Bacterial community diversity in sparse debris and cryoconite holes on nearby glaciers

Francesca Pittino[1], Roberto S. Azzoni [2,3], Mirko Rossi[1], Guglielmina Diolaiuti[2], Andrea Franzetti[1*], Roberto Ambrosini[2]

[1]Department of Earth and Environmental Sciences (DISAT) - University of Milano-Bicocca, Milano, Italy

[2] Department of Environmental Science and Policy, University of Milan, Milano, Italy

[3]Department of Earth Sciences "Ardito Desio", Milano, Italy

* correspondence to: andrea.franzetti@unimib.it

ABSTRACT

Supraglacial ecosystems concentrate their microbial communities mostly in cryoconite holes, small pits full of melting water with a sediment on the bottom. The geographical differences of their bacterial communities (among glaciers) are quite ascertained, especially at large scale. Furthermore, so far no data are available to confirm the hypothesis that bacterial communities inhabiting cryoconite holes are different from those that can be found in the sparse debris (the dry debris that is not immersed in the melting water), especially considering that the sparse debris can form cryoconite holes and vice versa. In this study we characterized bacterial communities of the sparse debris of three different glaciers belonging to a quite restricted area (maximum distance < 10 km) of the Ortles Cevedale Group (Italian Alps) and confirmed that bacterial communities differ among different glaciers, but not according to their geographic distance. Indeed, lithology seems to have an effect on their composition. Furthermore, we found that bacterial communities of the sparse debris are significantly different from those inhabiting cryoconite holes.

Keywords

Cryoconite, bacterial communities, supraglacial ecosystem, supraglacial sediment

1. INTRODUCTION

Cryoconite holes are small ponds full of melting water with a fine-grained sediment on the bottom (the cryoconite) characteristic of glaciers surface. Cryoconite origin is mostly atmospheric mainly from local sources [1–3]. They are hot-spots of biodiversity in glacier environments, hosting metabolically active microbial communities [4] dominated by Cyanobacteria [5], Betaproteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Bacteroidetes, and Proteobacteria [3, 6, 7].

On glaciers, bacterial communities composition depends on temperature, intense solar radiation, wind exposition, electrical conductivity, and pH [1, 8, 9]. Furthermore, cryoconite holes are oligotrophic microhabitats [10] and nutrient availability is a limiting factor for bacteria, especially total organic carbon (TOC), ammonium, and phosphorous [1, 9, 11–13].

Cryoconite holes can be found on glaciers all over the world, at global scale their structure have been demonstrated to show a typical decay-by-distance pattern of similarity [14]. At smaller spatial scale, cryoconite hole bacterial communities seem to differ from one glacier to a nearby one, and even within a glacier [15]. The first study that compared cryoconite holes bacterial communities from different glaciers in a restricted geographical area (c.ca 10 km), is by Edwards et al. [1]. Results showed that the ecological distance among their bacterial communities did not reflect their geographic distance. Other studies confirmed the above mentioned results [16] and found that different sampling areas can host different bacterial communities [17]. It therefore appears that variation in cryoconite holes bacterial communities is mostly due to local environmental conditions, while the decay-by-distance pattern appears only at larger spatial scales.

On temperate mountain glaciers, cryoconite holes are rather ephemeral structures that continuously form and are dismantled during the melting season [8]. Interestingly, the within-season temporal dynamic of the bacterial communities seems to proceed on the whole glacier surface independently of the timing of hole formation [8]. In other words, when a hole is dismantled by the ablation and the cryoconite is dispersed on the glacier surface the succession process proceeds once the cryoconite has formed a new hole [8].

Therefore, the cryoconite that is broadly dispersed and has not formed a hole, may host bacterial communities similar to those of cryoconite holes. However, this type of sediment has been poorly investigated to date. Indeed, few papers studied the sediment of debris cover glaciers, but it is mostly not in direct contact with ice [18, 19]. Two studies investigated dirt cones (type of depositional glacial feature) [2, 20], however, this sediment differs from cryoconite (thicker and typically originates in crevasses or moulins) [21].

So far, a comprehensive study that investigated and compared the bacterial community composition of supraglacial sparse debris of different glaciers of the same study area has not been conducted yet. To fill this gap, we investigated bacterial communities of the supraglacial environments of three glaciers in a rather small geographical area (max distance = 6 km) of Italian Alps.

Hence, we formulated two main hypotheses. First, if bacterial communities of supraglacial sparse debris derive, at least partly, from cryoconite holes that are known to differ among glaciers, we expect also bacterial communities of supraglacial sparse debris to differ from one glacier to another. Second, if cryoconite hole bacterial communities are released on glacier surface because of ablation, we also expect communities of supraglacial sparse debris to be more similar to those of cryoconite holes of the same glacier than to those of supraglacial sparse debris of nearby glaciers.

2. METHODS

2.1. Sampling site and samples collection

Both cryoconite and supraglacial sediment were sampled on three glaciers of the Stelvio National Park (Italy): West Zebrù (WZB), Gran Zebrù and Cedec (CED) (Fig. 1). West Zebrù is the northernmost glacier, covers 0.99 km^2 and ranges from 2816 m a.s.l. to 3268 m a.s.l. This glacier lays on a sedimentary bedrock (dolomite). Gran Zebrù is located about 3 km south from WZB, has a surface area of 0.79 km^2 and ranges from 2957 m to 3380 m a.s.l. This ice body has a particular geologic setting: its accumulation basin lies on the Zebrù tectonic line that divides sedimentary bedrocks to the North from metamorphic bedrocks to the South. Consequently, its supraglacial debris is composed of both limestone and mica schist. Moreover, this glacier is divided into two tongues by a rock ridge. In the present study, we considered samples collected on the two tongues of this glacier separately, and we called them, respectively West Gran Zebrù (WGZ) and East Gran Zebrù (EGZ). Cedec Glacier lies on a metamorphic bedrock about 3 km south-east from Gran Zebrù, covers an area of 2.07 km^2 with an altitudinal range from 2687 to 3761 m a.s.l. (Azzoni et al., 2018).

Supraglacial sparse debris samples were aseptically collected in sterile plastic bags every ~ 20 m along transects crossing the glacier tongues at approximately the same elevation (~ 3100). Cryoconite samples were aseptically collected and stored in 50 mL Falcon™ tubes in the ablation areas of each glacier. Cryoconite holes were rare on WGZ and EGZ, and sample size from these glaciers was insufficient for any statistical analyses (Tab. S1). All the samples were kept at 4 °C during transport to the laboratory (~ 8 hours), where they were stored at -20 °C.

2.2. Chemical analyses

For each sample, we estimated the pH value and the total organic carbon (TOC) content. Details on pH and TOC analyses are reported in supplementary Methods.

2.3. Molecular analyses and sequences elaboration

DNA was extracted from 0.7 g of sediment of each sample using the FastDNA Spin for Soil kit (MP Biomedicals, Solon, OH) according to the manufacturer's instructions. DNA samples and library preparation were performed as reported in Pittino et al. (2018) amplifying the V5-V6 hypervariable region of the 16S rRNA gene and sequencing was performed at "Consorzio per il centro di Biomedicina Molecolare (CBM)" (Trieste, Italy). Reads were demultiplexed according to the indexes. Sequences were grouped in Amplicon Sequence Variants (ASVs) using DADA2 [22] and classified with RDP classifier [23]. Cyanobacteria were not classified at order level because the RDP taxonomy does not report the order level for this phylum [24, 25]. When working at phylum level we therefore kept the Cyanobacteria/Chloroplast definition given by rdp. Since we removed those Cyanobacteria/Chloroplast that were classified as Chloroplast, the difference between phylum and order is attributed to the presence of Unclassified Cyanobacteria/Chloroplast, that are those that rdp is not able to distinguish and are therefore not classified at more specific taxonomic levels. Therefore, considering the phylum Cyanobacteria/Chloroplast algal ASVs were likely included, as algae are an important component in cryoconite [20].

