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Community change of microorganisms in the Muztagata and Dunde glacier and climatic and environmental implications

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Abstract. Microorganisms are continuously blown onto the glacier snow, and thus the glacial depth profiles provide excellent geographic archives of the microbial communities. However, it is uncertain about how the microbial communities respond to the climatic and environmental changes over the glacier ice. In the present study, the live microbial density, stable isotopic ratios, $^{18}O/^{16}O$ in the precipitation, and mineral particle concentrations along the glacial depth profiles were collected from ice cores from the Muztagata glacier and the Dunde ice cap. Six bacterial 16S rRNA gene clone libraries were established from the Dunde ice core. 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. Both ice core data showed a frequent association of dust and microorganisms in the ice. Genera *Polaromas* sp., *Pedobacter* sp, *Flavobacterium* sp., *Cryobacteriium* sp., and *Propionibacterium*/*Blastococcus* sp. frequently appeared at the six tested ice layers, and constituted the dominant species endemic to the Dunde ice cap, whereas some genera such as *Rhodoferax* sp., *Variovorax* sp., *Sphingobacterium*. sp., *Cyanobacterium* sp., *Knoellia* sp., and *Luteolibacter* sp. rarely presented in the ice. In conclusion, data present a discrete increase of microbial cell density in the warming seasons and biogeography of the microbial communities associated with the predominance of a few endemic groups in the local glacial regions. This reinforces our hypothesis of dust-borne and post-deposition being the main agents interactively controlling microbial load in the glacier ice.

Key words: dust-born deposition, post-deposition, glacier, microbial cell density, biogeography

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29 **1 Introduction.**

30 Microorganisms are continuously blown onto the glacier snow, and thus the glacial depth profiles
31 provide good geographic archives of the microbial communities during the course of global climatic and
32 environmental processes. However it is unclear how the microbial communities respond to the climatic
33 and environmental changes over the glacier ice. Recently, microbiological data have been collected from
34 ice cores extracted from the geographically different glaciers such as the Vostok Station ice core,
35 Antarctica (Abyzov et al., 1998; Christner et al., 2006; Priscu et al., 2008), the Malan Glacier (Yao et al.,
36 2006) and the Guoqu Glacier in the Mount Geladaindong on the central Tibetan Plateau (Yao et al., 2008).
37 All results of the ice cores have showed a high microbial abundance corresponds with a high concentration
38 of particles, which suggests a strong effect of aeolian activities on the influx of dust born microorganisms
39 in the glacier ice.

40 However, the obvious transition of microbial diversity structures between the surface and subsurface
41 snow suggests an importance of the post-deposition mechanisms on the microbial community succession
42 in the glacier ice. *Cyanobacteria* were dominant across the surface snow slope in the Kuytun 51 Glacier,
43 but rarely in the subsurface snow layers (Xiang et al., 2009b); Red *Chlamydomonas* were frequently
44 observed at the pink to red surface snow, sometimes 15 cm below the snow surface in New Zealand and
45 on the Harding icefield, Alaska (Thomas and Broady, 1997; Takeuchi et al., 2006). The visible community
46 transitions are good indications of cold-adapted bacterial growth and colonization in the ice, and thus
47 strengthen the important role of post-deposition on the biogeographically development of microbial
48 communities in the glaciers.

49 Previous DNA sequence analysis have showed a significant difference in bacterial communities, and

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demonstrated a zonal distribution of microorganisms across the Kuytun 51, Qiangyong, and Rongbuk glacier surface (Xiang et al., 2009b, 2010). Studies indicate the presence of cosmopolitan bacteria and the endemic species as well. The cosmopolitan *Comamonadaceae* and *Flavisolibacter* sp. appeared in both Kuytun 51 and Qiangyong Glaciers, while *Rhodoferax* sp. (*Betaproteobacteria*) were dominant in the Kuytun 51 Glacier, but less in the Qiangyong Glacier. This suggests the spatial biogeography of microorganisms in the glacier ice. We thus could expect the influences of post-deposition on the microbial depth profile and biogeography of microbial diversity in the ice over time.

In this study, we forwarded our previous concerns about possible influences of the climatic and environmental changes on the microbial distribution in the glacier ice. The consecutive microbial data of two ice cores recovered from the geographically different regions on the Tibetan Plateau. Field observations and previous data showed a good preservation of the seasonal temperature changes along the ice core depth profile from the Muztagata Glacier (Tian et al., 2006). This made it possible for us to explore the seasonal profile of microbial cell density and relate it to the climatic and environmental changes over the glacier. To investigate the temporal and spatial biogeography of microbial community, six clone libraries of the bacterial rRNA genes were established from the Dunde ice core and compared with the previous data from the geographically different glaciers.

2 Study area, data collection and methodology

Data discussed in this study were collected from the Muztagata Glacier (38°17'N, 75°04'E), and the Dunde ice cap (38°06'N, 96°24'E). As shown in the figure 1, the Muztagata Glacier is located in the most western margin of the Tibetan Plateau where precipitation is mainly derived from air masses originating in the arid, and semi-arid regions, including deserts Sary-Ishykotrau, Muyun Kum, Kyzyl Kum and Kara

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71 Kum, Taklimakan and Gurbantunnut (Wake et al., 1990). The Dunde ice cap is located in the northern
72 margin of the Qaidam Basin, and in the Qilian mountain region on the northeastern Tibetan Plateau, where
73 the winter precipitation results from the incursion of westerly depressions along the southern slopes of the
74 Himalayas (Murakami, 1987; Davis et al., 2005); while the summer precipitation is derived from the
75 monsoon circulation from the Bay of Bengal to the central Hymalaya, and further to the Qaidam Basin and
76 large depressions in Takalamakan Desert and Daidam Basin (Davis et al., 2005; Dregne, 1968; Chen and
77 Bowler, 1986).

78 Approximately here, Fig.1 Map illustrating the location of glaciers discussed in this study.

79 The ice core Muztagata (37-m-long) was extracted at 7010 m ASL (above see level) from the Muztagata
80 Glacier in the summer of 2003 (Tian et al., 2006). The Dunde ice core (9.5-m-long) was extracted at 5325
81 m ASL from the Dunde ice cap summit in October 2002 (Wu et al., 2009). The visible stratigraphic
82 features were recorded immediately after ice core drilling. All ice cores were returned frozen to the freezer
83 room (air temperature between -18 to -24°C) at the Key Laboratory of the Ice Core and Cold Regions
84 Environment of the Chinese Academy of Sciences. The ice core sections were split lengthwise into four
85 portions and stored in a refrigerated room at -18°C to -24°C.

86 A 10 ml aliquot of melt-water from the Muztagata and Dunde ice cores was used for analysis of the
87 mineral particle. Total microparticle concentrations were measured by using a Coulter counter Multisizer3
88 (Beckman).

89 A 10 ml aliquot of melt-water from the Dunde ice core was used for analysis of the stable isotopic ratios,
90 $^{18}O/^{16}O$ ($\delta^{18}O$) in the precipitation. A Finnegan MAT-252 mass-spectrometer was used to determine $\delta^{18}O$
91 values within ±0.5%. The Dunde ice core was dated by using seasonal $\delta^{18}O$ variations and annual visible
92 dust layers, and confirmed by the previous data (Takeuchi et al., 2009). The Muztagata ice core dating and

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93 $\delta^{18}O$ data were previously described by Tian et al. (2006).

