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Biogeosciences Discussions

Interactive comment on "Differences in community composition of bacteria in four deep ice sheets in western China" *by* L. An et al.

L. An et al.

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Subject: Response to Interactive comments on "Differences in community composition of bacteria in four deep ice sheets in western China" by L. An et al.

Dear Editor,

All of the reviewer's comments were seriously considered into the new version of the manuscript, and replied on behalf of all of the coauthors as follows:

Anonymous Referee 1 Received and published: 23 March 2010. General Comments Overall the manuscript is well presented and written. The methods used were appropriate and seemed to be well designed and executed. However, the materials and methods lacked some detail and some key information was missing from the manuscript

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Interactive Discussion

Discussion Paper

for comparison between different glacial sites. Finally, I believe that the authors' hypothesis of spatial and temporal variation in this system may need more supporting evidence.

Re: The issue of biogeography of microorganisms was initially explored by comparisons among the pooled bacterial sequences from geographically isolated glaciers in western China in the current study "Differences in community composition of bacteria in four deep ice sheets in western China" by L. An et al.". The data showed an evident seasonal variation in the proportion of main phylogenetic clusters along the Muztztag Ata glacier profile (Fig. 4). It was also an apparent for the community shift of microorganisms among the geographically isolated glaciers as observed in Figure 5 and indicated by the gray shaded areas within the members of Betaproteobacteia, Gammaproteobacteria, Bacteroidetes and Actinobacteria phylum (Figs. 3a, 3b, 3c, 3d, and 3e). The proposed hypothesis still needs to be tested by more consistent ice core data from the glaciers worldwide, which has also been discussed in this study (see the last paragraph in the Discussion section in the new version of the manuscript).

Specific comments Material and Methods - The primers used for generating 16S rRNA gene amplicons should be listed. Presumably they are in the other references cited, but as this is a critical aspect of the diversity that will be recovered in this study, I believe they should be presented.

Re: The primers used for the 16S rRNA gene amplification from the Muztag Ata Glacier were provided in the paragraph "Biomass analysis and clone library establishment of the bacterial 16S rRNA gene amplified from the Muztagata Glacier" in the Materials and Methods section of the new manuscript version.

- On line 23 the authors state "community composition was statistically analyzed using the Unifrac software package". It should be noted whether the weighted or in weighted model was used (although it was mentioned in Figure 5). This belongs in the methods.

Re: The information about UniFrac analysis was provided in the Materials and Methods

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in the new version of the manuscript as follows: “Differences between the clone libraries were estimated with the weighted Unifrac algorithm (Lozupone and Knight, 2005). The clone numbers of the tested sequences were also used for the UniFrac analysis. A sequence jackknifing technique was applied to each cluster to determine the sensitivity of the relationships to sample size.”

- Sequences recovered in this study were compared to each other and to sequences recovered from other sites. Sequence comparison in BLAST was used to determine if similar sequences were present in multiple samples. The methods should explicitly state what the sequence identity was that determined if a sequence was deemed to be present in more than one site.

-Results.

Re: To initially explore the biogeography of microorganisms at temporal and spatial scales, all the sequences from the four glaciers and other closest relatives obtained by Blast search (Altschul et al., 1990) were aligned with reference sequences obtained using ClustalX (Thompson et al., 1997). All the obtained sequences from the glaciers were identified by the recognized species, and related to the ecological clusters (e.g., Rhodoferax sp. and Variovorax sp. in the Betaproteobacteria subphyla. See the first paragraph “Statistical analysis of the bacterial communities in the four deep ice sheets in western China” in the Materails and Methods section of the new manuscript version).

-No physical or chemical data for the different cores is presented. This is critical information for this study. It may be that factors such as organic carbon or temperature might be more closely related to patterns of diversity than temporal and spatial patterns.

Re: It is still a challenging issue about the factors and processes that determine the patterns of microbial community in glacier ice, which has been discussed in the last second paragraph in the Discussion section under the subtitle “Climatic and environmental implications of microbial communities in glacier ice”.

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Also the relevant physical or chemical data for the different cores were provided in the new manuscript version. The ice-core borehole temperature from the four glaciers showed an extremely cold ice temperature ranging from <-3.7 to -26.17 °C (Thompson et al., 1990; Pu et al., 2002; Wu et al., 2003; Li et al., 2004, see the Materials and Methods section). The organic matter concentrations in the glacier ice are very low (see the Methodological considerations in the Discussion section).

-Unless the sites sampled have similar physical/chemical characteristics, patterns of distribution can not be assigned to temporal or spatial factors.