2.4. Statistical analyses

Analyses were performed with R 3.5.1 (R Core Team, 2014) with the VEGAN, BIODIVERSITYR, MULTTEST, MULTCOMP packages. Singletons (ASVs present only once in the dataset) were removed and Hellinger distance was used to compute the distance among the samples, which depends on the differences in ASVs proportion among them, decreases the importance of ASVs over their occurrence and avoids the double-zero problem [27, 28]. Alpha-diversity was investigated calculating the Shannon diversity index, which accounts for both the richness and the evenness of the species [29], and Gini inequality index which is an index of inhomogeneity largely used in economics [30]. Redundancy analysis (RDA) and variation partitioning (VP) were used to investigate the variation of community structures. Predictors were: glacier (four-level factor), the type of sediment (either cryoconite or sparse debris, two level factor) and TOC. More details are available in Supplementary Methods.

3. RESULTS

3.1. Chemical features of the sediments

Results from chemical analyses showed that both pH and TOC varied among glaciers and between type of sediment. In each glacier, pH and TOC were strongly collinear ($r > 0.8$) except on CED ($r = 0.3$). pH values of supraglacial debris were more acidic on CED and more alkaline on WGZ while WZB and EGZ had intermediate pH values, the cryoconite was more acidic on CED than on WZB (Fig. 2a). TOC content in supraglacial debris was higher in WZB samples than in the other glaciers, which did not differ to one another. Cryoconite TOC was also higher on WZB than on CED (Fig. 2b).

Cryoconite samples were consistently more acidic than supraglacial sediment samples on both CED ($t_{16.15} = -8.78$, $p < 0.001$) and WZB ($t_{10.96} = -6.283$, $p < 0.001$) (Fig. 2a), and TOC content was always higher in cryoconite samples than in supraglacial debris (CED: $t_{11.05} = 4.635$, $p < 0.001$; WZB: $t_{11.69} = 3.464$, $p = 0.005$; Fig. 2b).

3.2. Bacterial communities

The number of sequences obtained varied from 7,458 to 113,151 per sample. The orders with more than 30,000 sequences were considered as the most abundant and were: Burkholderiales, Actinomycetales, Sphingobacteriales, Enterobacteriales, Pseudomonadales, Sphingomonadales, Cytophagales. Cyanobacteria class sequences were also > 30,000 (Fig. 3). They were analysed together with the other orders since the RDP taxonomy does not provide the classification at order level for them (see Pittino et al. (2018) for a similar approach). Abundant phyla (i.e. > 30,000 reads) were: Proteobacteria, Actinobacteria, Cyanobacteria and Bacteroidetes (Fig. 4).

3.2.1. Bacterial communities of sparse supraglacial debris

RDA on supraglacial sediment samples and post-hoc tests showed that bacterial communities differed significantly among glaciers (Tab. 1), with significant differences between each pair of glaciers ($F_{2,40} \geq 4.57$, $P_{FDR} \leq 0.025$). In addition, TOC significantly affected bacterial community structures (Tab. 1, Fig. 5a). VP showed that glacier explained 49 % of variance, while TOC accounted for 1.7 % only (Fig. 5b).

Analyses of most abundant taxa showed that their relative abundance significantly changed among glaciers ($F_{3,40} \geq 7.419$, $P_{FDR} \leq 0.002$). Burkholderiales were more abundant on CED and WGZ ($F_{3,40} = 10.301$, $P_{FDR} < 0.001$; Fig. S1a), Actinomycetales on CED and WZB and less abundant on WGZ ($F_{3,40} = 17.646$, $P_{FDR} < 0.001$;Fig. S1b), Sphingomonadales ($F_{3,40} = 18.750$, $P_{FDR} < 0.001$;Fig. 1c) and Pseudomonadales were more abundant on WGZ ($F_{3,40} = 27.211$, $P_{FDR} < 0.001$;Fig. S1d) and Cytophagales on WZB ($F_{3,40} = 7.419$, $P_{FDR} = 0.002$;Fig. S1e). Abundance of Sphingobacteriales and Enterobacteriales did not change among glaciers ($F_{3,40} \leq 1.23$, $P_{FDR} = 1$). TOC did not affect the abundance of any of the most abundant taxa ($F_{1,40} \leq 8.65$, $P_{FDR} \geq 0.11$).

GLMs performed on the most abundant phyla showed that Proteobacteria were more abundant on WGZ and CED than on the other glaciers, ($F_{3,40} = 6.32$, $P_{FDR} = 0.004$; Fig. S2a), Actinobacteria were more abundant on CED and WZB ($F_{3,40} = 6.708$, $P_{FDR} = 0.004$; Fig. S2b), and Bacteroidetes on WZB and EGZ ($F_{3,40} = 6.857$, $P_{FDR} = 0.004$; Fig. S2c). Abundance of Cyanobacteria/Chloroplast did not change significantly among glaciers ($F_{3,40} = 1.083$, $P_{FDR} = 0.765$). Cyanobacteria/Chloroplast ($F_{1,40} = 8.651$, $P_{FDR} = 0.023$) increased with TOC (Fig. S3a), while Proteobacteria ($F_{1,40} = 9.706$, $P_{FDR} = 0.023$) decreased (Fig. S3b).

3.2.2. Comparison of bacterial communities of cryoconite holes and sparse supraglacial debris

The RDA performed on the Hellinger-transformed ASV abundances of cryoconite and supraglacial sediment samples from CED and WZB showed that bacterial communities varied according to type of sediment, glacier and their interaction (Tab. 2, Fig. 6). Partial adjusted R^2 showed that the type of sample explains 45.9% of the variance, the glacier 3.4%, and their combined effect 2.8%.

Even if in the RDA biplot (Fig. 6) cryoconite samples of the two glaciers look similar, post-hoc tests revealed that they were significantly different, indeed bacterial communities resulted different both between glaciers and between type of sediment ($F_{1,39} > 2.708$, $P_{FDR} < 0.038$).

GLMs performed on the most abundant orders and phyla according to type of sediment, glacier and their interaction showed that only relative abundance of Cyanobacteria varied according to the type of sediment ($F_{1,39} = 12.199$, $P_{FDR} = 0.022$) and that they were more abundant in cryoconite samples than in supraglacial sediment samples (Fig. S4).

3.3. Alpha diversity

GLMs performed on alpha diversity indexes according to the glacier and TOC, showed that the Shannon index ($F_{3,40} = 5.020$, $P = 0.005$) and Gini index ($F_{3,40} = 7.228$, $P < 0.001$) changed among glaciers but not with TOC ($F \leq 0.950$, $P \geq 0.336$). In particular, Shannon index was higher on EGZ and WGZ and lower on CED and WZB (Fig. S5a) and Gini index was higher on CED and WZB and lower on EGZ (Fig. S5b).

GLMs on the alpha diversity indexes according to type of sediment, glacier and their interaction showed that Shannon index changed significantly according to glacier only ($F_{1,39} = 47.17$, $P < 0.001$) with higher values on the WZB (Fig. S6a). Gini index changed significantly among glaciers ($F_{1,39} = 21.822$, $P < 0.001$), with higher values on CED (Fig. S6b). In addition, it varied also with the interaction between glacier and type of sample ($F_{1,39} = 8.071$, $P = 0.007$), with higher values in both cryoconite and sparse supraglacial debris of CED and the lowest value in cryoconite of WZB (Data not shown).

4. DISCUSSION

The investigated glaciers are all within a limited geographical area (max. distance = 6 km). The Ortles-Cevedale area is geologically heterogeneous, with a close contact between sedimentary and metamorphic rocks [31]. In particular, the Zebru` Tectonic Line separates Pre-Permian mica schist and paragneiss, in the Southern area, from the Rhaetian dolomite and limestone, outcropping in the Northern part of the region [31]. The local bedrock, and consequently the debris originating from it, exhibits different colours, which has different effects on the albedo and on heat absorption [32]; metamorphic rocks are mostly dark grey, brown, or reddish brown, whereas sedimentary rocks feature a light gray to whitish colour.