94 For microbial analysis, the Muztagata ice core sections were cut into 156 samples, while the Dunde ice
95 sections were cut into 37 in intervals of 12-30 cm using a band saw within walk-in freezers (-18 to -24° C).
96 The ice samples were cut between the visible dust layers, and ice layers were collected separately. The
97 outside layers of the ice core sections were moved out, and the inner sections were slowly melted at 4°C
98 by following the protocols previously described by Yao et al. (2006). The freshly melted water (10 ml)
99 from the Muztagata and Dunde ice cores was diluted 10 fold. 100 µl of diluted sample was added to the
100 known concentration of fluorescent-dyed bead solution Trucount (Becton Dickinson) mixture with cell
101 sorting marker carboxyfluorescein diacetate (cFDA) and propidium iodide (PI). Three groups of bacteria
102 could be identified based on the difference of the bound probes: cFDA-stained, cFDA/PI-double-stained
103 and PI-stained group, indicating viable, injured, and dead cells, respectively (Xiang et al., 2009b). The
104 cFDA and PI staining was separately prepared by following the method of Amor et al. (2002), except for
105 the cell suspensions which were incubated for 15 min in the dark at the room temperature (25°C) for cell
106 staining. The live and total cell numbers in the melt-water were determined with a FACSCalibur flow
107 cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California, U.S.A.) by following
108 manufacturer's instruction.

109 For DNA analysis, six clone libraries of the bacterial 16S rRNA genes were collected from the Dunde
110 ice cap. Approximately 400 ml of ice core melt-water was used for DNA extraction. DNA extraction and
111 further clone library establishment procedure were conducted by following the same protocols as
112 previously used in a microbial analysis of the Kuytun 51 Glacier samples (Xiang et al., 2009b). The 16S
113 rRNA gene amplicons used for the establishment of clone libraries from the Dunde ice core were
114 generated by PCR amplification with the bacterial universal primer pair 8f

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115 (5′-AGAGTTTGATCATGGCTCAG) and 1492R (5′-CGGTTACCTTGTTACGACTT) (Lane 1991;
116 Weisenburg et al., 1991). To avoid possible bias, the three PCR products were pooled and used to
117 establish clone library from each ice column. A total of 137 clones were selected for sequencing by
118 *Hae*III-based ARDRA (amplified rRNA restriction analysis) out of the 406 clones from the Dunde ice core.
119 Each sequence was named using the initial of Dunde ice cap (DD1, noted for one out of the 5 ice cores
120 drilled in October 2002, Wu et al., 2009), along with the ice depth (D84, D107, D238, D324, D386, and
121 D466: 84, 107, 238, 324, 386, and 466 cm below the snow surface) followed by the clone number (1 to
122 163). For example, clones DD1D84-9, DD1D107-55, and DD1D466-123 were the clone representatives of
123 the ice core DD1 at the depth 84, 107, and 466 cm below the snow surface. The accession numbers of the
124 cloned sequences obtained from the Dunde ice core in GenBank are: KU060881 - KU061017.

125 All 137 sequences from the Dunde ice cap were checked by DECIPHER (Wright et al., 2012,
126 sequence chemera check tool available: http://decipher.cee.wisc.edu/FindChimerasOutputs.html), and
127 aligned with the Blast references (Altschul et al., 1990) by using ClustalX (Thompson et al., 1997). A
128 Neighbor-Joining phylogeny for the aligned sequences was constructed using MEGA 6.0 (Tamura et al.,
129 2013: http://www.megasoftware.net/) pairwise deletion mode for gaps (with bootstrap analysis, 100
130 replicates) and subroutines Maximum Composite Likelihood (MCL) for substitutions. The archaeal 16S
131 rDNA sequences from *Methanosaeta harundinacea* strain 8Ac (accession no. AY817738) and
132 *Methanosaeta concilii* strain GP6 (accession no. NR102903) were used as outgroup references on all trees.
133 All the obtained sequences from the glaciers were identified by the recognized species, and related to the
134 ecological clusters (e.g., *Variovorax* sp. and *Herbaspirillum* sp. in the *Betaproteobacteria* subphyla).
135 Sequences obtained displaying similarities of >97% with known species were identified as the reported
136 species. Most of the obtained clones were related to known cultivated genera or genus clones (e.g.,

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137 *Ketogulonicigenium* sp., *Cyanobacterium* sp., *and Sphingobacterium.* sp.). A few clones had <97%
138 similarity with reported species, and thus were designated separately.

139 **3 Results**

140 **3.1 Changes in physical-chemical and biological records in the Muztagata ice cores**

141 There was a strong influence of aeolian activities on the physical and biological records along the ice
142 core extracted at 7010 m ASL of the Muztagata Glacier (Fig. 2). An apparent seasonal temperature change
143 was indicated by the proxy value of the stable isotopic ratios, $^{18}O/^{16}O$ ($\delta^{18}O$) with a low value in winter
144 and high value in summer (Fig. 2b). The live cell density was greatly variable at a range from 6.5×10^2 to
145 2.1×10^4 cells/ml during 1964 to 2000 (Fig. 2a), the total cell density varied from 4.4×10^4 to 8.7×10^5
146 cells/ml (Fig. 2c). Several live cell density peaks were formed during the summer seasons in 1969, 1970,
147 1973, 1979, 1982, 1983, 1988, 1990 and 1993 for a total of 9 events, A1 to A9 (open triangles in Fig. 2a),
148 respectively, while cell density peaks were found during the winter-spring (filled triangles in Fig. 2a). This
149 ice core also had an increased density of the total microorganisms in summer in 1978, 1988 and 1993
150 (open triangles B1, B2, and B3 in Fig. 2c), and in spring of 1995 and 2000 (B4 and B5 in Fig. 2c),
151 respectively, which was consistent with the live cell density patterns (Fig. 2a). The high microbial cell
152 density significantly correlated with the peaks of mineral particle concentrations and possessed a high R^2
153 value of 0.7 (Fig. 3).

154 Approximately here, Fig. 2 Bacterial cell density, mineral particles and $\delta^{18}O$ in the Muztagata ice core.

155 Fig. 3 Correlation between mineral particle concentration and total cell density in the Muztagata ice
156 core

157 **3.2 Changes in physical-chemical and biological records in the Dunde ice core**

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158 It was not successful for the seasonal analysis of Dunde ice core because of the limitation of sample
159 resolution (Fig. 4). Oxygen isotope ratios of the melt-water samples from the Dunde ice core showed a
160 temperature change from –10.78‰ to –8.24‰ (temperature proxy $^{18}O/^{16}O$, Fig. 4d), while microbial cell
161 density varied from 1.2×10^3 to 9.1×10^4 cells/ml (Fig. 4b) and 1.3×10^5 to 1.9×10^6 cells/ml (Fig. 4c) for
162 live and total cell density, respectively. Three peaks C2, C3 and C4 of the total cell density were found in
163 1988-1989, 1992, and 2000, only one peak C1 in 1985, respectively (Fig. 4c). The live cell density
164 response pattern was consistent with the total cell density tendency (the dash lines in Figs. 4b and 4c).
165 Abundance of microbial cells frequently occurred at the dirty ice layers (Cell density peaks C1, C3, and
166 C4 at the dust layers labeled as the dash lines in Fig. 4), rarely presented at the clean ice layer (small
167 density peak C2 at the A1 layer in Fig. 4).

