

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

**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 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 sequences data and previously published sequences from similar

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studies of three more 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 and in most cases the authors use the more accurate in my opinion 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 including the title.
- In the Materials and methods section the authors do not specify what was the diameter of each studied ice core and how long were the ice columns.
- 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.
- 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 certain phylogenetic cluster (group) may have common functional characteristics, functional versatility also exists within a single species or genus.
- 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 restricts the evidence supporting the authors' hypothesis for spatial biogeographic distribution. It is not clear why 13 sequences from the Puruogangri and Dunde glaciers

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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.

- One of the Discussion subsections is addressing methodological considerations related to the quality and reliability of the climatic ice core records, which is totally adequate. At the same time, it is surprising that the well known methodological constraints 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.

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).

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

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

p.1174, line 6 - The number of live cells in Table 1 ranges from  $4.28 \times 10^3$  to  $4.98 \times 10^3$  (not  $10^5$ , which would exceed the total number of cells).

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