CED supraglacial debris showed the lowest pH values, followed by EGZ and WZB, while WGZ had the highest values (Fig. 2a). These differences can be related to differences in the lithology of the rocks surrounding each glacier. CED is surrounded mainly by mica schist with chlorite and sericite. EGZ lays under the steep south face of Gran Zebrù mountain, which is composed of dolomite as the rocks surrounding WZB. Dolomite clasts therefore reach EGZ in large abundance (our personal observations), which may explain why pH values of EGZ supraglacial debris was similar to that of WZB. WGZ receives less dolomite clasts then EGZ, because of the rock ridge that separates them, and is surrounded by metamorphic rocks enriched in iron that gives them a red-brown colour. TOC content of supraglacial debris was higher on WZB than on all other glaciers, and showed also larger variance. Maybe carbonate debris can favour higher productivity, which may also explain the slightly higher TOC values of EGZ, whose debris is enriched in dolomite, than WGZ and CED. This is also consistent with the increase in Cyanobacteria/Chloroplast with TOC.

Generally, bacterial communities of both cryoconite from cryoconite holes and supraglacial sparse debris were dominated by Cyanobacteria, Burkholderiales, and Actinomycetales, Sphingobacteriales, Pseudomonadales, Clostridiales, Rhodospirillales, Cytophagales. These orders are typical of glacial environments and among the most abundant in cryoconite [6–8, 33]. Indeed, also in the few cryoconite samples of EGZ and WGZ we found these orders (Fig. 3).

Despite the taxonomic similarity, the structure of bacterial communities of supraglacial sparse debris differed among glaciers, and this held true also for EGZ and WGZ, which are two tongues of the same glacier. Interestingly, Figs. 3 and 5a show that bacterial communities of EGZ are more similar to those of WZB than to those of the

WGZ. Sparse debris of WZB and EGZ have similar pH values maybe because of the inputs of dolomite debris. WGZ and CED communities were also separated from those of the other glaciers and to one another. This latter difference can be explained by the difference in pH values among glaciers. Indeed, the first RDA axis seems to represent a gradient of the pH values of supraglacial debris of our glaciers (Fig. 5a), which, unfortunately, we could not include directly in the analysis because of its strong collinearity with TOC. In contrast, the second axis seems to separate glaciers with and without carbonatic inputs. WGZ, EGZ and WZB communities in the RDA are also along the TOC gradient of the glaciers, while CED ones seem to deviate. Indeed, CED supraglacial debris showed the lowest values of alpha diversity, the highest evenness (Fig. S5), and the highest relative abundances of Actinomycetales, Burkholderiales and Pseudomonadales. In contrast, WZB showed both low alpha diversity values and high relative abundance of Actinomycetales and Cytophagales. These orders are chemorganotrophic bacteria and their presence is consistent with the high TOC values present on this glacier that decrease also the presence of metals because of its sorption capacities, decreasing metals bioavailability for lithotrophic bacteria [34, 35]. Consistently, Acidimicrobiales, that are chemolithotrophic, were more abundant on the WGZ that had very low TOC values, and consequently more bioavailable metals [35]. WGZ showed also higher alpha diversity than the other glaciers, with abundant Sphingobacteriales and Burkholderiales that may have been favoured by the alkaline pH.

TOC was significantly related to bacterial community variation of supraglacial debris, but VP analysis showed that it explained 1.7% of variance only (Tab. 1). TOC is known to affect bacterial communities in soils of different environments [36–38]. However, the actual effects of TOC on bacterial communities of the supraglacial sparse debris are unknown. In our study, it had a significant effect on Cyanobacteria/Chloroplast and Proteobacteria only. In contrast, differences among glaciers accounted for 49% of observed variability in bacterial communities, which strongly suggests that, even within a small geographical area, bacterial communities are glacier-dependent.

The fact that Cyanobacteria/Chloroplast abundance is positively correlated with TOC might be due to their autotrophic activity that is important for cryoconite grains production [5]. Indeed, organic carbon in supraglacial environments can have three main sources: aeolian transport, the wash-away of supraglacial debris and biological carbon transformation mostly in cryoconite holes played by Cyanobacteria [39]. Furthermore, only a small fraction of organic carbon is consumed by the heterotrophic community, explaining the increase and accumulation of TOC in parallel with the high relative abundance of Cyanobacteria/Chloroplast [40].

Proteobacteria are a phylum characteristic of soils with a wide variety of metabolisms involved in the global carbon, nitrogen and sulfur cycles [41]. This phylum is one of the most abundant in many studies of soils in extreme environments [8, 42–46] and also as a pioneer phylum [47]. Their decrease with the increase in TOC is difficult to explain since this phylum is composed by a very heterogeneous group of bacteria, making hard to find an ecological explanation of their correlation with TOC [41].

These results show that the structure of bacterial communities of supraglacial sediment changes from one glacier to another. This is consistent with the results reported by Sommers et al. [3], which showed that the source of the sediment plays an important role in shaping supraglacial bacterial communities. This may occur because the sediment source determines its mineralogical and chemical composition, which, in turn, affect the structure of the bacterial communities that develop in cryoconite holes and supraglacial sediment.

Our results also corroborate the lack of a decay-by-distance pattern previously reported in other studies[1], consistently with the fact that other factors, likely edaphic variables, are mostly responsible for bacterial communities' composition in soils [1, 48].

The second aim of this study was to compare cryoconite collected in cryoconite holes and sparse debris. The low number of cryoconite holes on EGZ and WGZ allowed this comparison on CED and WZB only. Cryoconite collected in cryoconite holes showed lower pH and higher TOC values than the sparse debris of the same glacier. In addition, both CED cryoconite and sparse debris were more acidic than WZB ones, consistently with the different lithology of the surrounding rocks.

RDA showed that cryoconite communities from the two glaciers are closer to one another, while sparse debris communities look more different (Fig. 6), even if this was not supported by the results of post-hoc tests. The effect of the type of sediment (either cryoconite from cryoconite holes or sparse debris) was predominant (45.9% of variance explained) with respect to that of glacier (3.4%). This suggests that cryoconite holes provide a peculiar microhabitat different from the sparse supraglacial debris. Liquid water in cryoconite holes provides protection

from harsh wind, extremely cold temperatures and high UV radiation that characterize the glacier environment [49]. These conditions allow the establishment and development of bacterial communities typical of these microhabitats, which increase the organic carbon content of the sediment and decrease pH. This may explain the similarity between bacterial communities of cryoconite holes of these nearby glaciers, which however largely differ for the lithology of the surrounding mountains. Furthermore, the fact that bacterial communities of cryoconite holes of different glaciers are more similar than those of the sparse debris, brings support to the hypothesis that these communities (the cryoconite holes ones) inhabit a microhabitat that select similar taxa and brings the communities to a climax situation. This hypothesis was already supposed in Pittino et al. [8], where appears that in the late ablation season communities are more similar than in the early stages.

The higher alpha diversity and the higher evenness of cryoconite than sparse debris observed on WZB is consistent with this hypothesis. However, on CED, the opposite pattern was found. A possible explanation is that on this glacier cryoconite biodiversity is lower because of the extreme low pH of cryoconite samples (< 6). Alpha diversity was also generally higher on WZB than on CED. However, on CED alpha diversity was higher in the supraglacial debris, while on WZB it was higher in cryoconite (Fig. S6). Only Cyanobacteria abundance changed between the two type of samples, and they were more abundant in cryoconite samples (Fig. S4), consistently with the general assumption that this phylum is typical of cryoconite holes and a keystone taxon on these environments [5].

To the best of our knowledge, no studies so far compared bacterial communities between cryoconite holes and supraglacial sparse debris. Stibal et al. (2006) compared bacterial communities of cryoconite holes with those of supraglacial kames (characterized by a thicker sediment) and concluded that cryoconite is a more suitable environment for bacteria [20].