168 Approximately here, Fig. 4 Bacterial cell density, mineral particles and $\delta^{18}O$ in the Dunde ice core.

169 **3.3 Phylogenetic analysis of bacterial 16S rRNA gene amplified from the Dunde ice core**

170 The dominant bacteria in six ice layers of the Dunde ice core were investigated by 16S rRNA gene
171 clone library, as well as sequencing techniques, and BLAST and phylogenetic tools. A total of 24 bacterial
172 genera were identified in the Dunde ice core. They belonged to genera *Polaromonas* sp., *Rhodoferax* sp.,
173 *Variovorax* sp., *Burkholderiales*, *Herbaspirillum* sp., *Xanthomonadaceae*, *Ketogulonicigenium* sp.,
174 *Devosia* sp., *Bacteriovorax* sp., *Hymenobacter* sp., *Pedobacter* sp., *Flavobacterium* sp., *Flectobacillus* sp.,
175 *Cytophagales*, *Sphingobacteriaceae*, *Cryobacteria*, *Propionibacterium*/*Blastococcus* sp.,
176 *Salinibacterium*/*Frigoribacterium* sp., *Knoellia* sp., *Cyanobacteria*, *Luteolibacter* sp., *Paenibacillus* sp.,
177 *Anoxybacillus* sp., and TM7 candidates (Figs. 5a, 5b, and 5c). Three genus groups *Cryobacteria*,
178 *Salinibacterium*/*Frigoribacterium* sp., and *Propionibacterium*/*Blastococcus* sp. were clustered with
179 65%-76% similarity to the known species, but grouped with genus *Knoellia* sp. with 95% similarity in the

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family members of *Actinobacteria* (Fig. 5b). Only one clone DD1D107-100 was 100% similar to the uncultured *Bacteroidetes* clone AKYG1686 (Fig. 5c). All tested bacterial clones in the ice fell into members of bacteria phyla *Alpha, Beta, Gamma, and Delta-proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia*, and TM7 candidates.

Approximately here,

Fig. 5a Phylogenetic analysis of the 16S rRNA genes for *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria* and *Deltaproteobacteria* clones from the Dunde ice core and the closest relatives.

Fig. 5b Phylogenetic analysis of the 16S rRNA genes for *Actinobacteria, Cyanobacteria, Verrucomicrobia*, and *Firmicutes* clones from the Dunde ice core and the closest relatives.

Fig. 5c Phylogenetic analysis of the 16S rRNA genes for *Bacteroidetes* and TM7 candidate clones from the Dunde ice core and the closest relatives.

3.4 Changes in proportion of the main bacterial genera along the Dunde ice core profile

There was a great difference in proportion of the main phylogenetic groups along the Dunde glacier depth profile (Figs. 6b1-6b6). Various bacterial clones comprises five dominant genus groups *Polaromonas* sp., *Pedobacter* sp., *Flavobacterium* sp., *Propionibacterium*/*Blastococcus* sp., and *Cryobacterium* sp.; they accounted for more than 55% of the total 406 clones, and frequently appeared in the six tested ice layers from 1990 to 2000 (dashed lines in Fig. 6). Eight genus groups like *Rhodoferax* sp., *Variovorax* sp., *Burkholderiales*, *Flectobacillus* sp., *Cytophagales*, *Sphingobacteriaceae*, *Knoellia* sp., *Cyanobacteria* rarely occurred in the ice. Other opportunity bacterial clones occasionally appeared in the ice.

Fig. 6 Proportion of the main phylogenetic groups in the Dunde and Muztagata ice cores.

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202 **4 Discussion**

203 Our previous studies have documented a zonal distribution of microbial community at the surface snow,
204 indicating the spatial biogeography of microorganisms across the western mountain glaciers in China
205 (Xiang et al., 2009b, 2010). Similar zonal phenomena have also been found in the glacier depth profiles
206 from the Dunde ice core and Muztagata ice cores. However, the current data have presented a change of
207 the dominant endemic community composition, indicating an association of the microbial spatial
208 patterning with the presence/absence of the dominant species within the specific glaciers. The new data
209 have also presented seasonal response patterns of cell density in the Muztagata ice core. All results
210 reinforce the concept of interactive mechanisms, aeolian- and post-deposition of microorganisms on the
211 glacier surface.

212 **4.1 Dust deposition and microbial distribution along the glacial depth profiles**

213 Ice core data from the Muztagata and Dunde glacier showed a frequent association of microbial
214 abundance with high concentrations of particles (Fig. 3, and Fig. 4), which was consistent with previous
215 data from the Antarctic Glacier (Abysov et al., 1998; Priscu et al., 2008 the Malan Glacier (Yao et al.,
216 2006), and the Guoqu Glacier on the Tibetan Plateau (Yao et al., 2008). The trace elements Tb, Sr, Th, and
217 U, and rare earth elements (REE) including light REE La, Ce, Pr, Nd, Sm, and Eu, and heavy REE Gd, Tb,
218 Dy, Ho, Er, Tm, Yb, and Lu were extracted from the same series of ice core section (Wu et al., 2009). The
219 trace element and REE analyses revealed that the fine fractions in the Dunde dust were more similar to
220 those in the western Qaidam Basin, and Tarim Taklimakan Desert than those in the Badain Juran and
221 Tengger Desert, which implies the long range of transportation of the Dunde dust and dust-borne

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microorganisms from the western desert. These results suggest an aeolian driving effect on both dust and microbial deposition in the ice. One small cell density peak C2 presented at the clean ice layer A1 in Fig. 4a. This indicates that microbial loading onto the glacier surface does not always associate with the dust deposits or "dirty" wind, may transport with "clean" wind or snow, which implies influences of the processes like aerosol, and snow deposition (microbial deposition with snow, wet-deposition), and post-deposition and other factors.

4.2 Changes in glacial microbial density at variable temperatures

The present data sets from the Muztagata glacier 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. The direct evidences of positive temperature effect on the microbial growth at the snow were reported on the red *Chlamydomonas* growth at the surface snow in New Zealand and on the Harding icefield, Alaska in late spring and summer (Thomas and Broady, 1997; Takeuchi et al., 2006). Thus it is not surprise for the high live cell density in summer as a result of microbial growing in the surface snow. This is consistent with another independent microbial investigation on the Muztagata glacier (Liu et al., 2013). 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). In addition to those cell density peaks in summer, there were many density peaks in spring from 1963-2000 (filled triangles in Figs. 2a and 2c). The growth of red *Chlamydomonas* were also observed on the late spring snow (Thomas and Broady, 1997). So, the yearly discrete increasing pattern of microbial density presenting along the Dunde ice core profile could be

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attributed to microbial growth followed by the new snow cover during the spring-summer months. All results suggest the fundamental contribution of dry-aeolian and wet-deposition (with snow) to the basic population pool size of microorganisms, and the cascade effect of post-deposition of microorganisms by microbial growth, enhanced metabolic activity and increased population density in spring and summer.

4.3 Temporal and spatial biogeography of microbial community in the glacier ice

Under the same project guideline for microorganisms in glacier ice and the relation to climatic and environmental changes, microbiological data have been collected from the ice core depth profiles from the geographically different glaciers in western China over the last decade. The same methodological system has been used for the investigation on the labelled glaciers in Figure 1, which makes it possible to explore the geographical features of microorganisms in the glacier ice across western China. We here will initially discuss the temporal and spatial biogeography of microbial community in western China.

Our previous sequence data showed a clear phylogenetic distance with only 87% similarity among the bacteria *Polaromonas* sp. from the different geographically glaciers (An et al., 2010), although *Polaromonas* sp. were identified from all of the ice layers from the glaciers Dunde, and Muztagata, and Kuytun 51, Qiangyong, and Puruogangri (An et al., 2010; Xiang et al., 2009a, 2009b, 2010). Statistical analyses showed that the genetic distances among 43 unique glacier *Polaromonas* sequences were positively correlated with geographic distance among the glacier sites (Franzetti et al., 2013). These results indicate an evident biogeography of *Polaromonas* sp. Bacteria *Cryobacteria* more frequently presented in the Dunde ice cap than in the Muztagata glacier, while *Enterobacter* sp. appeared throughout the four tested ice layers of Muztagata glacier, but rarely in the Dunde ice cap (Figs 6a and 6b). The presence or absence of the dominant species indicates a clear spatial patterning of bacterial group endemic to the special glacier regions.

