16.1.15

Re: Manuscript bg-2014-289, entitled "Methane related changes in prokaryotes along geochemical profiles in sediments of Lake Kinneret (Israel)" by Bar-Or et al.

Dear Dr. Kirsten Küsel,

Please find enclosed a revised version of manuscript bg-2014-289, entitled "Methane related changes in prokaryotes along geochemical profiles in sediments of Lake Kinneret (Israel)". We would like to thank you and the anonymous reviewers for the thorough and constructive reviews of the manuscript. Their comments contributed significantly to improving the revised version. We have changed completely the manuscript and used larger dataset (454 sequencing) and added analysis of functional genes to improve our understanding of the microbial processes in the different depths. We are glad that they found this manuscript interesting, stimulating and of global importance. Hope that it will be interesting and satisfying.

Yours sincerely,

Itay Bar Or

Anonymous Referee #1
Received and published: 14 July 2014

The manuscript by Bar Or et al discusses the changes in microbial community (composition and diversity) alongside changes in electron donors and acceptors in a depth profile in the sediments of Lake Kinneret in Israel. The main conclusion of the paper is the link between Thaumarchaeota, which are typically known as marine ammonia oxidizers, and anaerobic methane oxidation. Unfortunately this important piece of information is well hidden within the text among other less significant / valid data.

While the above mentioned conclusion by the authors is very interesting, the paper in its current state requires, in the opinion of this reviewer, significant rewriting with respect to organization, language and to some extent data exclusion.

By reading the acknowledgments section it appears that this manuscript has already been under review. Since it has not passed through my hands, I understand that some of my comments may go against suggestion made by previous reviewers. Therefore, I will try to explain my comments as much as possible where I find it necessary.

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.

According to the reviewer advices, the result section was rewritten and most of the discussion was transferred to the discussion section.

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. Therefore we added a new 454 analysis. In addition, qPCR for functional genes (mcr, dsr and pmmo) 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 new MS the diversity indexes and the percentages of the sequences are not mentioned.

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. This should be applied from the Domain level onwards.

All the taxa were changed to italics.

Tatle: 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.

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 correctedP9814 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).

Sulfate concentrations are from recent study of Adler et al 2011. The nitrate concentration is cited form Serruya 1974 however the reviewer is right, 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 in Serruya 1974. Nishri et al (2000) show that nitrate is the dominant compound during the mixing time of the lake while in the stratified period ammonia becomes dominant (Nishri et al. reference was added to the MS). In addition in June 2014 we took porewater for nitrate profile and measured it 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, nitrate concentrations were below detection limit (measured also by ion chromatography). Therefore no profile was added and Serruya concentrations are still valid.

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 employ of less activity of manganese in the deep sediment then 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.

As the reviewer asked, 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).

The sampling was made every 3 to 4 month (this information was added to the MS). However different analysis were made on each sampling, only methane and ferrous measurements were made consistently on each sampling.

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 was right. The 8F 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 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 built prof however it can help finding the more suspicious sequences. However we added new 454 dataset to the MS with different methods for analysis using MOTHUR and SILVAngs pipeline (Quast et al., 2013).

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

The reviewer is right however new dataset was used and ARB wasn't used.

Results

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

The geochemical profiles of the sediment in station A were made long before this research and during this research. Adler et al,. 2011 shows 6 profiles out of 9 profiles that were made previous to this MS. During 2009-2014 about 20 profiles were made. Therefore over a dozen seems fit to the text.

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

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.

Sentence was corrected according to the reviewer 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 reviewer 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 result section was changed and made "cleaner" from "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.

The reviewer advice was taken 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.

The reviewer is right therefore 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 per-centage (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 a little bit 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.

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.

Discussion

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. However we used new 454 dataset and show higher taxonomy in the 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 is refer to the 10 cm of the upper part 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 maybe 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 mentioned earlier. 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.

The MS is focused on the methane cycle however there are some dominant phyla that 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 reviwer 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 microorganisms 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 compering the environment that they were found. All this 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. However we changed the dataset and used SILVA 119 database for classification.

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 we 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 laired redox tower zones is been shown to be not totally correct in many environments. 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 concentration 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 have made 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.

Response to anonymous referee #2

Received and published: 19 August 2014

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 advice.

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 in 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- However we changed the dataset therefore the figures were changed also.

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?

The sampling was made every 3 to 4 month (was added to the MS) from 2007 to 2013 from the same location. However different analysis were made on each sampling, only methane and ferrous measurements were made consistently on each one. 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. Also 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 column 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 we changed the dataset and it analysis.

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 $\delta^{13}C_{CH4}$ and $\delta^{56}Fe$ 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 used new dataset of 454 which gives better coverage of the microbial populations.

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 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 new dataset analysis show also sequences similarity to Halobacteria with close affiliation.

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 the word 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.

We have used two methods to analyze the 454 dataset. SILVA ngs pipeline were used for alpha diversity. Mothur pipeline were used for the beta diversity. Each pipeline has its own cutoffs as explained in the MS.

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 excess. Other studies showed denitrification in the water column. The rapid sedimentation rate (~4mm a year) allows assuming that some of the microorganisms that live in the denitrification zones could sink in to the sediment and could be found there.

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 use new 454 dataset. In addition we are discussing higher taxonomic levels (family and genus). Therefore those entire group 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 $\delta^{13}C_{CH4}$ to the profile in order to see the effect of methanotrophy in the deep sediment. Adding more profile to the same graph can some time puzzling. In our case we also tried to show the different process that occurs in the sediment by profile measurements of the reactant and products of different respirations. Also 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. However in the revised MS we more emphasize on higher taxonomic levels.