Our results are only partly consistent with those of the above mentioned study because we did not find a generally higher biodiversity in cryoconite holes than in sparse debris, but opposite patterns in the two glaciers. However, the debris considered by Stibal and colleagues was rather different than the sparse supraglacial debris we investigated in this study [20]. In addition, we found similar communities in the cryoconite holes of the two glaciers, and both communities differed from those of the supraglacial debris of the respective glacier, which also differed to one another. This is only partly consistent with our hypothesis that cryoconite released from cryoconite holes dismantled by ablation contributes to the bacterial communities of sparse debris, because a stronger similarity among cryoconite and sparse debris bacterial communities was expected in that case. Supraglacial sparse debris seems therefore to host more diverse bacterial communities, largely different from glacier to glacier, also within a small geographic area. These communities may derive partly from long-range transport, but also from the surrounding environments, and therefore be affected by the lithology of the glacier bedrock and of the surroundings, which affects sediment glacier lithology. On the other side, cryoconite holes seem to favour typical communities that benefit from the presence more protected conditions.

We can conclude that bacterial communities of the supraglacial sparse debris seem strongly variable and linked to the characteristic of the surrounding environment, including lithology. Cryoconite and supraglacial sparse debris host different bacterial communities. In particular, cryoconite samples, even from different glaciers, host bacterial communities that are more similar to one another than those of sparse debris, likely because of the peculiar feature of cryoconite hole microhabitat. Our hypothesis that bacterial communities of cryoconite holes may derive from those of the sparse supraglacial debris and vice versa found therefore little support. We can confirm that different glaciers host different bacterial communities in their supraglacial debris. Our results suggest that supraglacial sparse debris can host an even larger biodiversity than cryoconite holes. In a period of global warming, when glaciers are quickly disappearing, we may therefore be losing a larger amount of biodiversity than previously considered.

Funding The research was partially funded by University of Milano Bicocca (PhD fellowship to FP)

Conflicts of interest/Competing interests The authors have declared no conflicts of interest for this article.

Availability of data and material All data are available upon request.

References

1. Edwards A, Anesio AM, Rassner SM, et al (2011) Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. ISME J 5:150–160. https://doi.org/10.1038/ismej.2010.100

2. Franzetti A, Navarra F, Tagliaferri I, et al (2017) Potential sources of bacteria colonizing the cryoconite of an Alpine glacier. PLoS One 12:1–13. https://doi.org/10.1371/journal.pone.0174786

3. Sommers P, Darcy JL, Gendron EMS, et al (2018) Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes. FEMS Microbiol Ecol 94:1–11. https://doi.org/10.1093/femsec/fix167

4. Cook J, Edwards A, Takeuchi N, Irvine-Fynn T (2016) Cryoconite: The dark biological secret of the cryosphere. Prog Phys Geogr 40:66–111. https://doi.org/10.1177/0309133315616574

5. Takeuchi N, Nishiyama H, Li Z (2010) Structure and formation process of cryoconite granules on ürümqi glacier No. 1, Tien Shan, China. Ann Glaciol 51:9–14. https://doi.org/10.3189/172756411795932010

6. Franzetti A, Navarra F, Tagliaferri I, et al (2017) Temporal variability of bacterial communities in cryoconite on an alpine glacier. Environ Microbiol Rep 9:71–78. https://doi.org/10.1111/1758-2229.12499

7. Edwards A, Debbonaire AR, Sattler B, et al (2016) Extreme metagenomics using nanopore DNA sequencing: a field report from Svalbard, 78 N. DoiOrg 073965. https://doi.org/10.1101/073965

8. Pittino F, Maglio M, Gandolfi I, et al (2018) Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. 59:1–9. https://doi.org/10.1017/aog.2018.16

9. Mueller DR, Pollard WH (2004) Gradient analysis of cryoconite ecosystems from two polar glaciers. Polar Biol 27:66–74. https://doi.org/10.1007/s00300-003-0580-2

10. Takeuchi N, Kohshima S, Seko K (2010) Structure , Formation , and Darkening Process of Albedo-Reducing Material (Cryoconite) on a Himalayan Gl. Arctic, Antarct Alp Res 33:115–122. https://doi.org/10.1080/15230430.2001.12003413

11. Mindl B, Anesio AM, Meirer K, et al (2007) Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacieri. FEMS Microbiol Ecol 59:307–317. https://doi.org/10.1111/j.1574-6941.2006.00262.x

12. Grzesiak J, Górniak D, Świątecki A, et al (2015) Microbial community development on the surface of Hans and Werenskiold Glaciers (Svalbard, Arctic): a comparison. Extremophiles 19:885–897. https://doi.org/10.1007/s00792-015-0764-z

13. Zarsky JD, Stibal M, Hodson A, et al (2013) Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea. Environ Res Lett 8:. https://doi.org/10.1088/1748-9326/8/3/035044

14. Darcy JL, Gendron EMS, Sommers P, et al (2018) Island biogeography of cryoconite hole bacteria in Antarctica's Taylor Valley and around the world. Front Ecol Evol 6:1–9. https://doi.org/10.3389/fevo.2018.00180

15. Ambrosini R, Musitelli F, Navarra F, et al (2017) Diversity and Assembling Processes of Bacterial Communities in Cryoconite Holes of a Karakoram Glacier. Microb Ecol 73:827–837. https://doi.org/10.1007/s00248-016-0914-6

16. Sommers P, Darcy JL, Gendron EMS, et al (2018) Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes. FEMS Microbiol Ecol 94:. https://doi.org/10.1093/femsec/fix167

17. Ambrosini R, Musitelli F, Navarra F, et al (2017) Diversity and Assembling Processes of Bacterial Communities in Cryoconite Holes of a Karakoram Glacier. Microb Ecol 73:827–837. https://doi.org/10.1007/s00248-016-0914-6

18. Darcy JL, King AJ, Gendron EMS, Schmidt SK (2017) Spatial autocorrelation of microbial communities atop a debris-covered glacier is evidence of a supraglacial chronosequence. FEMS Microbiol Ecol 93:1–26. https://doi.org/10.1093/femsec/fix095

19. Franzetti A, Tatangelo V, Gandolfi I, et al (2013) Bacterial community structure on two alpine debris-covered glaciers and biogeography of Polaromonas phylotypes. ISME J 7:1483–1492. https://doi.org/10.1038/ismej.2013.48

20. Stibal M, Šabacká M, Kaštovská K (2006) Microbial communities on glacier surfaces in Svalbard: Impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb Ecol 52:644–654. https://doi.org/10.1007/s00248-006-9083-3

21. Swithinbank C (1950) The origin of dirt cones on glaciers. J Glaciol 1:461–465. https://doi.org/461-465. doi:10.3189/S0022143000012880

22. Callahan BJ, Mcmurdie PJ, Rosen MJ, et al (2016) DADA2 : High-resolution sample inference from Illumina amplicon data. 13:. https://doi.org/10.1038/nmeth.3869

23. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07

24. Garrity GM, Lilburn TG, Cole JR, et al (2007) Taxonomic Outline of the Bacteria and Archaea (Formerly the Taxonomic Outline of the Prokaryotes). 7:

25. Wilmotte A, Herdman M (2015) the Cyanobacteria Based on 16S rRNA Sequences. https://doi.org/10.1002/9781118960608.cbm00021.