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Several bacterial genus groups were frequently identified along the Dunde ice core depth, and became the dominant groups endemic to the local glacier regions (labeled as the dashed lines in Fig. 6b). Bacterial genus groups *Polaromonas sp.*, *Pedobacter* sp., *Flavobacterium* sp., *Propionibacterium*/*Blastococcus* sp., and *Cryobacterium* sp. were frequently found at the six tested ice layers of Dunde glacier from 1990-2000. Genera *Polaromonas* sp., and *Flavobacterium* sp. were also identified from the Dunde ice column AD 1780–1830 (Zhang et al., 2009). However, there were obviously different genus groups endemic to the Muztagata and Puruogangri glacier. Seven genus groups *Polaromonas* sp., *Enterobacter* sp., *Acinetobacter* sp., *Flexibacter* sp., *Thermus* sp., *Propionibacteria/Luteococcus* sp., and *Flavisolibacter* sp. were frequently identified at the four tested ice layers of Muztagata glacier from 1970-1988 (labeled as the dashed lines in Fig. 6b), while *Polaromonas* sp., and *Flexibacter* sp. were found at all of three tested ice columns of Puruogangri glacier from 1600 -1920 (Zhang et al., 2009; An et al., 2010). These results clearly indicate that the biogeography of microbial communities associates with the presence/absence of several dominant genus groups within the specific glacier region.

The changing composition of dominant groups endemic to the local glacier regions could be attributed to the climatic and environmental differences in the different geographical glacier regions. As shown in Figure 1, the precipitations over the Muztagata glacier is mostly influenced by the westerly depressions, while the precipitation over the Dunde ice cap and Puruogangri ice cap is mainly driven by the westerly depressions in winter and Indian monsoon in summer (Wake et al., 1990; Davis et al., 2005; Dregne, 1968; Murakami, 1987). The dramatic change of climatic and environmental processes across the Tibetan Plateau mountainous glaciers may lead to differences in the microbial communities uploaded onto the snow. Moreover, the heterogeneity of local conditions such as temperature, light intensity, meltwater availability and nutrient concentrations in the ice may drive the temporal and spatial patterning of

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288 microbial community by the effects on successful colonization and primary succession of the endemic
289 species dominant in the ice. More data on the meteorologic, physical and chemical characteristics of the
290 ice core will be helpful for better understanding the biogeography of microorganisms in the ice.

291 **5 Conclusions**

292 Physical-chemical and microbiological data sets from the Muztagata glacier showed a seasonal pattern
293 during the rapidly changing temperature phases. The cell density peaks are frequently associated with high
294 concentration of particles in the warming spring-summer. These suggest the importance of aeolian and
295 post-deposition on the microbial upload on to the glacier snow. Sequence analyses of 16S rRNA gene
296 clone libraries from the Dunde ice core showed an obvious difference in composition of the dominant
297 genus groups between the two glaciers Muztagata and Dunde. Five bacterial dominant genus groups
298 *Polaromonas*, *Pedobacter* sp., *Flavobacterium* sp., *Propionibacterium*/*Blastococcus* sp., and
299 *Cryobacterium* sp. frequently appeared at the six tested ice layers, constituting the dominant species
300 endemic to the Dunde ice cap, while Seven genus groups *Polaromonas* sp., *Enterobacter* sp.,
301 *Acinetobacter* sp., *Flexibacter* sp., *Thermus* sp., *Propionibacteria/Luteococcus* sp., and *Flavisolibacter* sp.
302 were frequently found at the four tested ice depths of Muztagata glacier. This study presented a discrete
303 seasonal increase pattern of microbial cell density, and community transition of dominant endemic
304 bacterial community among the different geographically glaciers. This strengthens the importance of
305 post-deposition, and reinforces our hypothesis of dust-borne and post-deposition being the main agents
306 interactively controlling microbial load in the glacier ice.

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Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016

311 **Reference:**

312 Abyzov, S. S., Mitskevich, I. N., and Poglazova, M. N.: Microflora of the deep glacier horizons of central
313 Antartica, Microbiol., 67 (4), 451–458, 1998.
314 Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J.: Basic local alignment search tool, J.
315 Mol. Biol., 215, 403–410, 1990.
316 Amor, K. B., Breeuwer, P., Verbaarschot, P., Rombouts, F. M., Akkermans, A. D. L., De Vos, W. M., and
317 Abee, T.: Multiparametric flow cytometry and cell sorting for the assessment of viable, injured, and
318 dead bifidobacterium cells during bile salt stress, Appl. Environ. Microbiol., 68, 5209–5216, 2002.
319 An, L. Z., Chen, Y., Xiang, S. –R., Shang, T. –C., and Tian, L.-D.: Differences in community composition
320 of bacteria in four glaciers in western China, Biogeosci. 7, 1937-1952, doi:10.5194/bg-7-1937-2010,
321 2010
322 Chen, K., and Bowler, J. M.: Late Pleisticene evolution of salt lakes in the Qaidam Basin, Qinghai
323 province, China, Paleogeog. Paleoclimat. Paleoeol., 54, 87–104, 1986.
324 Christner, B. C., Mosley-Thompson, E., Thompson, L. G., Zagorodnov, V., Sandman, K., and Reeve, J. N.:
325 Recovery and identification of viable bacteria immured in glacial ice, Icarus, 144, 479–485, 2000.
326 Davis, M. E., Thompson, L. G., Yao, T., and Wang, N. L.: Forcing of the Asian monsoon on the Tibetan
327 Plateau: Evidence from high-resolution ice core and tropical coral records, J. Geophys. Res., 110,
328 D04101. doi:10.1029/2004JD004933, 2005.
329 Dregne, H. E.: Surface materials of desert environments, in deserts of the world, edited by McGinnies, W.
330 G., Goldman, B. J., and Paylore, P., 286-377, 1968, University of Arizona Press, Tuscon.
331 Franzetti, A., Tatangelo, V., Gandolfi, I., Bertolini, V., Bestetti, G., Diolaiuti, G., D'Agata, C., Mihalcea, C.,
332 Smiraglia, C., and Ambrosini, R.: Bacterial community structure on two alpine debris-covered glaciers
333 and biogeography of *Polaromonas*phylotypes. SME J. 7: 1483–1492, doi:10.1038/ismej.2013.48, 2013.

