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

Anonymous Referee #1

Received and published: 23 March 2010

In the manuscript “Differences in community composition of bacteria in four deep ice sheets in western China”. By An et al., presents the microbial community as described by 16S rRNA sequence analysis from four glaciers in China. The sequences recovered from these four glaciers were compared to other studies that used similar methods to describe the microbial communities in other glacial systems. The authors hypothesize that the microbial populations in glacial ice show both spatial and temporal patterns of biogeography.

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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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. 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.
- 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.
- 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

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

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be in glacial ice. For example, an obligate thermophile, such as *Thermotoga* 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.

- 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.

- Figure 5. The “Malan” clone library used in this figure contains only contains ~ 7 clones. Can you truly make a comparison based on this little 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.

- 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.* 73:2360-2363) or ground ice (Steven et al., *Environ. Microbiol.* 10:3388-3403).

- 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.

- 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 cluster more closely together than samples from

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different times, then you demonstrate differences in temporal distribution.

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.

- Section 3.1 line 10, change OUTs to OTUs

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

BGD

7, C296–C299, 2010

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