1 Introduction

Mercury (Hg) is a heavy metal with high biotoxicity. The toxicity of Hg depends on its chemical form (Clarkson 1998, Li *et al.* 2017). Organic Hg is more toxic than inorganic Hg. One of the most important forms of organic Hg is methylmercury (MeHg), which is a neurotoxin that bioaccumulates in aquatic and terrestrial food chains and can have extreme impacts on wildlife and human health (Mahaffey *et al.* 2011) (Scheulhammer *et al.* 2007, Silver 1996). Elemental mercury (Hg0) is highly volatile and chemically stable and consequently can be dispersed via atmospheric circulation to areas far away from its source, with evidence of its deposition in non-populated regions such as the Qinghai-Tibet Plateau, high-altitude glaciers, the Antarctic and Arctic (Kang *et al.* 2016). Due to a "cold capture" effect, these specific locations may be global sinks for the accumulation of Hg and consequently important reservoirs in the global Hg cycle (Paudyal *et al.* 2017). Since the industrial revolution, the amount of Hg deposited in glaciers has increased sharply in line with increasing human activities (Kang *et al.* 2015, 2016). A study found that the total Hg content in high-altitude glaciers on the Tibetan Plateau increases with altitude, indicating that Hg deposited from the atmosphere may become trapped in glaciers, with the effect diminishing with increasing temperatures and decreasing altitudes (Huang et al. 2012). During glacier ablation, the proportion of Hg emitted into the atmosphere is very low, with the vast majority of Hg that is stored in snow and ice subsequently found in melt-waters that feed streams, rivers and lakes (Paudyal, *et al.* 2017; Kang *et al*., 2019). Indeed, many studies have shown that the Hg in many fish has exceeded or approached a critical value in the lakes fed by glacial meltwater. Zhang (2014) found that among 166 wild fish sampled from 13 lakes or rivers in the Qinghai-Tibet Plateau, MeHg in 5 samples exceeded the Chinese National Standard Limit (500 ng g^{-1}, GB2762-2005), and the MeHg concentration of *Pseudecheneis sulcatus* reached 1196 ng g^{-1}.

Most MeHg in the environment is formed by microbial catalyzed methylation of inorganic Hg (Hsu-Kim *et al.* 2013). The main Hg methylation microorganisms are Sulfate-Reducing Bacteria (SRB), followed by Iron-Reducing Bacteria (FeRB) and Methanogens (Gilmour *et al.* 2013). The genes responsible for the process are *hgcA*, encoding a corrinoid protein, and *hgcB*, encoding a ferredoxin. The process of Hg

33 methylation is achieved by transfer of a methyl group by the corrinoid protein and

34 reduction of the corrinoid cofactor by the ferredoxin (Parks *et al.* 2013). The presence

35 of *hgcAB* orthologues reflects the ability of microorganisms to methylate Hg

36 (Gilmour *et al.* 2013). Consequently, identification of these genes is valuable for

37 studying the process of microbial Hg methylation in the environment (Du *et al.* 2017,

38 Liu *et al.* 2014). There are two methods for analyzing the *hgcAB* gene in the

39 environment in published research. One is based on PCR technology. The *hgcAB* gene

40 sequence and abundance in the sample were obtained using *hgcAB* specific primers

41 (Bae *et al.* 2014, Bravo *et al.* 2018, Du *et al.* 2017, Liu *et al.* 2014, Schaefer *et al.*

42 2014, Xu *et al*. 2019). Another method is to screen out *hgcAB* homologous sequences

43 in the metagenomic data of the sample by a BLAST-like method (Gionfriddo *et al*.