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016

334 Lane, D. J.: 16S/23S rRNA sequencing, in: Stackebrandt, E. and Goodfellow, M., Nucleic acid techniques
335 in bacterial systematics, Academic Press, Chichester, England, 115–175, 1991.
336 Liu, Y. -Q., Yao, T. -D., Xu, B. -Q., Jiao, N. -Z., Luo, T. -W., Wu, G. -J., Zhao, H. -B., Shen, L., and Liu, X.
337 -B.: Bacterial abundance vary in Muztagata ice core and respond to climate and environment change in
338 the past hundred years. Quaternary Sciences, 33, 19–25, 2013.
339 Murakami, T.: Effects of the Tibetan Plateau; in Monsoon Meteorology, edited by C. P. Chang and T. N.
340 Krishnamurti, 235–270, Oxford University Pres, New York, 1987.
341 Price, P. B., and Bay, R. C.: Marine bacteria in deep Arctic and Antarctic ice cores: a proxy for evolution
342 in oceans over 300 million generations, Biogeosci. 9, 3799-3815. doi:10.5194/bg-9-3799-2012, 2012.
343 Priscu, J. C., Tulaczyk, S., Studinger, M., Kennicutt II, M. C., Christner, B. C., and Foreman, C. M.:
344 Antartic subglacial water: origin, evolution and microbial ecology, In Vincent, W. and J.
345 Laybourn-Parry (eds), Polar Limnology, Oxford University Press, pp. 119–135, 2008.
346 Takeuchi, N., Dial, R., Kohshima, S., Segawa, T., and Uetake J.: Spatial distribution and abundance of red
347 snow algae on the Harding Icefield, Alaska derived from a satellite image. Geophys. Res. Lett. 33,
348 L21502, doi:10.1029/2006GL027819, 2006.
349 Takeuchi, N., Dial, R., Kohshima, S., Segawa, T., Uetake J.: Spatial distribution and abundance of red
350 snow algae on the Harding Icefield, Alaska derived from a satellite image. Geophysical Research Letter,
351 33, L21502, doi:10.1029/2006GL027819, 2006.
352 Takeuchi, N., Takayuki, M., Nakazawa, H. N., Fujita, K., Saki, A., Nakawo, M., Fujiii, Y., Duan, K., and
353 Yao, T.-D.: A shallow ice core re-drilled on the Dunde Ice Cap, western China: recent changes in the
354 Asian high mountains, Environ. Res. Lett. 4, 045207, doi:10.1088/1748-9326/4/4/045207, 2009.
355 Tamura, K., Stecher G., Peterson, D., Filipski, A., and Kumar, S.: MEGA6: Molecular Evolutionary

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016

356 Genetics Analysis version 6.0. Mol. Bio. Evo. 30, 2725-2729, 2013.

357 Thomas, W. H., and Broady, P. A.: Distribution of coloured snow and associated algal genera in New
358 Zealand. New Zeal J. Bot. 35, 113–117, 1997.

359 Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G.: The CLUSTAL– X
360 Windows interface – flexible strategies for multiple sequence alignment aided by quality analysis tools,
361 Nucleic Acids Res., 24, 4876–4882, 1997.

362 Tian, L. –D., Yao, ., T. -D., Li, Z., MacClune, K., Wu, W. -J., Xu, B. Q., Li., Y. F., Lu, A. X., and Shen, Y.
363 P.: Recent rapid warming trend revealed from the isotopic record in Muztagata ice core, eastern Pamirs,
364 J. Geophys. Res., 111, D13103, doi:10.1029/2005JD006249, 2006.

365 Uetake, J., Kohshima, S., Nakazawa, F., Suzuki, K., Kohno, M., Kameda, T., Arkhipov, S., and Fujii, Y.:
366 Biological ice-core analysis of the Sofiyskiy Glacier in the Russian Altai mountains, Ann. Glaciol., 43,
367 70–78, 2006.

368 Wake, C. P., Mayewski, P. A., and Spencer, M. J.: A review of central Asian glaciochemical data, Ann.
369 Glaciol. 14, 301-306, 1990.

370 Weisenburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J.: 16S ribosomal DNA amplification for
371 phylogenetic study, J. Bacteriol., 173, 697–703. 1991.

372 Wright, E. S., Yilmaz, L. S., and Noguera, D. R.: DECIPHER, A search-based approach to chimera
373 identification for 16S rRNA sequences. App. Environ. Microbiol. 78 (3), 717-725,
374 doi:10.1128/AEM.06516-11, 2012.

375 Wu, G. -J., Zhang, C., Gao, S., Yao, T., Tian, L. -D., and Xia, D.: Element composition of dust from a
376 shallow Dunde ice core, northern China, Glob. Planet. Chang., 67, 186-192,
377 doi:10.1016/j.gloplacha.2009.02.003, 2009.

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016

Xiang, S. -R, Chen, Y., Shang, T., Jing, Z., and Wu, G.: Change of microbial communities in glaciers along a transition of air masses in western China, J. Geophys. Res. 115, G04014, doi:10.1029/2010JG001298, 2010.

Xiang, S. -R., Shang, T. -C., Chen, Y., and Jing, Z. -F.: Phylogenetic diversity of bacteria at the different habitats in the surface of Kuytun 51 Glacier, Tienshan Mountains, China, App. Environ. Microbiol. 75, 7287–7290, doi:10.1128/AEM.00915-09, 2009a.

Xiang, S. -R., Shang, T. -C., Chen, Y., Jing, Z. –F., and Yao, T. -D.: Changes in diversity and biomass of bacteria along a shallow snow pit from Kuytun 51 Glacier, Tianshan Mountains, China, J. Geophys. Res., 114, G04008, doi:10.1029/2008JG000864, 2009b.

Yao, T. -D., Liu, Y., Kang, S., Jiao, N., Zeng, Y., Liu, X., and Zhang, Y.: Bacteria variabilities in a Tibetan ice core and their relations with climate change, Glob. Biogeochem. Cycl., *22*, GB4017, doi:10.1029/2007GB003140, 2008.

Yao, T. -D., Xiang, S. -R., Zhang, X. -J., Wang, N. -L., and Wang, Y. -Q.: Microorganisms in the Malan ice core and their relation to climatic and environmental changes, Glob. Biogeochem. Cycl., 20, GB1004, doi:10.1029/2004GB002424, 2006.

Zhang, X.-J., Ma, X., Wang, N., and Yao, T.: New subgroups of Bacteroidetes and diverse microorganisms in Tibetan plateau glacial ice provide a biological record of environmental conditions, FEMS Microbiol. Ecol., 67, 21–29, 2009.

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016

397 **Figure Legends**

398 **Fig. 1** Map illustrating the location of glaciers discussed in this study.

399 **Fig. 2** Bacterial cell density, mineral particles and $\delta^{18}O$ in the Muzt ice core. Muzt (43-m-long) was
400 extracted at 7010 m ASL from the Muztagata Glacier in the summer of 2003. (a) Live cell density in the
401 ice. (b) The $\delta^{18}O$ value was measured by Finnegan MAT-252 mass-spectrometer (adapted from Tian et al.,
402 2006). Ice core was annually dated by using seasonal $\delta^{18}O$ variations and annual visible dust layers, and
403 the peak of beta radioactivity by the nuclear weapon test in 1963 was identified at a depth of 37.89 m (Fig.
404 2b, Tian et al., 2006). (c) Total bacterial cell density estimated by using flow cytometer and cFDA/PI-stain,
405 see the detailed in the materials and methods). The annual ice layers ranged from 50 to 136 cm, and the
406 years were indicated by the dash lines in the Figs. 2a, 2b, and 2c. The data presented here were only for
407 the ice core section in a depth range from 2.21 to 39.91 m since the annual layers become thinner below
408 35 m and the ice layer being near the bottom of the glacier (the depth of the glacier is 52.6 m, Tian et al.,
409 2006).

410 **Fig. 3** Correlation between mineral particle concentration and total cell density in the Muztagata ice core.
411 Total microparticle concentrations were measured by using a Coulter counter Multisizer3 (Beckman).

412 **Fig. 4** Bacterial cell density, mineral particles and $\delta^{18}O$ in the Dunde ice core. (a) Mineral particle
413 concentration along the depth profile. Total microparticle concentrations were measured by using a
414 Coulter counter Multisizer3 (Beckman). (b) Live cell density. (c) Total bacterial cell density. The live and
415 total bacterial cell density were estimated by using flow cytometer and cFDA/PI-stain. (c) $\delta^{18}O$ value. The
416 $\delta^{18}O$ value was measured by Finnegan MAT-252 gas stable isotope ratio mass-spectrometer. In this study,
417 the ice core section at 0.54 to 9.81 m depth was dated by using seasonal $\delta^{18}O$ variations and annual visible
418 dust layers, and confirmed by the 51-m-long Dunde ice core drilled at the same site and in the same year

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2002 (Takeuchi et al., 2009). The winter-spring seasons of 1992 and 2000 were identified and confirmed by the deep valleys of $\delta^{18}O$ and dust peaks, the summer of 1989 was identified by the peak of $\delta^{18}O$, and the summer of 1985 was confirmed by both of the $\delta^{18}O$ and dust peaks, respectively.