Re: The high quality of ice core records and overall extremely cold and oligotrophic glacier environments at the high altitudes ensures the preservation of microbial information in the ice, and makes it possible for a community comparison of microorganisms in the geographically isolated glaciers (see the Methodological considerations in the Discussion section).

- Figure 3a and 3b present phylogenetic dendograms of the recovered 16S rRNA gene sequences. I am not sure what the logic of choosing Methanosaeta. Presumably it was chosen as an archaeal outgroup. Choosing an outgroup that belongs to a different kingdom than the other sequence may hide details of the tree due to long-branch attraction. I would suggest a closer related outgroup from a bacteria not expected to be in glacial ice. For example, an obligate thermophile, such as Themotoga could be used. In addition, if a rooted tree is presented, the root should be preserved in the figure so that this distance is maintained for comparison.

Re: The reason for choosing Metahnoaeta outgroup was explained in the Materials and Methods section in the new version of the manuscript. The Thermotogae referent sequences AB039768 and U89768 collapsed the outgroup references into either one or two branches of the tree. We attempted to construct the phylogenetic trees without and with the Metahnoaeta outgroup, but the results showed no significant difference between the bacterial subphyla, which was consistent with previous results (Daubin et

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al., 2001). Also, the Metahnoaeta outgroup sequences were listed on the tree.

- Figure 5. The authors state “cluster showing the overall phylogenetic distances”. Unifrac does not provide phylogenetic distances, but a measure of community similarity that is based on phylogenetic difference.

Re: the phrase “Hierarchical cluster showing the overall phylogenetic distances” was corrected to “Hierarchical cluster showing the overall phylogenetic differences.....” in the new manuscript version.

- Figure 5. The “Malan” clone library used in this figure contains only contains 7 clones. Can you truly make a comparison based on this small amount of data? These 7 clones at best can only represent the most abundant species. With no abundance data and small clone libraries I don’t believe that these comparisons are valid, or at least should be confined to studies with a similar number of clones.

Re: The current available clone library technique can only recover the dominant bacteria in glacier ice. Only a limited clone library data are available from the database, which is the reason for consideration of the Malan clone libraries as references for the UniFrac analysis. The UniFrac has the advantage of considering the taxonomic species sequences and their abundance into the analysis of microbial communities. So the clone numbers of the tested sequences were used for the UniFrac analysis (see the last paragraph of Materials and Methods).

- The authors compare their results to other glacial environments, but what about other ice environments? For example, ice wedges (Katayama et al., Appl. Environ. Microbiol. 3:2360-2363) or ground ice (Steven et al., Environ. Microbiol.10:3388-3403).

Re: The current study focused on the clone library data from the mountain glaciers at the high altitude in western China, where two main hydrological circles, westerly and summer monsoon play an important role in the distribution of precipitation over the glaciers, and could be related to the community shift of microorganisms across the

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glaciers in western China. This was cautiously discussed in the current study. However, in the ice wedges, other processes such as repeated cycles of frost cracking followed by the infiltration of snow, meltwater, soil, and other material into open frost cracks (Mackay, 1972) might strongly influence the community distribution of microorganisms in ice.

- In section 4.2 line 13 the authors state “strengthens the concept of adaptation and acclimation of microorganisms to : : : glacier environments”. In this study no metabolic activities were measured, therefore it is not clear that the organisms described in this study are adapted to this environment. Instead they may be frozen dormant cells that are more resistant to freezing than other populations. This is an important point as cells like Firmicutes could have been present as spores.

Re: The sentence “strengthens the concept of adaptation and acclimation of microorganisms to : : : glacier environments” was changed to “The microorganisms present in ice may be dormant, representing a viable but metabolically inactive state. They may even metabolize within ice for maintenance but not growth (Price and Sowers 2004; Price 2007), which possibly makes them more resistant to freezing than other populations in mild environments.” in the new manuscript version.

-Finally, the authors propose two independent hypotheses, that the microbial communities display temporal biogeography and that they also show spatial biogeography. These are two independent hypotheses, and there is very little overlap between samples to demonstrate this. For example, Figure 5 may suggest spatial biogeography but the samples in space are also from different times. To truly show that there are both spatial and temporal patterns of distribution you need to have physically separated samples from the same time. If these clusters are more closer together than the samples from different times, then you demonstrate differences in temporal distribution.