44 2016, Liu *et al.* 2018, Podar *et al.* 2015). A significant positive correlation was

45 observed between *hgcA* abundance and MeHg concentrations in soils (Bae *et al.* 2014,

46 Du *et al.* 2017, Liu *et al.* 2018, Liu *et al.* 2014, Xu *et al.* 2019). *hgcAB* diversity and

47 distribution was studied in >3500 microbial metagenomes, encompassing a broad

48 range of environments, indicating that they have a high abundance and diversity in

49 dissolved permafrost, lake and river sediments, and are derived from many unknown

50 bacterial species in these environments (Podar *et al.* 2015). In the Antarctic, the

51 marine microaerophilic bacterium *Nitrospina* was identified as a potential mercury

52 methylator within sea ice and a source of MeHg in the Antarctic marine ecosystem

53 (Gionfriddo *et al.* 2016). While this study indicated that a cold environment is

54 important for microbial Hg methylation, the presence of microbial Hg methylation on

55 glaciers that feed freshwater systems has not been reported.

56 The glaciers of the Tibetan Plateau are the water reservoir for billions of people

57 living downstream in China, India and many other Asian countries (Immerzeel *et al.*

58 2010). Millions of people living downstream are dependent on melt water from these

59 glaciers as the most important sources of fresh water. The accumulated Hg in the

60 glacier can be released and subsequently pollute downstream ecosystems, causing

61 potential risks to human and the ecosystem health (Zhang *et al.* 2014). The rapid

62 economic development of Asian countries has enhanced the emission of

63 anthropogenic Hg to the environment (Chakraborty *et al.* 2013, Streets *et al.* 2011).

64 The Laohugou No.12 glacier is located in the western part of the Qilian Mountains in

65 the northeast of the Qinghai-Tibet Plateau, accounting for 53.6% of the total area of

66   the Laohugou glacier. Its glacial meltwater is an important source of the Changma

67   River. To identify potential microbially mediated Hg transformations within the ice,

68   we combined Hg speciation measurements and metagenomic analysis of microbial

69   community DNA extracted from glacial samples, focusing on the *hgcA* and *hgcB*

70   genes. This study indicates the potential importance of microbial Hg methylation in

71   glaciers. The objectives of the study were to: 1) Determine whether Hg methylation

72   microorganisms are present on the glacier; and 2) Assess where microbial-mediated

73   mercury methylation is more likely to occur.

74   2 Material and methods

75   2.1 Site description and Sampling

76   The Qilian Mts. are located in the northeastern edge of the Tibetan Plateau.

77   Laohugou Glacier No. 12 is the largest glacier in the Laohugou Valley which is

78   located in the northern slope of the western section of the Qilian Mountains (96°31′E,

79   39°30′N). The area of the glacier is approximately 20.49 km^2 and the length is about

80   10.1 km. The annual average temperature is -5.90 °C and the mean annual

81   precipitation is 358.6 mm (Zhang *et al.* 2016).

82   The samples were collected at the downstream ablation area (altitude 4250 m) of

83   Laohugou Glacier No. 12 in October 22th 2017. Six sample types were collected:

84   fresh snow (FS), supraglacial ice (SI), supraglacial cryoconite (SC, the area with dark

85   debris on the surface of the glacier), dusty layers (DL, the internal area with dark

86   debris), clean layers (CL, the internal area with little or no dark debris) and glacial

87   meltwater (GM). Ice and snow samples were collected with high density polyethylene

88   spatulas. The spatulas were soaked in 20% HNO_3 for 24 h and washed three times

89   with sterile ultrapure water before use. The glacial meltwater was collected, avoiding

90   contamination by measures such as wearing sterile polyethylene gloves. Five samples

91   of each type were collected with sample sites more than 1 meter apart. Fresh snow

92   samples were collected from the surface of the glacier; it had snowed the day before

93   sample collection. The samples of dusty layers ice and clean layers ice were collected

94   from glacier transects resulting from glacier ablation. Ten cm of glacier transect was

95   removed before sampling. One litre per sample was collected. The samples were

96   placed into sterile Nasco Whirl-Pak sample bags. Samples were kept below zero

97 during shipment and then were stored at −20 °C until the analyses were performed.

98 Samples used for total mercury (THg), methylmercury (MeHg) analysis were stored

99 in 50 mL polypropylene BD Falcon® centrifuge tubes with 250 μL BV-III grade

100 (CMOS) HCl (Beihua Chemical, China) added immediately (the ice and snow

101 samples were added after melting).

