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Interactive comment on "Community change of microorganisms in the Muztagata and Dunde glacier and climatic and environmental implications" *by* Yong Chen et al.

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Response to interactive comments on BG2015-637 "Changes of the bacterial functional groups in the Dunde and Muztagata glacier" by Yong Chen et al.

-previous title: Community change of microorganisms in the Muztagata and Dunde glacier and climatic and environmental implications

Dear Dr. Minhan Dai,

I here wrote you on behalf of all co-authors. After we seriously considered the two anonymous reviewer's comments, we reorganize our manuscript from a chronological to hierarchy order: Firstly, we proposed the question of why the specific microorganisms live in the geographically glaciers where they are, and the new idea of biogeography of functional groups in glaciers. We expect that the apparent shifts in microbial communities in the glaciers associated with isolated habitat changes are most possibly related to the influences of geographic differences on the climates and environments over glaciers across the geographically different regions. . .. . . We hypothesized that the geographic locations control the glacier habitats of microbial communities by changing the climatic patterns, and determining the dominant colonizers, and their main contributions to the microbial feedback to the global and local climatic and environmental changes. . . ..(introduction section in the new manuscript version). Comparisons of microbial communities across the geographically different glaciers Dunde, Muztagata and Puruogangri allowed us to infer the impact of functional groups on the biogeography of microbial communities in the ice.

We put the question how the live bacterial cells response the dynamics of seasonal temperature changes in the secondary. The live bacterial data were examined and related to the physical-chemical parameters from the two ice cores Muztagata and Dunde (Introduction section in the new manuscript version). The Muztagata ice core presented seasonal response patterns for both live and total cell density with high cell density occurring in the warming spring and summer (in the result and discussion section).

We addressed the questions in the result and discussion sections of the new manuscript version. It was possibly the first attempt to explore the biogeography of functional groups in glaciers, although our team colleagues found differences of the microbial communities across the mountain glaciers (Yao et al., 2008; Xiang et al., 2009; 2010; An et al., 2010), and proposed "the prevalent bacteria" in the local regions based on the findings that several bacterial genera frequently occurred throughout the depth profile from the Muztag Ata Glacier and the Rongbuk glacier of Himalaya (An et al, 2010; Zhang et al., 2008). However, this present data showed a similarity of functional group members in the related ice cores at a historical sale, indicating that the micro-biogeography associated with geographic differences was mainly influenced by a few dominant taxonomic groups. This study supports our hypothesis of geographic differences in climatic patterns controlling the biogeography of microbial communities at a taxonomical group level in glaciers.

We made some modifications after we seriously considered the two anonymous reviewer's comments into the new MS version. In this new MS version, we tried to answer the reviewer's questions point by point as follows:

Anonymous Referee 1 Received and published: 19 February 2016 General comments: The paper "Community change of microorganisms in the Muztagata and Dunde glacier and climatic and environmental implications" by Chen et al. describes the microbial abundance and community structure in two short ice cores, collected from two Tibetan ice caps/glaciers, using flow cytometry and sequencing analysis of 16S rDNA clone libraries. The microbial data are related to dust concentrations and temperature record inferred from oxygen isotope analysis. The authors show a correlation between dust and microbial cells and conclude that wind deposition combined with post-depositional processes controls the microbial community structures in glacier ice in the region.

Re: This present data showed a similarity of functional group members in the related ice cores at a historical sale, indicating that the micro-biogeography associated with geographic differences was mainly influenced by a few dominant taxonomic groups. This study supports our hypothesis of geographic differences in climatic patterns controlling the biogeography of microbial communities at a taxonomical group level in glaciers (in the result and discussion section). The Muztagata ice core presented seasonal response patterns for both live and total cell density with high cell density occurring in the warming spring and summer (in the result and discussion section).

We reorganize our manuscript from a chronological to hierarchy order:

The primary goal of this study was to evaluate how the biogeography of microbial communities at a taxonomic group level is controlled by the geographically climatic patterns

across the mountain glaciers in western China. Two different glaciers, Muztagata in the westerly region and Dunde in the westerly and monsoon regions were investigated (Wake et al., 1990; Davis et al., 2005). To validate the bigoegraphical effect of climatic patterns on the microbial communities at a taxonomic group level, six structures of bacterial communities were established from the Dunde ice core columns in 1990 to 2000 by using the clone library, and bacterial 16S rRNA gene techniques. Comparisons of the main taxonomic groups across the geographically different glaciers Dunde, Muztagata and Puruogangri allow us to infer the impact of functional groups on the biogeography of microbial communities in the ice. Additionally, we also evaluate the live populations of microbial communities respond to the climatic and environmental changes. The live bacterial data were examined and related to the physical-chemical parameters from the two ice cores Muztagata and Dunde (Introduction section in the new manuscript version).