26. Team RC (2014) R: A language and environment for statistical computing, Vienna. R Found Stat Comput Vienna http//wwwR-project.org/

27. De Cáceres M, Legendre P, Moretti M (2010) Improving indicator species analysis by combining groups of sites. Oikos 119:1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

28. Legendre P, Gallagher DE (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280. https://doi.org/10.1007/s004420100716 Pierre

29. Shannon CE (1948) A mathematical theory of communication. Bell Syst Tech J 27:379–423. https://doi.org/10.1145/584091.584093

30. Gini C (1912) Variabilità e mutabilità. Contributo allo studio delle distribuzioni e delle relazioni statistiche. Paolo Cuppini, Bologna

31. Montrasio A, Berra F, Cariboni M, et al (2008) Note illustrative della Carta Geologica d'Italia: foglio 024. Bormio, ISPRA, Serv Geol d'Italia, Roma

32. Hall K, Lindgren BS, Jackson P (2005) Rock albedo and monitoring of thermal conditions in respect of weathering: some expected and some unexpected results. Earth Surf Process Landforms 30:801–811. https://doi.org/https://doi.org/10.1002/esp.1189

33. Sommers P, Darcy JL, Porazinska DL, et al (2019) Comparison of microbial communities in the sediments and water columns of frozen cryoconite holes in the McMurdo Dry Valleys, Antarctica. Front Microbiol 10:1–10. https://doi.org/10.3389/fmicb.2019.00065

34. Reith F, Brugger J, Zammit CM, et al (2012) Influence of geogenic factors on microbial communities in metallogenic Australian soils. 2107–2118. https://doi.org/10.1038/ismej.2012.48

35. Risueno Y, Petri C, Conesa HM (2020) The importance of edaphic niches functionality for the sustainability of phytomanagement in semiarid mining impacted ecosystems. J Environ Manage 266:. https://doi.org/10.1016/j.jenvman.2020.110613

36. Banerjee S, Baah-acheamfour M, Carlyle CN, et al (2016) Determinants of bacterial communities in Canadian agroforestry systems. Environ Microbiol 18:1805–1816. https://doi.org/10.1111/1462-2920.12986

37. Zeng J, Liu X, Song L, et al (2016) Nitrogen fertilization directly affects soil bacterial diversity and

indirectly affects bacterial community composition. Soil Biol Biochem 92:41–49. https://doi.org/10.1016/j.soilbio.2015.09.018

38. Song H, Li Z, Du B, et al (2011) Bacterial communities in sediments of the shallow Lake Dongping in China. J Appl Microbiolgy 112:79–89. https://doi.org/10.1111/j.1365-2672.2011.05187.x

39. Stibal M, Lawson EC, Lis GP, et al (2010) Organic matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet. Ann Glaciol 51:1–8. https://doi.org/DOI: 10.3189/172756411795931958

40. Anesio AM, Sattler B, Foreman C, et al (2010) Carbon fluxes through bacterial communities on glacier surfaces. 51:32–40. https://doi.org/https://doi.org/10.3189/172756411795932092

41. Spain AM, Krumholz LR, Elshahed MS (2009) Abundance , composition , diversity and novelty of soil Proteobacteria. ISME J 3:992–1000. https://doi.org/10.1038/ismej.2009.43

42. Connon SA, Lester ED, Shafaat HS, et al (2007) Bacterial diversity in hyperarid Atacama Desert soils. J Geophys Res 112:1–9. https://doi.org/10.1029/2006JG000311

43. Bastida F, Torres IF, Moreno JL, et al (2016) The active microbial diversity drives ecosystem multifunctionality and is physiologically related to carbon availability in Mediterranean semi-arid soils. Mol Ecol 25:4660–4673. https://doi.org/10.1111/mec.13783

44. Nogales B, Moore ERB, Abraham W, Timmis KN (1999) Identification of the metabolically active members of a bacterial community in a polychlorinated biphenyl- polluted moorland soil. Environ Microbiol 1:199–212. https://doi.org/10.1046/j.1462-2920.1999.00024.x

45. Azzoni RS, Tagliaferri I, Franzetti A, et al (2018) Bacterial diversity in snow from mid-latitude mountain areas : Alps , Eastern Anatolia , Karakoram and Himalaya. 1–11. https://doi.org/10.1017/aog.2018.18

46. Edwards A, Pachebat JA, Swain M, et al (2013) A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. Environ Res Lett 8:. https://doi.org/10.1088/1748-9326/8/3/035003

47. Esposito A, Ciccazzo S, Borruso L, et al (2013) A Three-Scale Analysis of Bacterial Communities Involved in Rocks Colonization and Soil Formation in High Mountain Environments. Curr Microbiol 67:472–479. https://doi.org/10.1007/s00284-013-0391-9

48. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631. https://doi.org/10.1073/pnas.0507535103

49. Takeuchi N, Kohshima S, Yoshimura Y, et al (2000) Characteristics of cryoconite holes on a Himalayan glacier, Yala Glacier Central Nepal. Bull Glaciol Res 17:51–59

Tables

Table 1 RDA of Hellinger-transformed bacterial ASVs abundances of supraglacial sediment samples according to the glacier and TOC.

Variable	df	Variance	F	P
Glacier	3	0.207	14.974	< 0.001
TOC	1	0.011	2.394	0.018
Residuals	40	0.184		
$F_{4,40}$ = 11.829, P < 0.001, Adjusted R^2 = 0.496				

Table 2 RDA of Hellinger-transformed bacterial ASV abundances of both cryoconite and supraglacial sediment samples of Cedec and Zebrù Est according to the glacier, type of sample and their interaction.

Variable	Df	Variance	F	P	Partial Adjusted R^2
Glacier	1	0.014	3.684	< 0.001	0.034
Type	1	0.137	36.992	< 0.001	0.460
Glacier × Type	1	0.012	3.172	< 0.001	0.028
Residuals	39	0.144			
$F_{3,39}$ = 14.42, P < 0.001, Adjusted R^2 = 0.489					

Figures caption

Figure 1 Location map of the studied glaciers with 2015 glacier limits (Stelvio Park, Ortles-Cevedale Group, Lombardy Sector, Italian Alps). The yellow line indicates the Zebru` Tectonic Line that divides sedimentary bedrocks in the North from metamorphic bedrocks in the South.

Figure 2 Boxplots representing pH (a) and TOC (b) of supraglacial sediment on WGZ (brown), EGZ (orange), WZB (blue) and CED (black), and of cryoconite of WZB (blue) and CED (black). The thick lines represent the median, boxes upper and lower limits the 25th and the 75th percentiles respectively, whiskers the data that go beyond the 5th percentile (lower whisker) and the 75th percentile (upper whisker), dots represent the outliers and different letters indicate significant differences (P < 0.05) between the mean values of different groups at post hoc-tests.

Figure 3 Barplot showing the relative abundance of the orders in each glacier in cryoconite and in supraglacial sediment. Orders less abundant than 1 % were grouped in others. Cyanobacteria *are reported among the orders since rdp does not provide the classification at order level for them. Cryoconite samples from GZ were included here but not in statistical analyses because of the low number of samples.*

Figure 4 Barplot showing the relative abundance of the phyla in all the glaciers in cryoconite and supraglacial sediment. Phyla less abundant than 1 % were grouped in others. Cryoconite samples from GZ were included here but not in statistical analyses because of the low number of samples.

*Figure 5 (a) Biplot from RDA on Hellinger-transformed bacterial ASVs abundances in the supraglacial sediment of glacier and TOC. The percentage of variance explained by each axis and their significance (***: P < 0.001) is reported. (b) Results from the Variation Partitioning (VP) showing the amount of variance explained by each predictor of the RDA. The joint contribution of glacier and TOC was null.*

*Figure 6 Biplot from RDA on Hellinger-transformed bacterial ASV abundances on glacier and type of sample. The percentage of variance explained by each axis and their significance (***:P < 0.001) is reported.*