2.2 Measurement of sample chemical characteristics

103   The snow and ice samples were melted at room temperature in a laminar flow

104 hood before analysis. Chemical analyses of all samples include THg, MeHg,

105 dissolved organic carbon (DOC), total nitrogen (TN), pH and major soluble ions (Ca^{2+},

106 Mg^{2+}, K^+, Na^+, NH_4^+, Li^+, SO_4^{2-}, NO_3^-, NO_2^-, Cl^- and F^-). We followed the US EPA

107 Method 1631 (version E; US EPA, 2002) for THg analysis in melted snow/ice

108 samples. Analysis for Hg was performed by using a Tekran 2600 mercury analyzer in

109 a Class 1000 metal-free laboratory cleanroom at the State Key Laboratory of

110 Cryospheric Sciences, Chinese Academy of Sciences, Lanzhou, China. Analysis of

111 MeHg was determined using the EPA Method 1630, which involves distillation, water

112 ethylation, purging, trapping and cold atomic fluorescence (EPA, 1998). Total MeHg

113 was determined using a Tekran 2700 fully automated methylmercury analyzer. The

114 instrument method detection limit is 0.002 ng·L^{-1} (Sun *et al.* 2018). Quality assurance

115 and quality control for THg and MeHg determination were performed as *Sun* et al.

116 (2018). The range of recovery rate for THg standard solution (5.00 ng·L^{-1}) was from

117 93%-105%. The THg concentrations of field blanks and sampling container blanks

118 were <0.10 ng·L^{-1}. The range of recovery rate for MeHg standard solution (0.05

119 ng·L^{-1}) was from 82%-121%. The MeHg concentrations of field blanks and sampling

120 container blanks were <0.0.002 ng·L^{-1}. The DOC and TN contents were quantified

121 with a TOC-VCPH carbon analyzer (Shimadzu Corp., Japan). The pH values were

122 analyzed with a pH meter (PT-10, Sartorius, Göttingen, Germany). Analysis of

123 soluble cations was perfomed by ion chromatography DX320 (Dionex Corp., USA),

124 and of soluble anions by ion chromatography ICS-500 (Dionex Corp., USA)

2.3 DNA extraction and sequencing

126   The melted water samples were filtered through a 0.22 μm filter, discarding the

127 large stones at the bottom. The fine sediment and the filter members were combined,

128 and the DNA was extracted using a DNeasy PowerMax Soil Kit (QIAGEN, Corp.,

129   Germany), following the manufacturer's instructions. Since the DNA concentration of

130   a single sample did not meet the minimum standards for metagenomic sequencing, we

131   combined samples of the same type for sequencing. Sequencing was performed using

132   an Illumina HiSeq 2500 PE150 (Illumina Corp., USA).

133   **2.4 Metagenome analyses**

134   We used software cutadapt (v1.9.1) to remove the linker and low-quality reads

135   from the raw reads (150 bp in length) to obtain clean data for subsequent information

136   analysis (Table S1). Based on the optimized clean data, SOAPdenovo (v2) software

137   was used for assembly analysis. For each sample, different K-mers (49, 55, 61) were

138   assembled, and the N50 maximum scaffolds result was selected as the result of the

139   assembly. Coding genes were predicted using MetaGeneMark (v3.26) software. The

140   predicted gene sequences were further deduplicated using the sequence clustering

141   software CD-HIT (v4.5.6). Using BWA (version 0.7.12) comparison software, the

142   pre-processed reads were aligned to the constructed non-redundant gene set unigene

143   sequence, and then based on the number of reads and gene length on each unigene

144   alignment, unigene abundances were obtained for each sample. The unigene

145   sequences were blasted against the NR database using MEGAN (v6.4.4) software to

146   obtain the community composition of all samples. The metagenomic sequences have

147   been deposited with the NCBI under accession number PRJNA560154.