The paper is interesting and brings data from very remote (and high!) locations in central Asia; however, it suffers from several weaknesses that preclude publication in C1 BGD Interactive comment Printer-friendly version Discussion paper the present form. My main concerns are the following:

Question: First, what hypothesis/es is/are being tested in the paper? This/these should be clearly stated in the introduction, and addressed in the results and/or discussion sections.

Re: In this study, we hypothesized that the geographic locations control the glacier habitats of microbial communities by changing the climatic patterns, and determining the dominant colonizers, and their main contributions to the microbial feedback to the global and local climatic and environmental changes. We test this hypothesis by comparing bacterial communities across the geographically different glaciers Muztagata, Dunde, and Puruogangri, to determine the dominant colonizers in the three glaciers where the precipitation is controlled by the westerly and monsoon circulations. Furthermore, we test this hypothesis by analyzing the structures of bacterial communities from the related ice cores to determine the impact of dominant functional groups on the biogeography of microbial communities in glaciers.

Question: What are the novel aspects of this ms compared with previous papers such as Yao et al. 2008 GBC, Zhang et al. 2008 BG, An et al. 2010 BG, Liu et al. 2013 Quat Sci, some of which were conducted at the same sites? The authors claim that "the current data have presented a change of the dominant endemic community composition, indicating an association of the microbial spatial patterning with the presence/absence of the dominant species within the specific glaciers" (line 206-8), which is rather unclear. Could this be elaborated on/clarified? Did the authors find this phenomenon for the first time? This is not likely (see the above mentioned papers).

Re: It was possibly the first attempt to explore the biogeography of functional groups in glaciers, although our team colleagues found differences of the microbial communities across the mountain glaciers (Yao et al., 2008; Zhang et al., 2008; Xiang et al., 2009; 2010; An et al., 2010), and proposed "the prevalent bacteria" in the local regions based on the findings that several bacterial genera frequently occurred throughout the depth profile from the Muztagata Glacier, and the Rongbuk glacier of Himalaya (An et al, 2010; Zhang et al., 2008). However, this present data showed a similarity of functional group members in the related ice cores at a historical sale, indicating that the micro-biogeography associated with geographic differences was mainly influenced by a few dominant taxonomic groups. This study supports our hypothesis of geographic differences in climatic patterns controlling the biogeography of microbial communities at a taxonomical group level in glaciers.

The present data sets from the Muztagata glacier at 7010 m above sea level (38°17'N, 75°04'E), revealed clear seasonal patterns with high microbial cell density occurring in the warming spring (filled triangles in Fig. 2) and summer (open triangles Fig. 2), which indicated the positive temperature effects on the microbial density patterns. This was consistent with another independent microbial investigation on the Muztagata glacier at 6300 m above sea level (38°17'N, 75°06'E, Liu et al., 2013). The high repeatability of both ice cores from the Muztagata glacier confirmed reliability of the data sets discussed here.

The sentence "the current data have presented a change of the dominant endemic community composition, indicating an association of the microbial spatial patterning with the presence/absence of the dominant species within the specific glaciers" was corrected to "this present data showed a similarity of functional group members in the related ice cores at a historical sale, indicating that the micro-biogeography associated with geographic differences was mainly influenced by a few dominant taxonomic groups. " (in the discussion section of the new manuscript version).

Question: The authors also claim that "the new data have also presented seasonal response patterns of cell density in the Muztagata ice core" (208-9); however, one of the conclusions in Yao et al. 2008 is that "bacterial genetic diversity also changes seasonally" so this is hardly novel.

Re: In this study, the dynamic changes of live cells was related to the temperature parameter, the oxygen isotope ratios, and showed a clear seasonal response of live cells to the climatic changes. This was also the first attempt on the live cell density in this ice. Yao's study showed a tendency of annual change of total bacterial abundance without the related temperature information (Yao et al. 2008, GBC).

Question: Second, the authors say that "there was a strong influence of aeolian activities on the physical and biological records along the ices core" (141-2) but this is not quite supported by the data. A significant correlation is claimed to have been found between dust and microbial abundance (151-3). This is potentially important as concurrent analysis of cells and dust particles in ice cores is scarce. The authors should show this correlation explicitly, e.g. as an additional panel of Fig. 3. But it is still not an unequivocal proof of aeolian control, as post-depositional processes may have occurred (e.g. nutrient leaching). In addition, no statistically significant relationship between microbial abundance and the oxygen isotope signal is presented to support the seasonal temperature effect (though of course it makes sense).