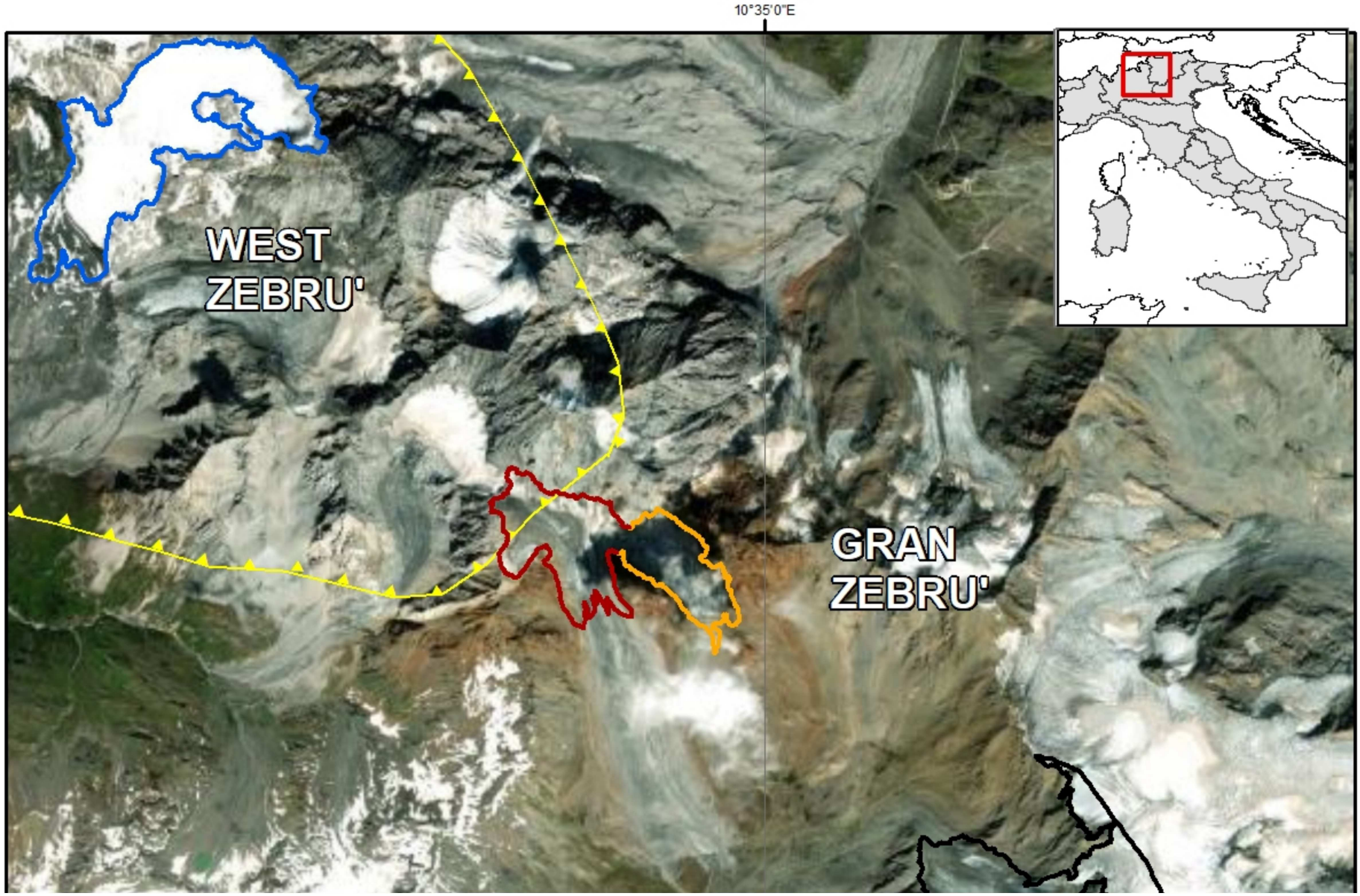
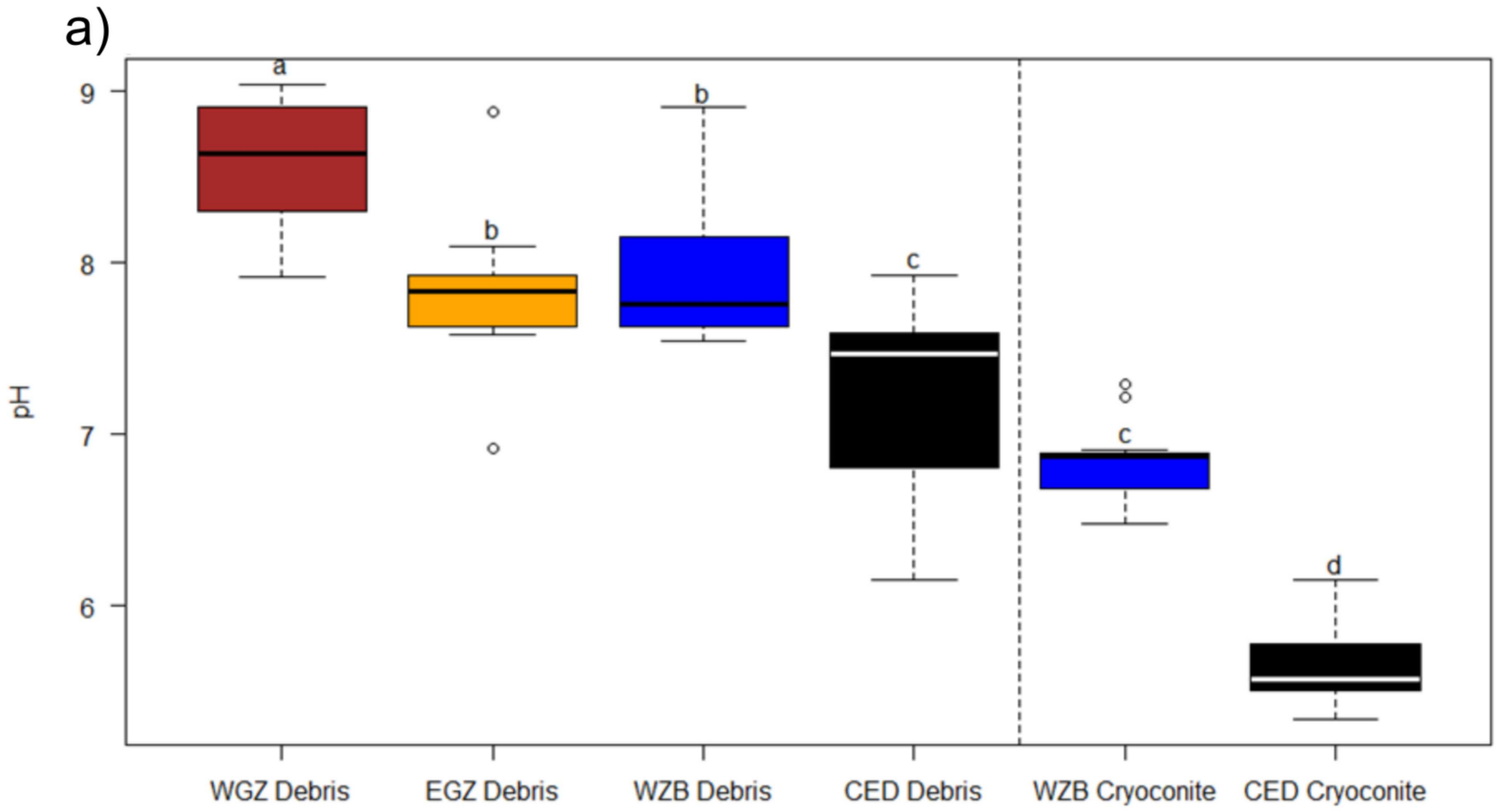


Figure 1

Figure 2


a)
pH
9
8
7
6
a
b
b
c
c
d
WGZ Debris
EGZ Debris
WZB Debris
CED Debris
WZB Cryoconite
CED Cryoconite

