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

methylation is achieved by transfer of a methyl group by the corrinoid protein and reduction of the corrinoid cofactor by the ferredoxin (Parks *et al.* 2013). The presence of *hgcAB* orthologues reflects the ability of microorganisms to methylate Hg (Gilmour *et al.* 2013). Consequently, identification of these genes is valuable for studying the process of microbial Hg methylation in the environment (Du *et al.* 2017, Liu *et al.* 2014). There are two methods for analyzing the *hgcAB* gene in the environment in published research. One is based on PCR technology. The *hgcAB* gene sequence and abundance in the sample were obtained using *hgcAB* specific primers (Bae *et al.* 2014, Bravo *et al.* 2018, Du *et al.* 2017, Liu *et al.* 2014, Schaefer *et al.* 2014, Xu *et al*. 2019). Another method is to screen out *hgcAB* homologous sequences in the metagenomic data of the sample by a BLAST-like method (Gionfriddo *et al*. 2016, Liu *et al.* 2018, Podar *et al.* 2015). A significant positive correlation was observed between *hgcA* abundance and MeHg concentrations in soils (Bae *et al.* 2014, Du *et al.* 2017, Liu *et al.* 2018, Liu *et al.* 2014, Xu *et al.* 2019). *hgcAB* diversity and distribution was studied in >3500 microbial metagenomes, encompassing a broad range of environments, indicating that they have a high abundance and diversity in dissolved permafrost, lake and river sediments, and are derived from many unknown bacterial species in these environments (Podar *et al.* 2015). In the Antarctic, the marine microaerophilic bacterium *Nitrospina* was identified as a potential mercury methylator within sea ice and a source of MeHg in the Antarctic marine ecosystem (Gionfriddo *et al.* 2016). While this study indicated that a cold environment is important for microbial Hg methylation, the presence of microbial Hg methylation on glaciers that feed freshwater systems has not been reported.

The glaciers of the Tibetan Plateau are the water reservoir for billions of people living downstream in China, India and many other Asian countries (Immerzeel *et al.* 2010). Millions of people living downstream are dependent on melt water from these glaciers as the most important sources of fresh water. The accumulated Hg in the glacier can be released and subsequently pollute downstream ecosystems, causing potential risks to human and the ecosystem health (Zhang *et al.* 2014). The rapid economic development of Asian countries has enhanced the emission of anthropogenic Hg to the environment (Chakraborty *et al.* 2013, Streets *et al.* 2011). The Laohugou No.12 glacier is located in the western part of the Qilian Mountains in the northeast of the Qinghai-Tibet Plateau, accounting for 53.6% of the total area of

the Laohugou glacier. Its glacial meltwater is an important source of the Changma River. To identify potential microbially mediated Hg transformations within the ice, we combined Hg speciation measurements and metagenomic analysis of microbial community DNA extracted from glacial samples, focusing on the *hgcA* and *hgcB* genes. This study indicates the potential importance of microbial Hg methylation in glaciers. The objectives of the study were to: 1) Determine whether Hg methylation microorganisms are present on the glacier; and 2) Assess where microbial-mediated mercury methylation is more likely to occur.

2 Material and methods

2.1 Site description and Sampling

The Qilian Mts. are located in the northeastern edge of the Tibetan Plateau. Laohugou Glacier No. 12 is the largest glacier in the Laohugou Valley which is located in the northern slope of the western section of the Qilian Mountains (96°31′E, 39°30′N). The area of the glacier is approximately 20.49 km^2 and the length is about 10.1 km. The annual average temperature is -5.90 °C and the mean annual precipitation is 358.6 mm (Zhang *et al.* 2016).

The samples were collected at the downstream ablation area (altitude 4250 m) of Laohugou Glacier No. 12 in October 22th 2017. Six sample types were collected: fresh snow (FS), supraglacial ice (SI), supraglacial cryoconite (SC, the area with dark debris on the surface of the glacier), dusty layers (DL, the internal area with dark debris), clean layers (CL, the internal area with little or no dark debris) and glacial meltwater (GM). Ice and snow samples were collected with high density polyethylene spatulas. The spatulas were soaked in 20% HNO_3 for 24 h and washed three times with sterile ultrapure water before use. The glacial meltwater was collected, avoiding contamination by measures such as wearing sterile polyethylene gloves. Five samples of each type were collected with sample sites more than 1 meter apart. Fresh snow samples were collected from the surface of the glacier; it had snowed the day before sample collection. The samples of dusty layers ice and clean layers ice were collected from glacier transects resulting from glacier ablation. Ten cm of glacier transect was removed before sampling. One litre per sample was collected. The samples were placed into sterile Nasco Whirl-Pak sample bags. Samples were kept below zero

during shipment and then were stored at −20 °C until the analyses were performed. Samples used for total mercury (THg), methylmercury (MeHg) analysis were stored in 50 mL polypropylene BD Falcon® centrifuge tubes with 250 μL BV-III grade (CMOS) HCl (Beihua Chemical, China) added immediately (the ice and snow samples were added after melting).