148   Homologous sequences for *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP* were

149   screened from metagenomic raw read set with HMMER 3.2.1

150   (http://www.hmmer.org/). The HMM using Profile hidden Markov models (HMMs)

151   is more sensitive and accurate in identifying homologs compared with BLAST or

152   other similar methods. First, the HMM profile was constructed with the known

153   complete sequences of the genes *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP*. The

154   sequences of *hgcA* and *hgcB* were from Liu *et al.* (2018). The *merA*, *merB*, *merR* and

155   *merP* were from Boyd and Barkay (2012). Using the EMBOSS toolset (Madeira *et al.*

156   2019), the reads were translated using the bacterial translation table into amino acid

157   sequences for each possible ORF. This amino acid sequence set was searched using

158   HMMsearch, and reads below a threshold e-value of 0.01 were removed to obtain the

159   homologous gene sequence set. False positive sequences in HMMsearch results based

160   on *hgcA* HMM profiles were determined by aligning the conserved domain (cap helix

161 and transmembrane regions) of the *hgcA* gene. The resulting *hgcA* homolog sequences

162 were further validated by BLAST searches. The closest taxon distribution of *hgcA*

163 homology sequences were annotated by BLAST with the NCBI NR database and

164 known Hg methyl microbial sequences. In order to compare the abundance of *hgcA*,

165 *hgcB*, *merA*, *merB*, *merR* and *merP* between samples, we normalized them to

166 metagenome size (Gbp).

2.5 Statistical analyses

168   The statistical analyses were performed with SPSS 19.0. Comparisons of THg

169 and MeHg concentrations among the 6 kinds of sample were performed using

170 two-way analysis of variation (ANOVA) followed by Tukey's multiple comparison

171 tests. The relationship between chemical characteristics, or chemical characteristics

172 with the relative abundance of *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP* or THg,

173 MeHg with microbial community or KEGG pathway were performed using

174 Spearman's correlation. Spearman correlation coefficients were corrected using the

175 False Discovery Rate (FDR).

3 Results

3.1 Concentration levels of THg and MeHg in the glacier terminus and their relationship with other ions

179   In the six types of samples at the glacier terminus, the concentration of THg

180 ranged from 22.4 ng/L to 172.1 ng/L. The concentration of THg in SC and DL were

181 significantly higher than FS, SI, CL and GM (Figure 1). The mean values of THg in

182 SC (149.1 ng/L) and DL (141.8 ng/L) were two times greater than in other sample

183 types. The site with the lowest THg concentration was GM3, and the site with the

184 highest value was SC3 (Table S2). The concentration of MeHg ranged between 0.005

185 ng/L to 0.465 ng/L. There was no significant difference in MeHg concentrations in

186 the six environments. However, in SC and DL, the MeHg concentrations exhibited the

187 greatest variation. In SC, the MeHg concentration of SC1 is 2.2 times the average

188 value, and in DL, for DL2 it is 2.6 times the average value (Figure 1, Table S2).

189 Therefore, the ratio of MeHg to THg changed drastically (Table S2). The mean ratio

190 of MeHg to THg was FS>SI>GM>CL>SC>DL. Compared with glacier meltwater,

191 runoff and wetland in the Zhadang-Qugaqie Basin, which is located in the

192   south-central Tibetan Plateau, the concentration of THg in this study was higher, the
193   MeHg concentration were lower, and ratio of MeHg to THg was lower (Sun *et al*.
194   2018). Compared with snow samples from the Xiao Dongkemadi Glacier in the
195   central Tibetan Plateau, the THg concentration in this study was lower than in snow
196   from August, but higher than in snow from May, June, July, September and October
197   (Paudyal *et al*. 2017).

198   Through Spearman correlation analysis, we found the concentrations of THg in
199   the samples from the glacier terminus were positively correlated with K^+
200   concentrations, in particular, and less so with Ca^{2+} and Mg^{2+} concentrations (Figure S1,
201   Table S3).

202   **3.2 The microbial communities of the glacier terminus and their relationship with**
203   **THg and MeHg**

204   At the terminus of the Laohugou glacier, the proportion of unclassified
205   sequences was very high, with a mean relative abundance of 52.6% in the 6 kinds of
206   sample type, and as high as 65% in the SC samples. Other sequences belonged to 66
207   bacterial phyla, 22 eukaryotic phyla and 2 archaeal phyla. The most abundant phylum
208   was Proteobacteria with a mean relative abundance of 41.2%. The following is
209   Bacteroidetes, and its mean relative abundance was 3.1%. The most abundant
210   eukaryota were Chordata and Arthropoda, with mean relative abundances were 0.91%
211   and 0.03%, respectively (Figure 2).