Re: The present data sets from the Muztagata glacier at 7010 m above sea level (38°17'N, 75°04'E) revealed a significant correlation between dust and microbial abundance, which indicated a strong influence of aeolian activities on the physical and biological records along the ices core. This was also consistent with another independent microbial investigation on the Muztagata glacier at 6300 m above sea level (Liu et al., 2013). The high repeatability of both ice cores from the Muztagata glacier re-confirmed reliability of the data sets discussed here. We showed this correlation between dust and microbial abundance as an additional panel of Fig. 3 with high R2 value of 0.7. The strong association of microorganisms with dust was also fund in previous data from the Antarctic Glacier (Abysov et al., 1998; Priscu et al., 2008) the Malan Glacier (Yao et al., 2006, in the result and discussion sections of the new manuscript version).

The temperature effect question was answered in the last question.

Question: Third, no blanks or controls are mentioned in the methods. How did the authors account for possible contamination? In such low-abundance samples this is not negligible, and for example Herbaspirillum sequences, also found in this study, have previously been identified as potential contaminants in glacier ice samples (Cameron et al. C2 BGD Interactive comment Printer-friendly version Discussion paper 2016 FEMS). Why did the authors use clone libraries? If it was to compare their data to older datasets then it should be stated explicitly and the direct comparisons shown.

Re: Because the outer surfaces of ice core and reagents for DNA analysis were cautiously decontaminated, and all the procedures were undertaken within a sterile, positive pressure laminar flow hood, the DNA samples from the inner core columns we used are unlikely to have been contaminated. Only small DNA fragments (<100 bp) were detected from our procedure control by the autoclaved sterile water, which were not considered for further sequence analysis in this study. It should be noted that Herbaspirillum sequences, also found in this study, have previously been identified as

potential contaminants in glacier cryoconite debris and surface ice samples (Cameron et al., 2016). However, the procedures used by Cameron were completely different from ours in this present study. Their procedures used for glacier cryoconite debris and surface ice samples. They did not necessarily decontaminate the outside of the samples to avoid transfer, by entrainment, of surface contaminants towards the inner ice samples (in the methodological considerations of discussion section of the new manuscript version).

Question: Also, data from other parts of the world are largely ignored. For example, a recent paper from the Greenland ice sheet (Stibal et al. 2015 Front Microbiol) addresses the relationship between microbial abundance and dust and other environmental factors and their possible causes.

Re: With the application of molecular techniques, CFM with cell stains cFDA, PI and SYTOX have been used to investigate viable bacteria (Amor et al. 2002; Schumann et al., 2003; Xiang et al., 2009). These investigations help us to investigate the abundance of live cells and the potential metabolic activities of microorganisms in an environment. However, CFM approach suffers from certain limitations because of interference from dust particles or spurious abiotic autofluorescence, and underestimation of the accurate cell counts under the typical SYSTEM parameters (Stibal et al., 2015). Despite the limitations, the background noisy signs and underestimate can be counterweighed by data series from the ice core profiles. In this study, the apparent seasonal tendency presented here suggests that our analyses were based on a substantial fraction of bacterial densities (in the methodological considerations of discussion section of the new manuscript version).

Question: The ms would also benefit from a language correction.

Re: The job on language correction was done in the new manuscript version.

Question: Specific comments: 1-2 The title should be changed. The ms does not show any climatic and environmental implications.

Re: The title was changed to “Changes of the bacterial functional groups in the Dunde and Muztagata glacier” in the new manuscript version.

Question: 25, 305 dust-borne what? Re: We moved the “dust-born” out of the new manuscript version.

Question: 43-5, 241-2 snow algae are not quite relevant to this.

Re: The direct evidences of positive temperature effects were the growth of red Chlamydomonas at the surface snow in New Zealand. Another evidence of temperature effects on bacterial growth, colonization and community transition were reported on Kuytun 51 Glacier, where bacterial Cyanobacteria were dominant across the surface snow slope in the warming spring-summer, but rarely in the subsurface, the winter-snow-layers (Xiang et al., 2009)

Question: 54 biogeography is by definition spatial

Re: We corrected the error in the new version of the manuscript

Question: 75 Himalaya

Re: It was corrected to “Himalaya” in the new manuscript version.

Question: 76 Taklimakan

Re: It was corrected to “Takalimakan” in the new manuscript version

Question: 158-9 this is unclear, please rephrase

Re: It was corrected to “Oxygen isotope ratios of the melt-water samples from the Dunde ice core showed a change range from –10.78‰ to –8.24‰$. . .

. . .

.” in the new manuscript version.

Question: 198 opportunistic?

Re: It was corrected to “opportunistic” in the new manuscript version.

Anonymous Referee 2 Received and published: 13 March 2016

Question: It is difficult to understand that reason why the authors compared two glaciers on their cell density and 18O/16 O in the precipitation, but only analyzed the microbial composition on one of these glaciers. It will be more persuasive if both glaciers were compared not only in their numbers but also in their microbial composition.

