioned in the MS), however the denitrification can be seasonally but for a short period as nitrate is very low concentration in the top 1 cm of the sediment.

P9828 L23: Archaeal communities are responsible for many environmental processes. This sentence is meaningless unless you specify which processes.

The reviewer is right, therefore the sentence was deleted.

P9828 L29: similarity at the phyla level is almost meaningless and the authors clearly state that this is not valid at the OTU level. Keep the discussion to meaningful data. It does not make sense to provide information regarding similarity of taxonomic units to which one cannot (practically or potentially) assign a defined functional role.

The reviewer is right, therefore the text was changed and the role of the microorganisms is discussed in the family level and higher. This was made using similarity to cultured microorganisms and environments related to uncultured microorganisms that might indicate to the role of our sequences in our environment.

P9829 L3: Why did the authors use such a low cutoff (90%) for their similarity? Please have a look at the paper by Rosello-Mora and Amann: The species concept of prokaryotes FEMS Microbiol Rev. 2001 Jan;25(1):39-67. The paper shows the correlation between DNA-DNA hybridization (i.e. genomic similarity) and 16S similarity. 90% is quite far off to say anything about the functional similarity of the organisms from which the sequence was obtained.

The reviewer is right - the cut off is low. However, using 97% cut off didn't give us a lot of similarity results of cultured microorganisms. Most of the environmental microorgan-

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isms are unculturable as the reviewer probably knows. 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 comparing the environment that they were found. 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.

P9829 L 25-26: The authors make a factual statement citing a reference from 1992. The use of old reference regarding what is happening in the lake during the course of the present study is done quite often in this paper. If the authors believe the lake remained unchanged since the 70' 80' 90' or so, they should provide evidence for this and state this clearly at the beginning of the manuscript.

The reviewer is right, there are some changes during 40 years in the lake as this is nature. However, our research is based on new data (Adler et al,. 2009) and long monitoring of the lake (http://kinneret.ocean.org.il/nitur_grp.aspx) and research (Eckert and Conrad,. 2007. Nusslein et al., 2001 and Schwarz et al., 2007). We also emphasizing our analyses on the deep sediment (∼30 cm=∼70 years) which the old data is still relevant.

P9829 L29- It is more common to say that the newer study supports the older one and not vice versa.

The advice of the reviewer was taken in to account and the MS was changed accordingly.

P9830 L5: It may be true that generally sulfate reduction outcompete methanogenesis, however the concept of the sequential redox tower has been discussed recently as more and more "miss fitting" bacteria are found in the wrong place e.g. sulfate reducers in areas of oxygenic phototrophy. For the case mentioned here, have a look at MEPS 107, 177-18 (1994) where co culturing of methanogens and sulfate reducers has been

shown.

The concept of microbial sequential redox tower zones is been shown to be into simplified in many environments (e.g. see our data in Sivan et al., 2014). However here the dominant process is sulfate reduction as studied by Eckert and Conrad in 2007.

P9830 L15: The same comment as above.

The geochemical evidences show that more methane is been produced in the zone and that sulfate concentrations are depleted. Therefore it is a good assumption that methanogens are more active in this zone.

P9832 L5-6 The same comment – old reference for an actual value of a substrate in the lake. Don't you provide Mn data yourself in Fig 2?

The reference is focused on the presence of Mn in the solid state. We conducted profiles of dissolved Mn. The sources of the Mn to the lake sediment did not changed in the last 4 decades therefore the concentration should be similar.

P9834 L10-15 Too long sentence. Split and write explicitly to which enzyme you refer to.

The sentence was changed and we explained which enzyme might be involved.

Figures

Fig 2: Panels A and B are inverted with respect to the text. There is room to move panel D up to the same line as the other panels. At least in my version the figures are of low quality the text is not readable and the fonts too small.

The figures which we submitted are in high resolution and with the correct labeling. This remark will be noted to the editor. In order to move panel D in to the same line the size of all the panels need to be smaller thus making them less readable (unless the panels are vertical). Therefore it is better to keep it as it is.

Figures 3 and 4 – should be done at the family level and restricted to main families not all the observed ones. The latter should be supplied as a supplementary table.

The reviewer is right and the new analyses were added to the MS and more figures with higher taxonomy were added.

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