2.2 Measurement of sample chemical characteristics

The snow and ice samples were melted at room temperature in a laminar flow hood before analysis. Chemical analyses of all samples include THg, MeHg, dissolved organic carbon (DOC), total nitrogen (TN), pH and major soluble ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+, Li^+, SO_4^{2-}, NO_3^-, NO_2^-, Cl^- and F^-). We followed the US EPA Method 1631 (version E; US EPA, 2002) for THg analysis in melted snow/ice samples. Analysis for Hg was performed by using a Tekran 2600 mercury analyzer in a Class 1000 metal-free laboratory cleanroom at the State Key Laboratory of Cryospheric Sciences, Chinese Academy of Sciences, Lanzhou, China. Analysis of MeHg was determined using the EPA Method 1630, which involves distillation, water ethylation, purging, trapping and cold atomic fluorescence (EPA, 1998). Total MeHg was determined using a Tekran 2700 fully automated methylmercury analyzer. The instrument method detection limit is 0.002 $ng \cdot L^{-1}$ (Sun *et al.* 2018). Quality assurance and quality control for THg and MeHg determination were performed as *Sun* et al. (2018). The range of recovery rate for THg standard solution (5.00 $ng \cdot L^{-1}$) was from 93%-105%. The THg concentrations of field blanks and sampling container blanks were <0.10 $ng \cdot L^{-1}$. The range of recovery rate for MeHg standard solution (0.05 $ng \cdot L^{-1}$) was from 82%-121%. The MeHg concentrations of field blanks and sampling container blanks were <0.0.002 $ng \cdot L^{-1}$. The DOC and TN contents were quantified with a TOC-VCPH carbon analyzer (Shimadzu Corp., Japan). The pH values were analyzed with a pH meter (PT-10, Sartorius, Göttingen, Germany). Analysis of soluble cations was perfomed by ion chromatography DX320 (Dionex Corp., USA), and of soluble anions by ion chromatography ICS-500 (Dionex Corp., USA)

2.3 DNA extraction and sequencing

The melted water samples were filtered through a 0.22 μm filter, discarding the large stones at the bottom. The fine sediment and the filter members were combined, and the DNA was extracted using a DNeasy PowerMax Soil Kit (QIAGEN, Corp.,

Germany), following the manufacturer's instructions. Since the DNA concentration of a single sample did not meet the minimum standards for metagenomic sequencing, we combined samples of the same type for sequencing. Sequencing was performed using an Illumina HiSeq 2500 PE150 (Illumina Corp., USA).

2.4 Metagenome analyses

We used software cutadapt (v1.9.1) to remove the linker and low-quality reads from the raw reads (150 bp in length) to obtain clean data for subsequent information analysis (Table S1). Based on the optimized clean data, SOAPdenovo (v2) software was used for assembly analysis. For each sample, different K-mers (49, 55, 61) were assembled, and the N50 maximum scaffolds result was selected as the result of the assembly. Coding genes were predicted using MetaGeneMark (v3.26) software. The predicted gene sequences were further deduplicated using the sequence clustering software CD-HIT (v4.5.6). Using BWA (version 0.7.12) comparison software, the pre-processed reads were aligned to the constructed non-redundant gene set unigene sequence, and then based on the number of reads and gene length on each unigene alignment, unigene abundances were obtained for each sample. The unigene sequences were blasted against the NR database using MEGAN (v6.4.4) software to obtain the community composition of all samples. The metagenomic sequences have been deposited with the NCBI under accession number PRJNA560154.