Re: Structures of bacterial communities were compared between the Dunde and Muztagata glaciers in detail (Figures 6a and 6b). Furthermore, comparisons of microbial communities across the geographically different glaciers Dunde, Muztagata and Puruogangri allowed us to infer the impact of functional groups on the biogeography of microbial communities in the ice. The present data sets from the Muztagata glacier at 7010 m above sea level (38°17’N, 75°04’E), revealed clear seasonal patterns with high microbial cell density occurring in the warming spring (filled triangles in Fig. 2) and summer (open triangles Fig. 2), which indicated the positive temperature effects on the microbial density patterns. This was consistent with another independent microbial investigation on the Muztagata glacier at 6300 m above sea level (38°17’N, 75°06’E, Liu et al., 2013). The high repeatability of both ice cores from the Muztagata glacier confirmed reliability of the data sets discussed here.

Seasonal analysis of cell densities in the Dunde ice core was not successful because of limitation of the sampling resolution.

Question: If the authors could do more comparison between two glaciers, they might find out some common rule in the relationship between cells distribution and 18O/16 O ratio (or climate events) .

Re: The present data sets from the Muztagata glacier at 7010 m above sea level (38°17’N, 75°04’E), revealed clear seasonal patterns with high microbial cell density occurring in the warming spring (filled triangles in Fig. 2) and summer (open triangles Fig. 2), which indicated the positive temperature effects on the microbial density patterns. This was consistent with another independent microbial investigation on the Muztagata glacier at 6300 m above sea level (38°17'N, 75°06'E, Liu et al., 2013). The high repeatability of both ice cores from the Muztagata glacier confirmed reliability of the data sets discussed here.

Similar phenomenon of temperature positive effect on the cell density was also found across the glaciers (Uetake et al.,2006; Price and Bay, 2012;Yao et al., 2008),which was discussed in the discussion section 4.3 Changes in microbial density at variable temperatures in the new manuscript version.

Question: Line 304-305, The authors stated that “This strengthens the importance of postdeposition”, but in the discussion session, there is no more discussion to indicate how does these results strengthen the importance of post-deposition. I could see any direct link between them. Line

Re: We moved the phrase “This strengthens the importance of postdeposition” out of the new manuscript version. We discussed “the post-deposition impact on the cell densities of microorganisms by microbial growth in the warming seasons”, namely "positive temperature effect on the dynamics of microbial density" in the discussion section 4.3 Changes in microbial density at variable temperatures in the new manuscript version:

The present data sets from the Muztagata glacier at 7010 m above sea level (38°17'N, 75°04'E), revealed clear seasonal patterns with high microbial cell density occurring in the warming spring (filled triangles in Fig. 2) and summer (open triangles Fig. 2), which indicated the positive temperature effects on the microbial density patterns. This was consistent with another independent microbial investigation on the Muztagata glacier at 6300 m above sea level (38°17'N, 75°06'E, Liu et al., 2013). .... It is not surprise for the high live cell density in summer as a result of microbial growing in the surface snow. Uetake et al. (2006) also found that high microbial abundance was present in the warming spring-summer seasons in the Sofiyskiy Glacier in the south Chuyskiy range of the Russian Altai, so did Price and Bay (2012). The positive relationship between microbial abundance and temperature was very evident in the Guoqu Glacier in the Geladaindong mountain regions (Yao et al., 2008). ...... The yearly discrete increasing pattern of microbial density along the Dunde ice core profile could be attributed to microbial growth followed by the new snow cover during the spring-summer months. All results suggest the fundamental contribution of dust-microbe deposition to the basic population pool size, and the post-deposition of microorganisms by microbial growth in the warming seasons.

Question: 177ïijN It can’t be named as “groups Cryobacteria, ËĞ . . ...”, because that the similarity is only 65

Re: It was corrected to “Cryobacteria related” or “Cryobacteria-r” in the new manuscript version.

Question: It was not clarified what the purpose of showing the Changes in physical-chemical and biological records in the Muztagata ice cores? There is no any microbial analysis in this study for Muztagata ice cores. Therefore, it is nonsense to present the physicalchemical property of Muztagata ice cores.

Re: Additionally, we also evaluate the live populations of microbial communities respond to the climatic and environmental changes. The live bacterial data were examined and related to the physical-chemical parameters from the two ice cores Muztagata and Dunde (Introduction section in the new manuscript version). In this study, the dynamic changes of live cells showed a clear seasonal response of live cells to the climatic changes (result and discussion section in the new manuscript version).

Question: Line 333, it should be “ISME J” but not “SME J”.

Re: It was corrected to “ISME J” in the new manuscript version

Question: Line 399 the title of Figure 2 was wrong. The figure is nothing related with mineral paticles.

Re: phrase “mineral particles” was move out of the Figure legends section in the new manuscript version.

Thanks for your consideration of the publication of this manuscript.

Best regards

Sincerely yours

Shu-Rong Xiang

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