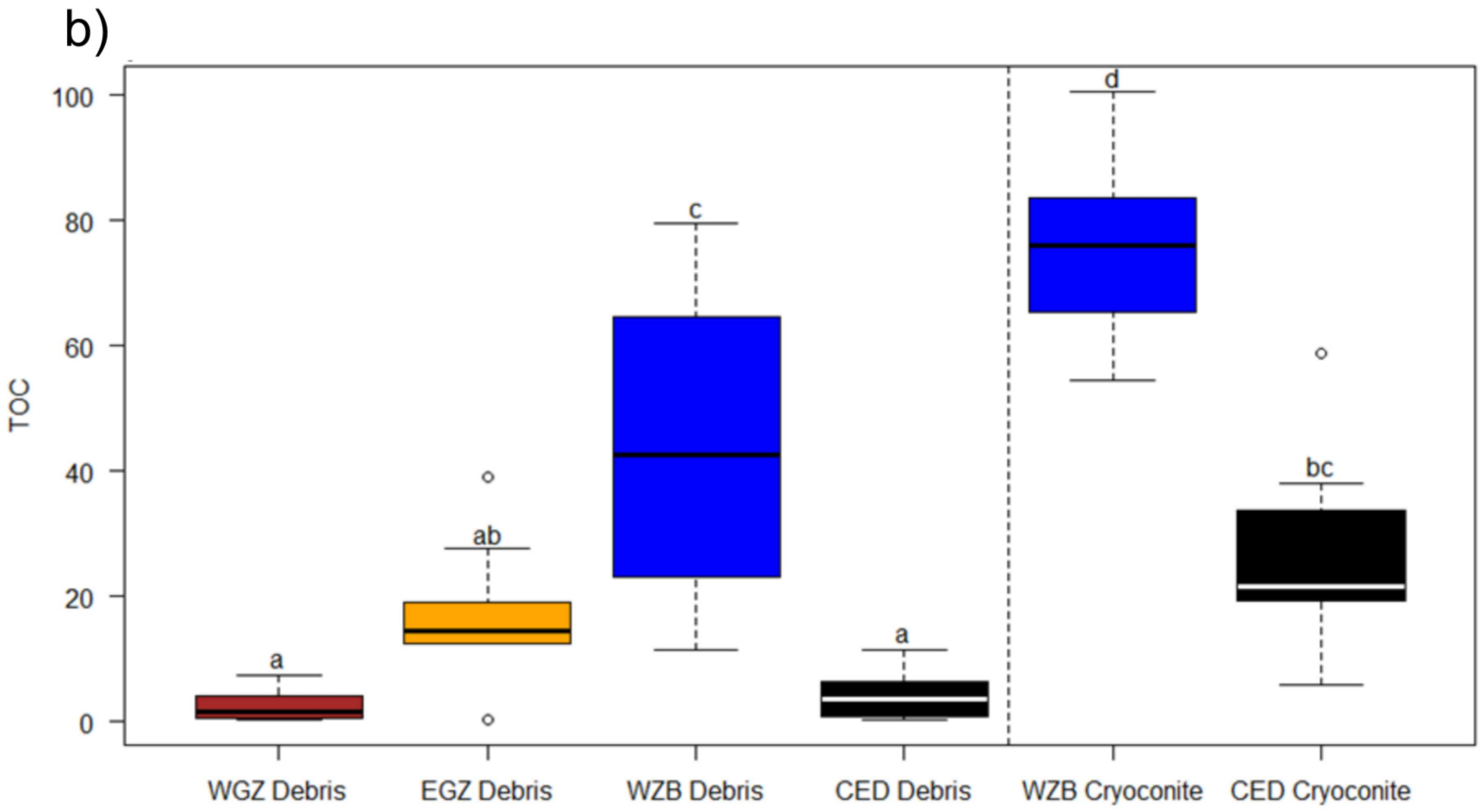

b)
TOC
100
80
60
40
20
0
a
ab
c
a
d
bc
WGZ Debris
EGZ Debris
WZB Debris
CED Debris
WZB Cryoconite
CED Cryoconite

Figure 3

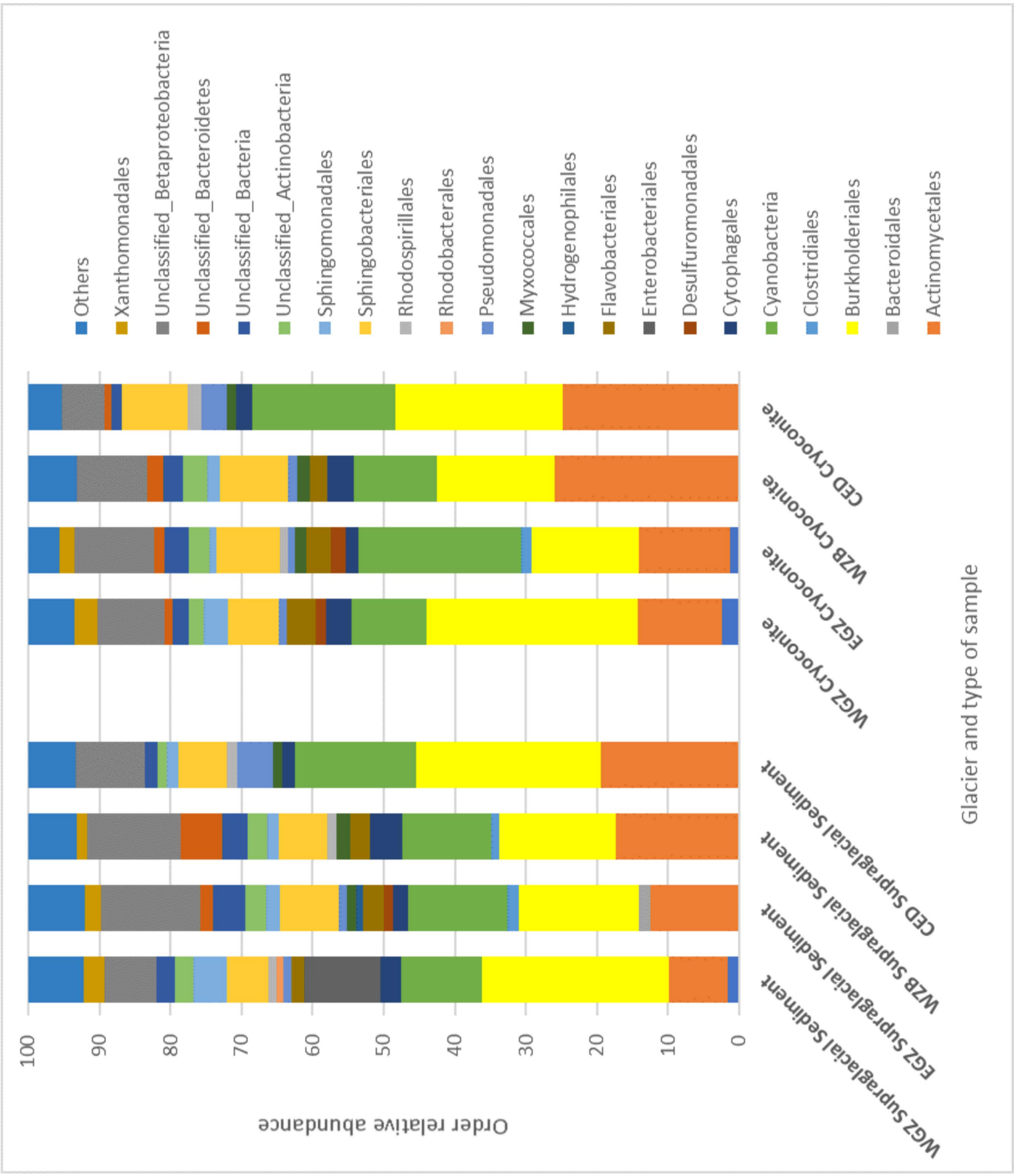

Others
Xanthomonadales
Unclassified_Betaproteobacteria
Unclassified_Bacteroidetes
Unclassified_Bacteria
Unclassified_Actinobacteria
Sphingomonadales
Sphingobacteriales
Rhodospirillales
Rhodobacterales
Pseudomonadales
Myxococcales
Hydrogenophilales
Flavobacteriales
Enterobacteriales
Desulfuromonadales
Cytophagales
Cyanobacteria
Clostridiales
Burkholderiales
Bacteroidales
Actinomycetales
Order relative abundance
0
10
20
30
40
50
60
70
80
90
100
WGZ Supraglacial Sediment
EGZ Supraglacial Sediment
WZB Supraglacial Sediment
CED Supraglacial Sediment
WGZ Cryoconite
EGZ Cryoconite
WZB Cryoconite
CED Cryoconite
Glacier and type of sample