Homologous sequences for *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP* were screened from metagenomic raw read set with HMMER 3.2.1 (http://www.hmmer.org/). The HMM using Profile hidden Markov models (HMMs) is more sensitive and accurate in identifying homologs compared with BLAST or other similar methods. First, the HMM profile was constructed with the known complete sequences of the genes *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP*. The sequences of *hgcA* and *hgcB* were from Liu *et al.* (2018). The *merA*, *merB*, *merR* and *merP* were from Boyd and Barkay (2012). Using the EMBOSS toolset (Madeira *et al.* 2019), the reads were translated using the bacterial translation table into amino acid sequences for each possible ORF. This amino acid sequence set was searched using HMMsearch, and reads below a threshold e-value of 0.01 were removed to obtain the homologous gene sequence set. False positive sequences in HMMsearch results based on *hgcA* HMM profiles were determined by aligning the conserved domain (cap helix

and transmembrane regions) of the *hgcA* gene. The resulting *hgcA* homolog sequences were further validated by BLAST searches. The closest taxon distribution of *hgcA* homology sequences were annotated by BLAST with the NCBI NR database and known Hg methyl microbial sequences. In order to compare the abundance of *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP* between samples, we normalized them to metagenome size (Gbp).

2.5 Statistical analyses

The statistical analyses were performed with SPSS 19.0. Comparisons of THg and MeHg concentrations among the 6 kinds of sample were performed using two-way analysis of variation (ANOVA) followed by Tukey's multiple comparison tests. The relationship between chemical characteristics, or chemical characteristics with the relative abundance of *hgcA*, *hgcB*, *merA*, *merB*, *merR* and *merP* or THg, MeHg with microbial community or KEGG pathway were performed using Spearman's correlation. Spearman correlation coefficients were corrected using the False Discovery Rate (FDR).

3 Results

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

In the six types of samples at the glacier terminus, the concentration of THg ranged from 22.4 ng/L to 172.1 ng/L. The concentration of THg in SC and DL were significantly higher than FS, SI, CL and GM (Figure 1). The mean values of THg in SC (149.1 ng/L) and DL (141.8 ng/L) were two times greater than in other sample types. The site with the lowest THg concentration was GM3, and the site with the highest value was SC3 (Table S2). The concentration of MeHg ranged between 0.005 ng/L to 0.465 ng/L. There was no significant difference in MeHg concentrations in the six environments. However, in SC and DL, the MeHg concentrations exhibited the greatest variation. In SC, the MeHg concentration of SC1 is 2.2 times the average value, and in DL, for DL2 it is 2.6 times the average value (Figure 1, Table S2). Therefore, the ratio of MeHg to THg changed drastically (Table S2). The mean ratio of MeHg to THg was FS>SI>GM>CL>SC>DL. Compared with glacier meltwater, runoff and wetland in the Zhadang-Qugaqie Basin, which is located in the

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

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

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

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

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

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

Microbial Hg methylation *hgcA* homolog fragments were detected in FS, SC, DL, CL and GM samples from the Laohugou glacier terminus, but were absent in SI samples. In these six kinds of environment, the relative abundance of *hgcA* was highest in DL. Next highest were SC, CL, GM and FS, respectively (Figure 4). Overall, *hgcA* abundance was highest in sediment-bearing samples. *hgcB* homolog fragments were found in all samples with a much higher relative abundance than for *hgcA*. The abundance of *hgcB* was highest in CL, followed by GM, DL, SC, SI and FS (Figure S2).

The *hgcA* gene sequences from all samples were derived from 8 phyla (Firmicutes, Chloroflexi, Proteobacteria, Elusimicrobia, Euryarchaeota, Nitrospirae, Spirochaetes and Bacteroidetes) and several unclassified bacterial species with potential for Hg methylation at the terminus of the Laohugou glacier (Figure 5). Overall, sequences from the Firmicutes and Proteobacteria had the highest diversity. In DL, the main taxon from which *hgcA* sequences were derived was *Clostridium*. In SC samples, *hgcA* sequences were derived from unclassified species belonging to Proteobacteria, Bacteroidetes and other unclassified bacteria. In CL, the main representative taxa were *Acetonema* and *Geobacter*, whereas in GM, they were *Geobacter* and *Methanomassiliicoccus*. In FS, only *hgcA* sequences from the genus *Clostridium* were found.

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

The *merA*, *merB*, *merP* and *merR* resistance and transport genes were detected in all samples. The variation in their relative abundance (in the raw data per GB) is indicated in Figure 6. The abundance of *merA* is significantly higher than for the other genes. Its relative abundance in the six environments was FS>CL>SI>GM>SC>DL. The relative abundance of *merB* in FS was significantly higher than in others sample types. The relative abundance of *merP* in the six environments was FS>GM>CL>SI>DL>SC. The relative abundance of *merR* was FS>SI>CL>GM>DL>SC. Overall, all four genes were most abundant in FS, contrasting with the lowest abundance of *hgcA* sequences in this sample type.