212   The composition of the microbial community in FS was the lowest microbial
213   diversity. The Proteobacteria phylum accounted for 81.8% of all, and unclassified
214   sequences accounted for 17.1% (Figure 2). Hierarchical cluster analysis shows that
215   microbes in FS are significantly different from other environmental types (Figure 3).
216   The relative abundance of Cyanobacteria in SC is the highest, at 4.4%. The Chordata
217   phylum was highest in DL, with a relative abundance of 3.7%. The number of
218   microbial phyla in GM was 75, higher than in any other sample type. Through
219   Spearman correlation analysis, we only found a significant correlation between the
220   *Stenotrophomonas* genus with THg concentration (p=0.971, FDR=0.036).

221   **3.3 Diversity and distribution of *hgcA* and *hgcB* genes at the glacier terminus**

222   Microbial Hg methylation *hgcA* homolog fragments were detected in FS, SC, DL,

223   CL and GM samples from the Laohugou glacier terminus, but were absent in SI

224   samples. In these six kinds of environment, the relative abundance of *hgcA* was

225   highest in DL. Next highest were SC, CL, GM and FS, respectively (Figure 4).

226   Overall, *hgcA* abundance was highest in sediment-bearing samples. *hgcB* homolog

227   fragments were found in all samples with a much higher relative abundance than for

228   *hgcA*. The abundance of *hgcB* was highest in CL, followed by GM, DL, SC, SI and

229   FS (Figure S2).

230   The *hgcA* gene sequences from all samples were derived from 8 phyla

231   (Firmicutes, Chloroflexi, Proteobacteria, Elusimicrobia, Euryarchaeota, Nitrospirae,

232   Spirochaetes and Bacteroidetes) and several unclassified bacterial species with

233   potential for Hg methylation at the terminus of the Laohugou glacier (Figure 5).

234   Overall, sequences from the Firmicutes and Proteobacteria had the highest diversity.

235   In DL, the main taxon from which *hgcA* sequences were derived was *Clostridium*. In

236   SC samples, *hgcA* sequences were derived from unclassified species belonging to

237   Proteobacteria, Bacteroidetes and other unclassified bacteria. In CL, the main

238   representative taxa were *Acetonema* and *Geobacter*, whereas in GM, they were

239   *Geobacter* and *Methanomassiliicoccus*. In FS, only *hgcA* sequences from the genus

240   *Clostridium* were found.

241   **3.4 The relative abundance of *merA*, *merB*, *merP* and *merR* genes**

242   The *merA*, *merB*, *merP* and *merR* resistance and transport genes were detected in

243   all samples. The variation in their relative abundance (in the raw data per GB) is

244   indicated in Figure 6. The abundance of *merA* is significantly higher than for the other

245   genes. Its relative abundance in the six environments was FS>CL>SI>GM>SC>DL.

246   The relative abundance of *merB* in FS was significantly higher than in others sample

247   types. The relative abundance of *merP* in the six environments was

248   FS>GM>CL>SI>DL>SC. The relative abundance of *merR* was

249   FS>SI>CL>GM>DL>SC. Overall, all four genes were most abundant in FS,

250   contrasting with the lowest abundance of *hgcA* sequences in this sample type.