Figure 4

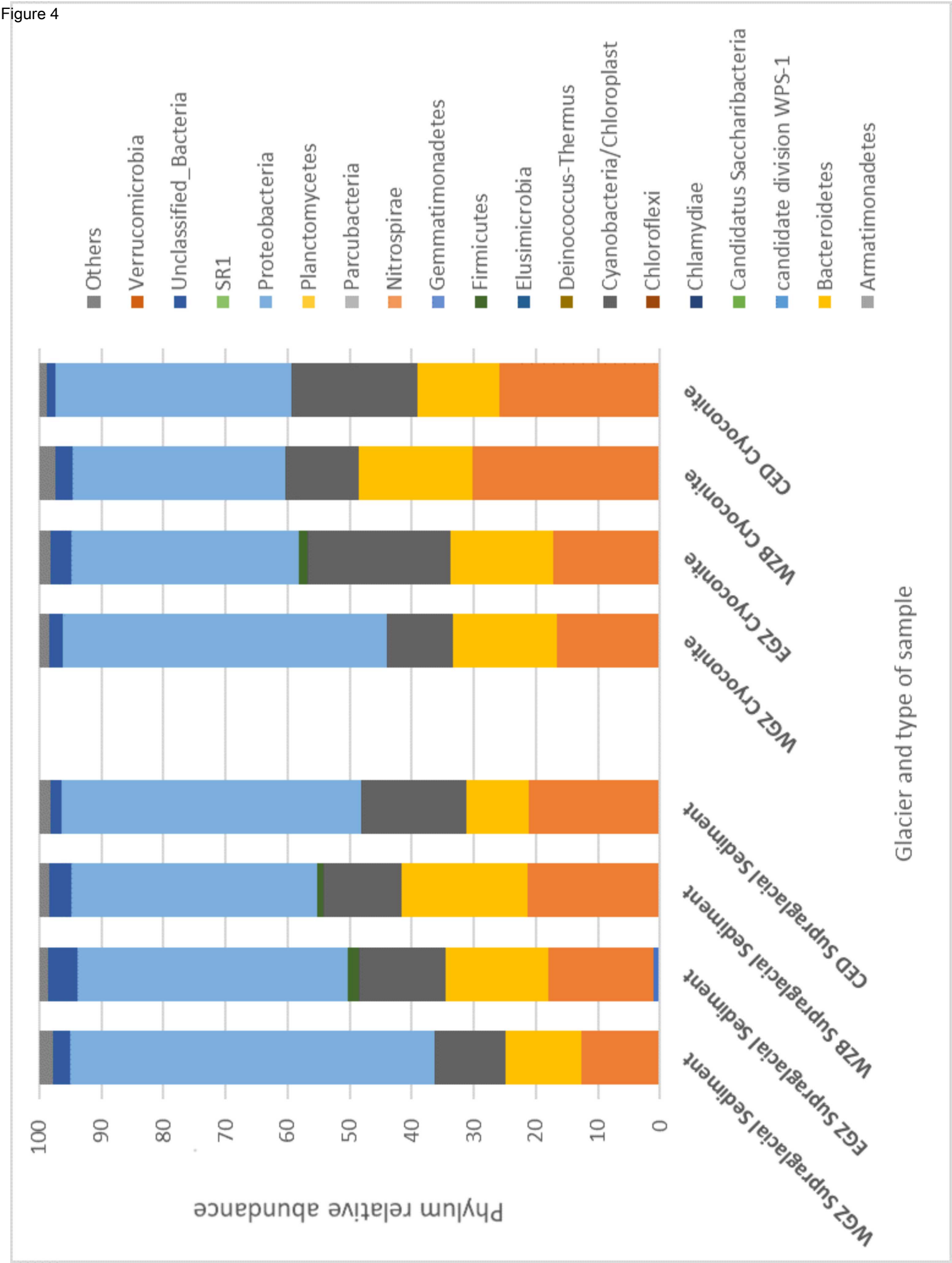
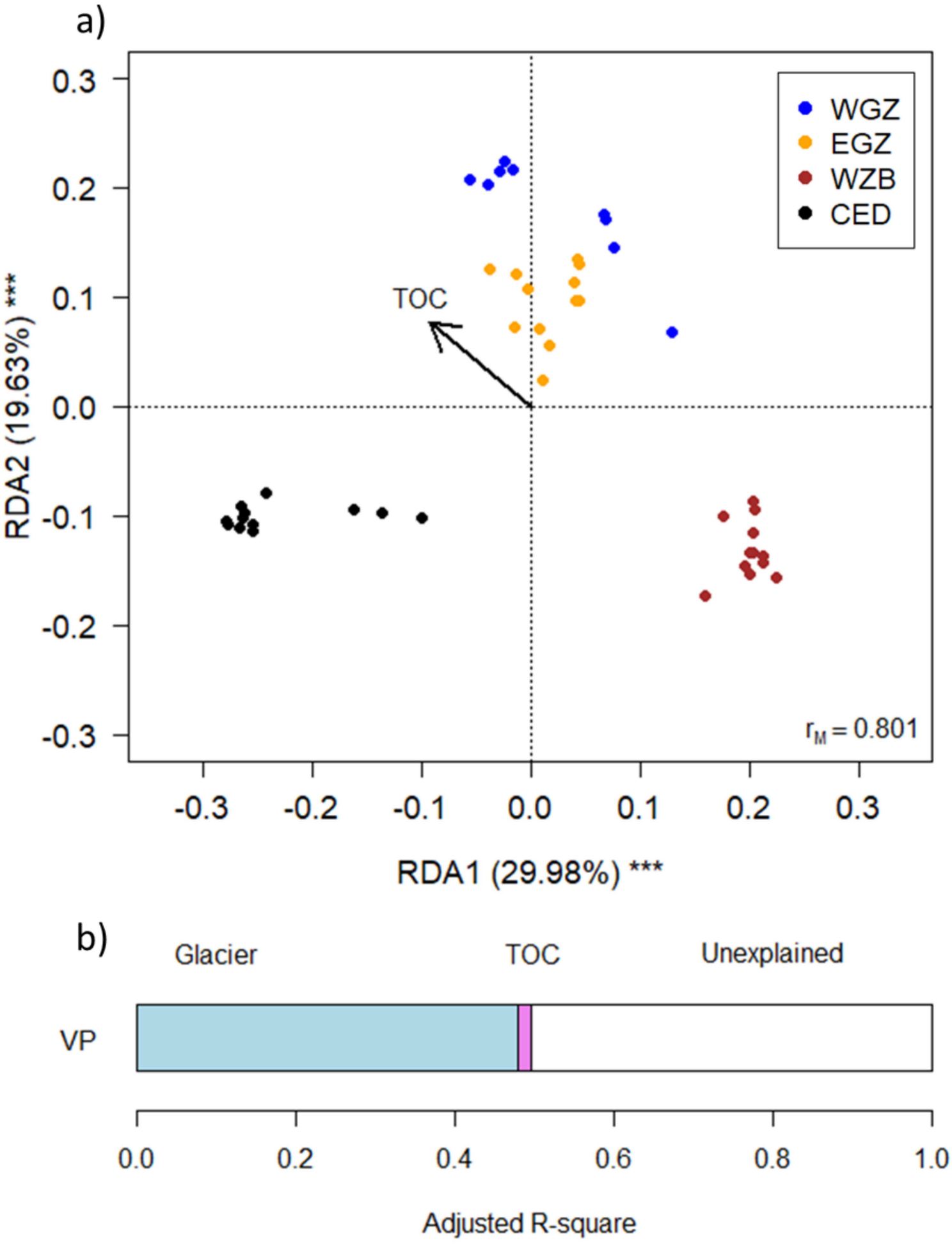

Others
Verrucomicrobia
Unclassified_Bacteria
SR1
Proteobacteria
Planctomycetes
Parcubacteria
Nitrospirae
Gemmatimonadetes
Firmicutes
Elusimicrobia
Deinococcus-Thermus
Cyanobacteria/Chloroplast
Chloroflexi
Chlamydiae
Candidatus Saccharibacteria
candidate division WPS-1
Bacteroidetes
Armatimonadetes
100
90
80
70
60
50
40
30
20
10
0
Phylum relative abundance
WGZ Supraglacial Sediment
EGZ Supraglacial Sediment
WZB Supraglacial Sediment
CED Supraglacial Sediment
WGZ Cryoconite
EGZ Cryoconite
WZB Cryoconite
CED Cryoconite
Glacier and type of sample

Figure 5


a)
WGZ
EGZ
WZB
CED
TOC
$r_M = 0.801$
RDA2 (19.63%) ***
RDA1 (29.98%) ***
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
b)
Glacier
TOC
Unexplained
VP
0.0
0.2
0.4
0.6
0.8
1.0
Adjusted R-square