Re: The main concern was corrected towards the general biogeography of microorganisms in glacier ice, which was initially evaluated mainly at a seasonal scale, and

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also somewhat discussed at a spatial scale. The current findings that the proportion of main phylogenetic clusters varied with seasons (Fig. 4) and microbial communities were different in the geographically isolated glaciers in western China (Fig. 5) were only preliminary results. Also it should be noticed that the bacterial sequences from the geographically isolated glaciers were pooled together and then compared. These results showed a clear separation of microbial communities corresponding to the spatial patterns of glaciers, which suggested that the spatial isolation might have a stronger influence than temporal stresses on the distribution of microbial community in glacier ice. The patterns of microbial communities in the mountain glaciers might be related to the variations in the air masses over the glaciers on the Tibetan plateau at the different time scale. More consistent ice core data from the glaciers worldwide are necessary before a definite conclusion can be drawn on the biogeography of microorganisms in ice at both time and spatial scale (See the limitation discussed in the last paragraph of the new manuscript version).

-Technical comments - Table 1, the first column reads clone library. This should be changed to drill core or something similar, as the numbers presumably do not refer to the number of cells in the clone library. I also think the number of clones sequence per site should be added to this table or be made explicit somewhere in the text.

Re: The mentioned technique error was corrected in the new manuscript version.

Section 3.1 line 10, change OUTs to OTUs.

Re: the “OUTs” was already corrected to “OTUs”.

-Anonymous Referee 2

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-General comments:

-The manuscript of Ann et al. presents a comparative study of the bacterial diversity in four glaciers in Western China. It is a new contribution within a broader long-term

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research of this team on the microbial community composition of a large number of glaciers from the Tibetan plateau that has been reported in previous publications. It also adds to the growing worldwide data pool of microbial diversity in glacial ice as a unique extreme environment. The major achievement of this work is the detailed analysis of four 16S rRNA gene clone libraries from the MuztB core drilled from the Muztag Ata glacier corresponding to different annual layers and seasons, which are characterized by specific aerosol fluxes. The authors performed their phylogenetic analyses using new sequence data and previously published sequences from similar studies of three additional geographically distant Tibetan glaciers in order to find meaningful correlations with climate and biogeography. The methods are adequate, the results are well presented and illustrated in one Table and five figures and the overall structure of the manuscript is clear.

-Specific comments: - In the title the authors use the term "ice sheets" referring to four well-known glaciers in western China. Further in the text "ice sheets" are mentioned only three times. Generally, the authors use the more accurate term "glacier", which is commonly used in the literature to designate specific Tibetan glaciers. My suggestion is to avoid this inconsistency and use the term glaciers throughout the manuscript, including the title.

Re: The term “ice sheets” were already changed to “glaciers” in the new manuscript version.

- In the Materials and methods section the authors do not specify the diameter of the studied ice cores and how long they were in the ice columns.

Re: The diameter of each studied ice core and length of the ice columns were provided in the new version of manuscript (See the second paragraph in the study sites and sample collection in the Materials and Methods section): All the four ice cores with 10-cm diameter were drilled from the glaciers at the high altitudes, >5300 m above sea level (a.s.l.). . .. . .. All ice core sub-samples (around 30 to 50 cm-long ice columns)

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were always handled at temperatures below 20°C within a sterile and positive-pressure laminar flow hood by following the previously described procedure (Xiang et al., 2005).

- Although the authors refer to an earlier publication for the experimental procedures some details are still necessary such as the PCR primers used or the model of the flow cytometer.

Re: The primer pair used in this study and the model of the flow cytometer (FCM) used for the analysis of microbial biomass was provided in the new version of manuscript (See the third and fourth paragraphs in the Materials and Methods section): The 16S rRNA gene amplicons used for the establishment of clone libraries from the Muztag Ata Glacier were generated by PCR amplification with the universal bacterial primer pair 8f (5-AGAGTTTGATCATGGCTCAG) and 1492R (5-CGGTTACCTTGTTACGACTT) (Lane 1991; Weisenburg et al., 1991). Samples were analyzed with a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California, U.S.A.).

- The authors should be more careful when linking functional and phylogenetic clusters based solely on cloned 16S rRNA gene sequences (p.1181, 1182, 1183). Actually they do not discuss any possible functions of the detected species, which in this case would be hypothetical. While microbial species belonging to a certain phylogenetic cluster group may have common functional characteristics, functional versatility also exists within a single species or genus.

Re: The term “function” was changed to “phylogenetic” throughout the new manuscript version.

- The sequence comparisons of the 151 16S rRNA gene clones from MuztagB with those from similar clone libraries from three other glaciers are based on a limited number of sequences (e.g. 14 from Dunde, 18 from Malan and 39 from Puruogangri), which restrict the evidence supporting the authors’ hypothesis for spatial biogeographic distribution.