4 Discussion

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

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

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

presence of mercury methylating bacteria in glacier samples. This can be attributed to the conserved [4Fe-4S] binding motif in HgcB which is shared with many other ferredoxins. In contrast, *hgcA* sequences were most abundant in the particulate layers in which THg is at highest concentration, but also in quite high abundance in clean layers and glacial meltwater. However, these sequences were derived from different profiles of bacterial phyla and genera in the different sample types. In cryoconite samples, the majority of sequences were from unclassified Bacteroidetes, Proteobacteria or unclassified bacteria. In dusty layer samples, the majority were from various Proteobacteria and Firmicutes. Whereas species of *Geobacter* were represented in all these different sample types, and have previously been associated with Hg methylation in paddy field soils (Liu *et al.* 2018), species of Nitrospirae were specific to meltwater. Comparative genomics indicates a strong evolutionary link between nitrite-oxidising *Nitrospira* and *Nitrospina* (Lücker *et al.* 2013), the latter being associated with Hg methylation in Antarctic sea ice (Gionfriddo, *et al.* 2016).

The genes of the microbial *mer* operon confer Hg resistance and are involved in Hg transport and volatilization (Boyd and Barkay 2012). The abundance of these genes was highest in fresh snow samples; indeed, if the presence of *merB* sequences is a reliable indicator, mercury-resistant bacteria are clearly a lot more abundant in fresh snow compared to other sample types. This is consistent with another study indicating that Hg-resistant bacteria accounted for up to 31% of the culturable bacteria in snow, but only 2% in freshwater and brine (Moller *et al.* 2011). These bacteria are most likely to come from snowfall but could contribute to the volatilization of dissolved Hg in this layer. Although bacteria with the *mer* operon cannot convert inorganic mercury to methylmercury directly, they can also play an important role in transformation of mercury speciation. *merA* encode a mercuric reductase which can catalyze Hg(II) to be Hg(0). *merB* encode an organomercury lyase. There are also genes that allow bacteria to transport extracellular mercury into cells (Boyd and Barkay 2012). Therefore, microbes with a *mer* operon can influence the form and distribution of Hg in glaciers. Changes in the concentration of Hg(II) in local areas can affect mercury methylation by microorganisms. It may be more likely to occur in particulate layers with higher mercury and nutrient.

Mercury methylation bacteria in particulate layers likely contributes to the mobilization of glacier-sequestered particulate-bound mercury. The particulate layers and microbial activity therein reduce the glacier surface albedo and accelerate melting of glacier ice (Anesio and Laybourn-Parry 2012, Cook *et al*. 2015, Musilova *et al*. 2016, Takeuchi *et al*. 2014). MeHg has higher water solubility and migration capacity than particulate-bound mercury. In these conditions, we postulate that metabolically active bacteria identified in this study can methylate Hg, effectively mobilizing it to run off in the melt water. As a consequence, we found no direct correlation between MeHg concentrations and Hg-methylating bacteria in particulate glacier samples. A prediction is that, with climate change, this process will be accelerated, with consequent negative impact on downstream freshwater ecosystems and human health. The gravity of this scenario warrants further investigation into the role of Hg-methylating bacteria as glaciers retreat.

Conclusions

This is the first study of microbial Hg methylation in a high altitude mountain glacier. In the terminus of LHG glacier, the Hg concentrations in SC and DL samples which contain considerable debris and dust is higher than in FS, SI, CL and GM. In addition, MeHg concentrations were highest in some of these SC and DL samples. Bacterial *hgcA* Hg methylation genes were present in all samples except supraglacial ice but were of highest abundance in SC and DL. This suggested that microbial Hg methylation is most likely to occur in SC and DL. There were 8 phyla (Firmicutes, Chloroflexi, Proteobacteria, Elusimicrobia, Euryarchaeota, Nitrospirae, Spirochaetes and Bacteroidetes) and some unclassified of potential Hg methylation microorganism. 37% of the sequences cannot be classified into any known genus. Most of the *hgcA* sequences were closely related to sequences from previously reported Hg methylating genera within the Deltaproteobacteria and Firmicutes, and the common Methanomicrobia was absent in glacial samples. The relative of *merA*, *merB*, *merP* and *merR* genes in fresh snow are higher than that in other samples. This indicates that such microorganisms in glaciers are most likely to come from snowfall.

Author Contributions

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

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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

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

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

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

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

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