Fig. 5a Phylogenetic analysis of the 16S rRNA genes for *Alphaproteobacteria*, *Betaproteobacteria* *Gammaproteobacteria*, and *Deltaproteobacteria* clones from the Dunde ice core and the closest relatives. The tree was generated by the Neighbour-Joining method after sequence alignment, and rooted with two *Methanosaeta* strains (accession no. AY817738 and NR102903). Bootstrap values (100 replications) were specified for each Node. Cut-off value for the condensed tree was 55%. Numbers of the obtained snow-ice clones (had the same ARDRA pattern to the sequenced representatives listed on the tree) and relative sequence affiliations corresponding to GenBank accession number were provided in parentheses. The sequences discussed in this study were noted bold. See a detailed description for the assigned sequence references and numbers in materials and methods.

Fig. 5b Phylogenetic analysis of the 16S rRNA genes for the *Actinobacteria*, *Cyanobacteria*, *Verrucomicrobia*, and *Firmicutes* clones from the Dunde ice core and the closest relatives. The tree was constructed by following the protocol as described in Fig. 5a.

Fig. 5c Phylogenetic analysis of the 16S rRNA genes for the *Bacteroidetes* and TM7 candidate clones from the four geographically isolated glaciers and the closest relatives. The tree was constructed by following the protocol as described in Fig. 5a.

Fig. 6 Proportion of the main phylogenetic groups in the Dunde and Muztagata ice cores. Muztagata ice core dada was adapted from our previous report (An et al., 2010).

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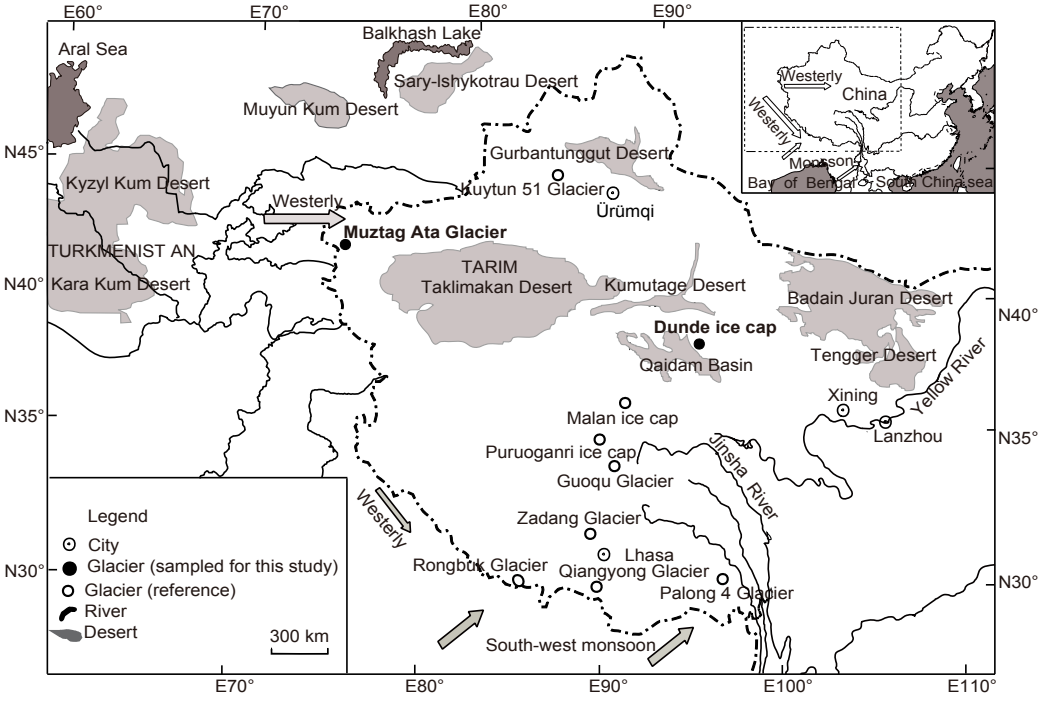

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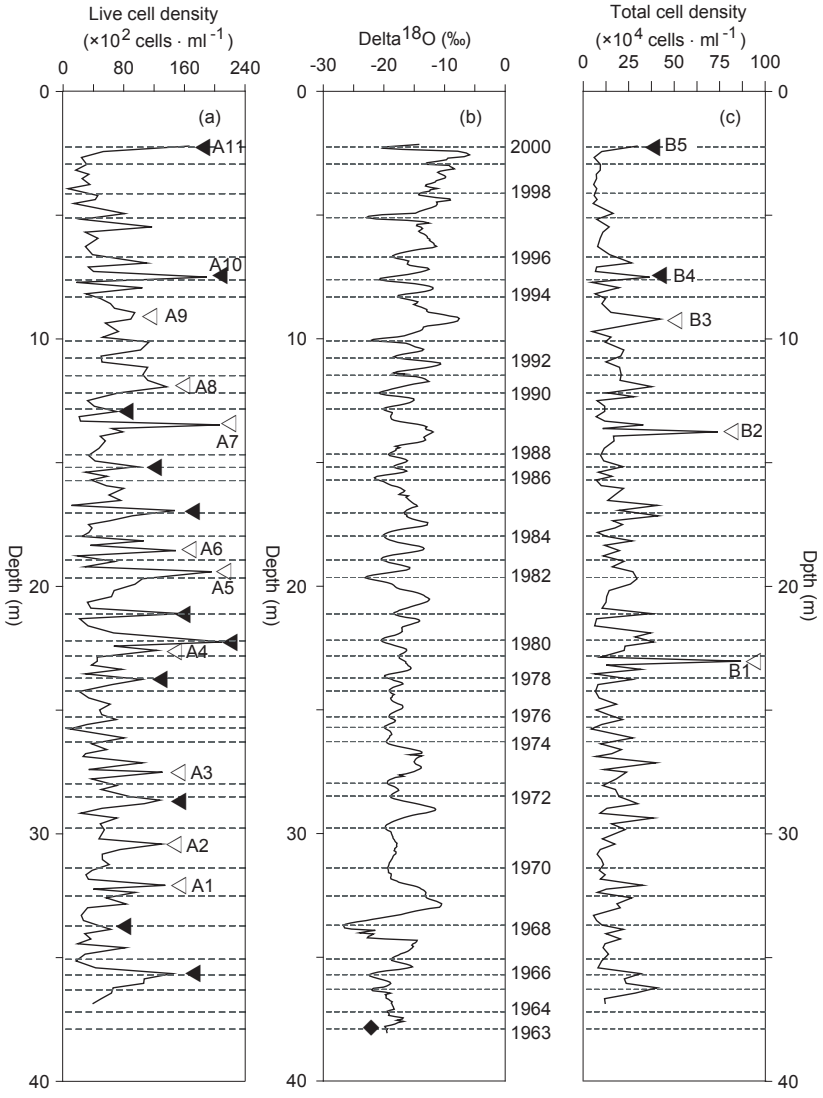