251   **4 Discussion**

252   Our analysis of the terminus of Laohugou Glacier No. 12 indicated that THg was
253   most abundant in particulate layers (DL and SC). A positive correlation between
254   concentrations of THg and other cations typically associated with particulate
255   depositions indicated that the accumulation of Hg in this glacier could be due to
256   anthropogenic generation, atmospheric dispersal and subsequent deposition of
257   Hg-particulates.   A previous study into the origins of cryoconite deposited at
258   Laohugou Glacier No. 12 indicated an influence by regional industrial emissions
259   reflected in a the high black carbon content, as the glacier is near the Hexi Corridor in
260   Gansu province with many large cities and many petroleum, iron and steel industries,
261   (Dong et al, 2016). Hg is mainly in the gas phase in warm environments with low
262   aerosols, but in the particle phase in cold environments with high aerosols (Zhou *et al*.
263   2018). Our results are also consistent with another study into the spatial distribution of
264   mercury in glaciers in the Tibetan plateau, which confirm the primary association
265   with particulate matter (Huang *et al.* 2012). This study also reported increases of THg
266   with increasing altitude, indicating that these glaciers act as a sink for global Hg
267   cycling. Furthermore, whereas dissolved Hg in snow is preferentially released due to
268   snowmelt, particulate-bound Hg is more likely to be retained (Sun *et al.* 2018),
269   contributing to the sink effect.

270   The sediment in these particulate layers is typically composed by mineral (85–
271   95% of total mass) and organic matter (Baccolo *et al.* 2017, Cook *et al.* 2016). It is a
272   vital material for supporting growth of microbial organisms (Takeuchi *et al.* 2001,
273   Takeuchi *et al.* 2010). A previous study of Tibetan glaciers, including the Laohugou
274   Glacier, identified that these layers are dominated by Cyanobacteria, Chloroflexi,
275   Betaproteobacteria, Bacteroidetes and Actinobacteria (Liu *et al.* 2017), but
276   communities varied significantly between different geographical locations. With the
277   exception of Actinobacteria, these phyla were also well represented in our study.
278   But, our study highlights the abundance of unclassified species, representing over
279   50% of species in all glacier samples except the relatively simple community in fresh
280   snow.

281   Confirming conclusions of other studies assessing the distribution of *hgcAB*
282   genes (Gionfriddo*, et al.* 2016, Liu*, et al.* 2018, Podar*, et al.* 2015), we found that the
283   presence of *hgcB* –like sequences in metagenomes was an unreliable predictor of the

284   presence of mercury methylating bacteria in glacier samples. This can be attributed to

285   the conserved [4Fe-4S] binding motif in HgcB which is shared with many other

286   ferredoxins. In contrast, *hgcA* sequences were most abundant in the particulate layers

287   in which THg is at highest concentration, but also in quite high abundance in clean

288   layers and glacial meltwater. However, these sequences were derived from different

289   profiles of bacterial phyla and genera in the different sample types. In cryoconite

290   samples, the majority of sequences were from unclassified Bacteroidetes,

291   Proteobacteria or unclassified bacteria. In dusty layer samples, the majority were from

292   various Proteobacteria and Firmicutes. Whereas species of *Geobacter* were

293   represented in all these different sample types, and have previously been associated

294   with Hg methylation in paddy field soils (Liu *et al.* 2018), species of Nitrospirae were

295   specific to meltwater. Comparative genomics indicates a strong evolutionary link

296   between nitrite-oxidising *Nitrospira* and *Nitrospina* (Lücker *et al.* 2013), the latter

297   being associated with Hg methylation in Antarctic sea ice (Gionfriddo*, et al.* 2016).

298   The genes of the microbial *mer* operon confer Hg resistance and are involved in

299   Hg transport and volatilization (Boyd and Barkay 2012). The abundance of these

300   genes was highest in fresh snow samples; indeed, if the presence of *merB* sequences is

301   a reliable indicator, mercury-resistant bacteria are clearly a lot more abundant in fresh

302   snow compared to other sample types. This is consistent with another study indicating

303   that Hg-resistant bacteria accounted for up to 31% of the culturable bacteria in snow,

304   but only 2% in freshwater and brine (Moller *et al.* 2011). These bacteria are most

305   likely to come from snowfall but could contribute to the volatilization of dissolved Hg

306   in this layer. Although bacteria with the *mer* operon cannot convert inorganic mercury

307   to methylmercury directly, they can also play an important role in transformation of

308   mercury speciation. *merA* encode a mercuric reductase which can catalyze Hg(II) to

309   be Hg(0). *merB* encode an organomercury lyase. There are also genes that allow

310   bacteria to transport extracellular mercury into cells (Boyd and Barkay 2012).