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Re: As discussed above, the current findings that the proportion of main phylogenetic clusters varied by seasons (Fig. 4) and microbial communities were different in the geographically isolated glaciers in western China (Fig. 5) were only preliminary results. More consistent ice core data from the glaciers worldwide is necessary before a definite conclusion can be drawn on the biogeography of microorganisms in ice at both time and spatial scales (See the limitation discussed in the last paragraph of the new manuscript version). It should also be noted that the discussion on the spatial biogeography of microbial community in glacier ice was based on a comparison of bacterial 16S rRNA gene sequences pooled from the geographically isolated glaciers.

- It is not clear why 13 sequences from the Puruogangri and Dunde glaciers are shown separately on Fig. 3e rather than in the corresponding trees representing the major phylogenetic groups (Fig. 3a, b, c, d). In addition, some sequence references are inaccurately presented such as the accession numbers AY121823-AY121830 on p. 1172 and in Fig. 5 legend are from Zhang et al. 2009 and not from Zhang et al. 2002. Similarly the number of clone libraries established from the four glaciers is changed from 9 to 13 on p.1184.

Re: All of the bacterial sequences from the four glaciers are partial of the 16S rRNA gene sequences, and most of them contain the forward portion of the 16S rRNA gene sequences, corresponding to regions 8-800 of the Escherichia coli 16S rRNA molecule (Figs. 3a, 3b, 3c, and 3d). However, 9 sequences from the clone library P80 from the Puruogangri Glacier and 4 sequences from the TD clone library from the Dunde Glacier contain the later portion of the 16S rRNA gene sequences, corresponding to regions 800-1452 of the Escherichia coli 16S rRNA molecule. The phylogenetic tree of the 13 sequences and the closest relatives was separately established (Fig. 3e). The references for sequences AY121823-AY121830 were checked, and corrected to Zhang et al. 2009. Re: The sentence “the number of clone libraries established from the four glaciers” was moved out of the new manuscript version.

- One of the Discussion subsections addresses methodological considerations related

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to the quality and reliability of the climatic ice core records, which is completely adequate. At the same time, it is surprising that the well known methodological constrains of the molecular methods used in microbial diversity studies (as this one) are not mentioned. This is particularly relevant to this comparative molecular analysis of sequences from clone libraries obtained over a 5-6 year time period.

Re: The disadvantages of polymerase chain reaction (PCR)-derived methods including resolution limitations of the highly conserved 16S rRNA genes, PCR bias, and sequence artifacts were briefly discussed in the method considerations in the Discussion section. The improvement of PCR-dervived method and comparability of bacterial sequence data from the geographically isolated glaciers were the main reasons for choosing 16S rRNA gene clone library method used in this study.

Technical comments: p. 1168, line 5 – The total number of sequences from the Muztag glacier is 152 in the Abstract and 151 in the text (p.1172, line 20).

Re: The number of sequences from the Muztag Ata Glacier was corrected to 151 in the new version of the manuscript.

p. 1171, Line11 - Is the temperature for handling the ice cores below 20°C or below -20°C?

Re: Each ice column of the obtained ice cores was split lengthwise into four sections within a walk-in freezer, and stored in a refrigerated room at -18°C to -24°C. All ice core sub-samples (around 30 to 50 cm-long ice columns) for further microbial analysis were handled at temperatures below 20°C within a sterile and positive-pressure laminar flow hood by following the previously described (procedure Xiang et al., 2005)

p.1172, Line 3 - Change "151 clones were sequenced by HaeIII ARDRA" to "151 clones were selected for sequencing by HaeIII ARDRA"

Re: The phrase “151 clones were sequenced by HaeIII ARDRA” was changed to “151 clones were selected for sequencing by HaeIII-based ARDRA” in the new manuscript

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version.

p.1174, line 6 - The number of live cells in Table 1 ranges from 4.28 to 4.98 (x 10^3) (not 10^5).

Re: In the text of the new manuscript version, the number of live cells in Table 1 was corrected to 4.28 to 4.98 (x 10^3).

Other modifications were also made as follows:

Abstract:

The sentence in the Abstract section “the sequences from the same glacier formed a distinct cluster.” was changed to “the sequence clusters from the same glacier more closely grouped together than those from the separated glaciers.”. The sentence "the findings . . .. . .” was changed to “the findings provide preliminary evidence of zone distribution of microbial community, and suggest biogeography of microorganisms in glacier ice.”.

Figure legends:

Figure 1: the map source “Map was adapted from Xiang et al., unpublished” was provided in the new version of manuscript. The locations of Malan and Puruogangri ice cores were corrected in the new manuscript version.

The relevant references were also provided in the new manuscriptversion.

Best regards

Sincerely yours

Shu-Rong Xiang

Interactive comment on Biogeosciences Discuss., 7, 1167, 2010.