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
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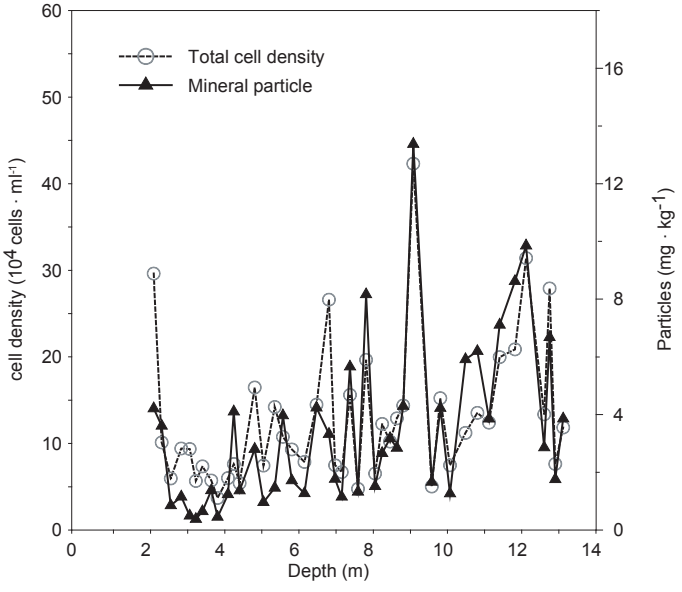

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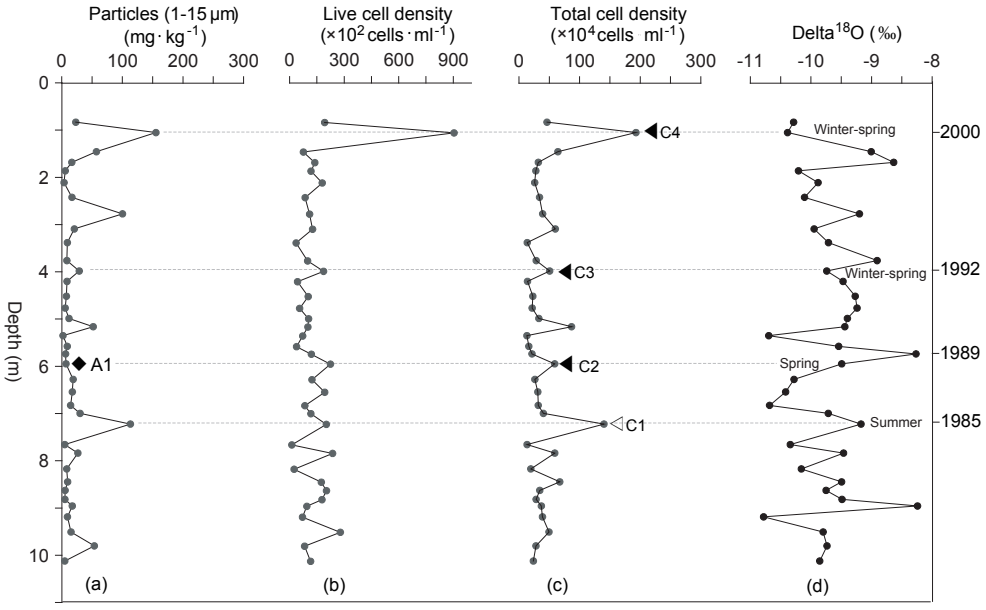
Manuscript under review for journal Biogeosciences
Published: 18 January 2016



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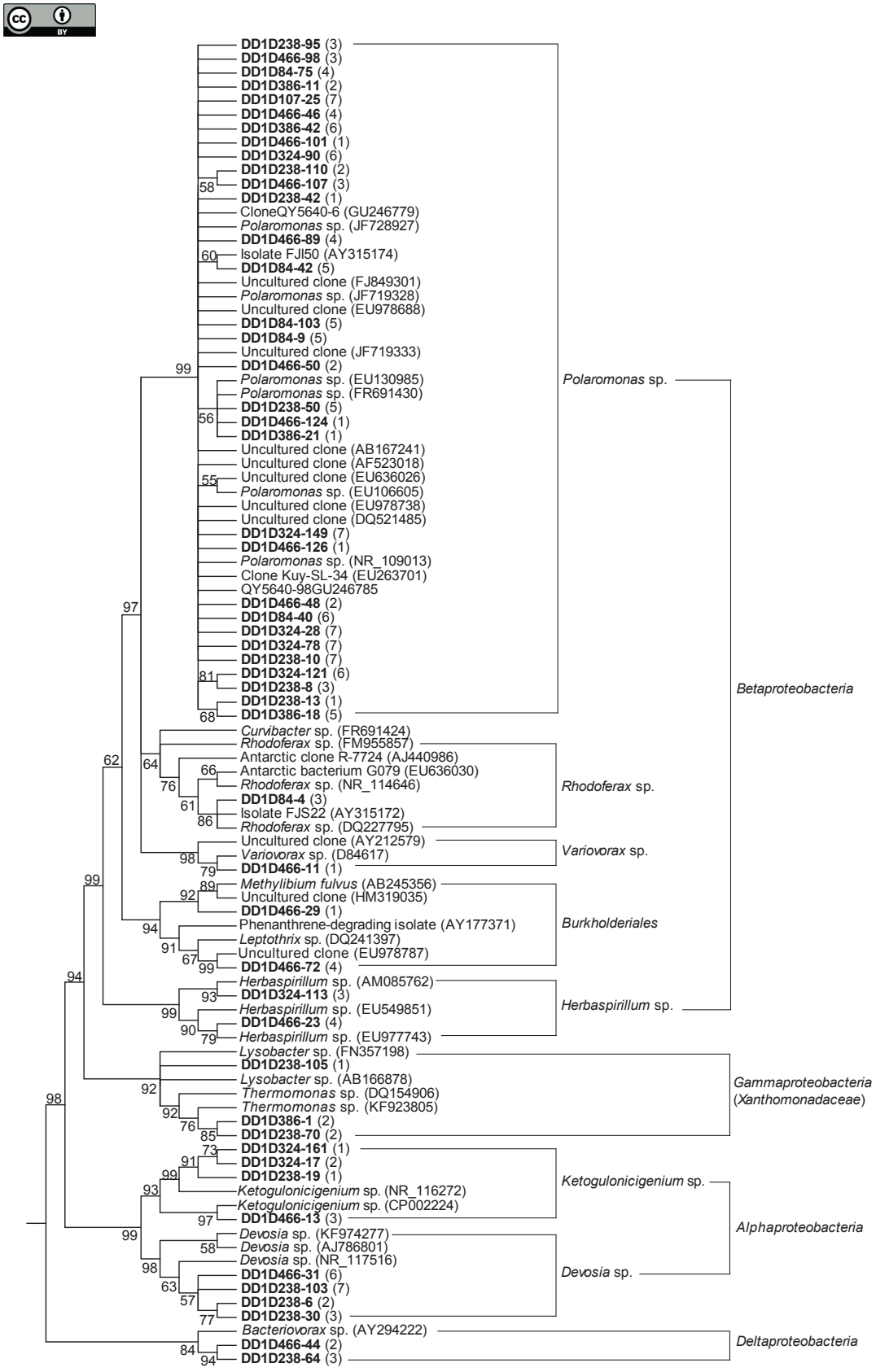