311   Therefore, microbes with a *mer* operon can influence the form and distribution of Hg

312   in glaciers. Changes in the concentration of Hg(II) in local areas can affect mercury

313   methylation by microorganisms. It may be more likely to occur in particulate layers

314   with higher mercury and nutrient.

315   Mercury methylation bacteria in particulate layers likely contributes to the

316   mobilization of glacier-sequestered particulate-bound mercury. The particulate layers

317   and microbial activity therein reduce the glacier surface albedo and accelerate melting

318   of glacier ice (Anesio and Laybourn-Parry 2012, Cook *et al*. 2015, Musilova *et al*.

319   2016, Takeuchi *et al*. 2014). MeHg has higher water solubility and migration capacity

320   than particulate-bound mercury. In these conditions, we postulate that metabolically

321   active bacteria identified in this study can methylate Hg, effectively mobilizing it to

322   run off in the melt water. As a consequence, we found no direct correlation between

323   MeHg concentrations and Hg-methylating bacteria in particulate glacier samples. A

324   prediction is that, with climate change, this process will be accelerated, with

325   consequent negative impact on downstream freshwater ecosystems and human health.

326   The gravity of this scenario warrants further investigation into the role of

327   Hg-methylating bacteria as glaciers retreat.

328   **Conclusions**

329   This is the first study of microbial Hg methylation in a high altitude mountain

330   glacier. In the terminus of LHG glacier, the Hg concentrations in SC and DL samples

331   which contain considerable debris and dust is higher than in FS, SI, CL and GM. In

332   addition, MeHg concentrations were highest in some of these SC and DL samples.

333   Bacterial *hgcA* Hg methylation genes were present in all samples except supraglacial

334   ice but were of highest abundance in SC and DL. This suggested that microbial Hg

335   methylation is most likely to occur in SC and DL. There were 8 phyla (Firmicutes,

336   Chloroflexi, Proteobacteria, Elusimicrobia, Euryarchaeota, Nitrospirae, Spirochaetes

337   and Bacteroidetes) and some unclassified of potential Hg methylation microorganism.

338   37% of the sequences cannot be classified into any known genus. Most of the *hgcA*

339   sequences were closely related to sequences from previously reported Hg methylating

340   genera within the Deltaproteobacteria and Firmicutes, and the common

341   Methanomicrobia was absent in glacial samples. The relative of *merA*, *merB*, *merP*

342   and *merR* genes in fresh snow are higher than that in other samples. This indicates

343   that such microorganisms in glaciers are most likely to come from snowfall.

344   **Author Contributions**

345　　　BZ, TC and SK designed the study. JG, MW and RY performed field
346　observation and sampling activities. BZ and XC analyzed Illumina sequencing data.
347　BZ, GZ and WZ made statistical analysis. BZ, TC, GL, SK and PD interpreted the
348　results and wrote the manuscript.

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Conflict of Interest Statement

355　　　The authors declare that the research was conducted in the absence of any

356　commercial or financial relationships that could be construed as a potential conflict of

357　interest.

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490   Figure 1 Boxplot showing the concentration of THg and MeHg in the ablation area of the
491   LHG glacier

492   Figure 2 Analysis of community composition at the phylum level in in the ablation area of the
493   LHG glacier

494   Figure 3 Comparison of microbiology community composition at the genus level in the
495   ablation area of the LHG glacier

496   Figure 4 Relative abundance of *hgc*A genes (from shotgun metagenomic sequencing)
497   normalized to metagenome size

498   Figure 5 Closest phylum and genera assignments for *hgcA* gene/fragments for metagenomic
499   data sets containing *hgcA*. Circle sizes represent the relative abundance of *hgcA* sequences
500   assigned to a specific genus in each sample.

501   Figure 6 Relative abundance of *mer*A, *mer*B, *mer*P, and *mer*R genes (from shotgun
502   metagenomic sequencing) normalized to metagenome size.

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