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Manuscript under review for journal Biogeosciences
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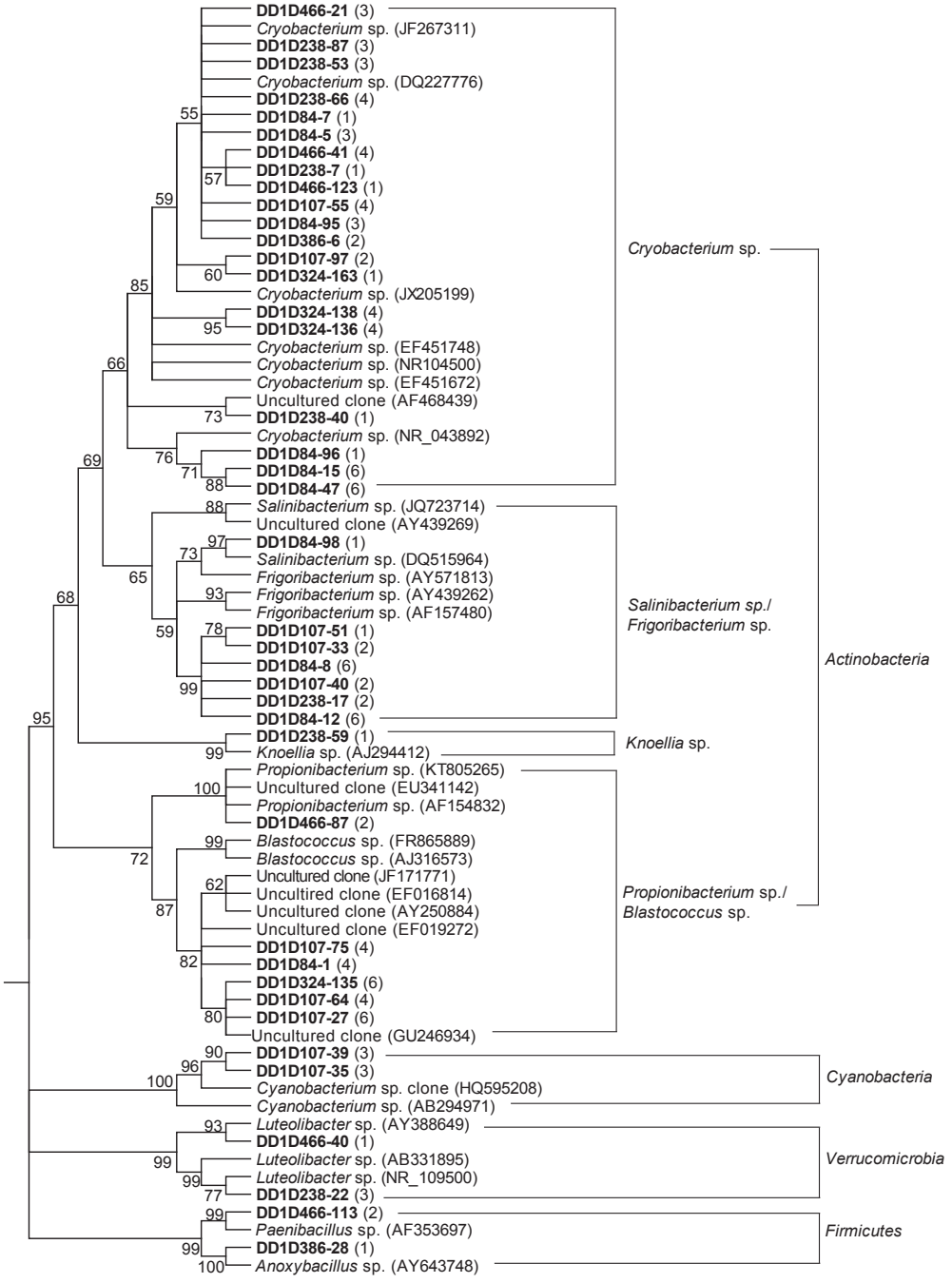

Biogeosciences Discuss., doi:10.5194/bg-2015-637, 2016
Manuscript under review for journal Biogeosciences
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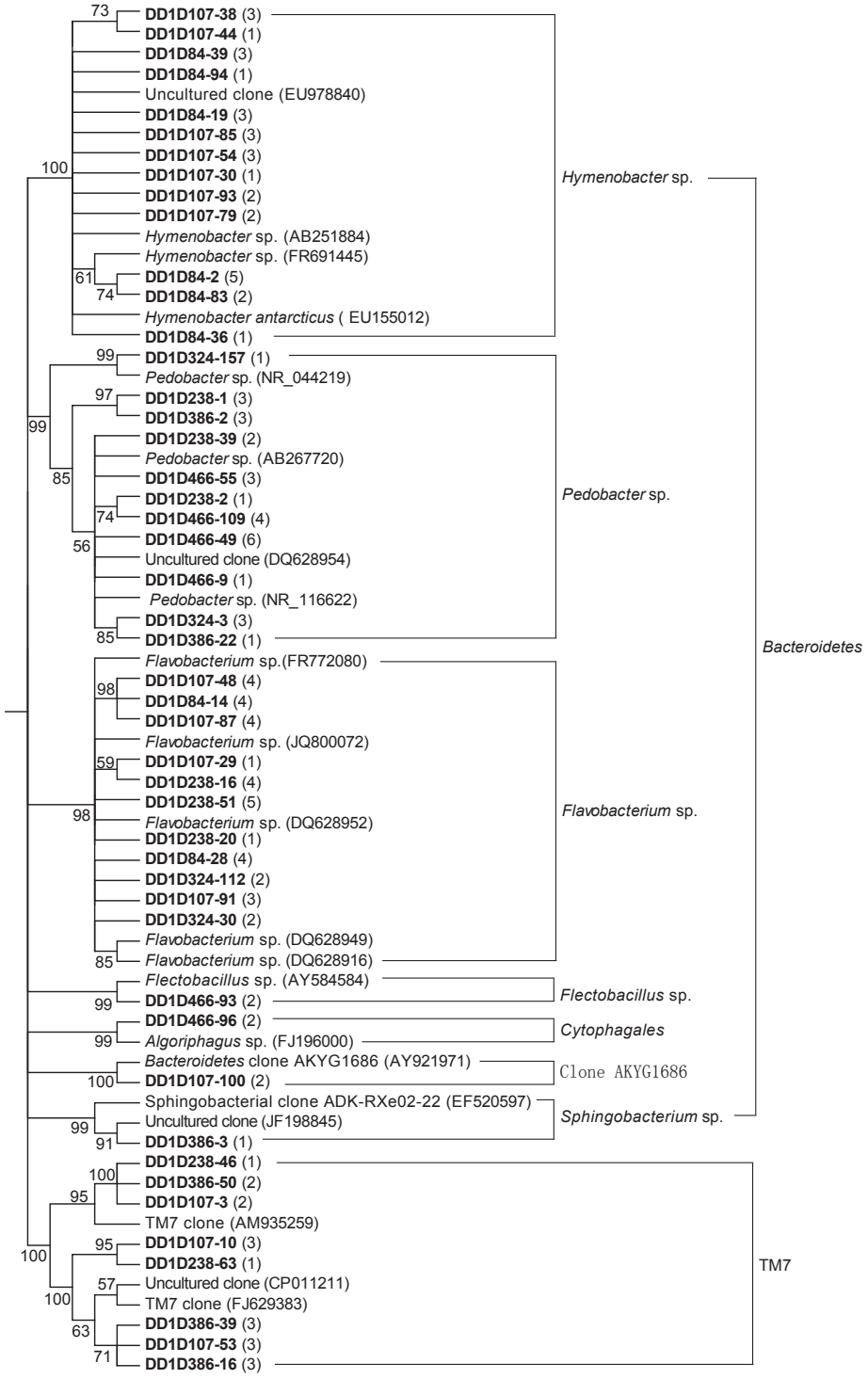

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Manuscript under review for journal Biogeosciences
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