MICROBIOLOGY

Metaproteomic comparison of cryoconite communities from Caucasian and Novaya Zemlya glaciers

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Abstract

Small cylindrical holes (cryoconites) appear in the early stages of glacier melt. While the microbiome of cryoconite is well described, differences in community compositions between various glaciers, especially between more or less polluted ones, still have not been fully understood. Here we performed the comparison of the cryoconite communities from the less polluted Caucasian (Kabardino-Balkaria) glaciers and from highly polluted glaciers in Novaya Zemlya by the shotgun metaproteomics approach which had not been used for cryoconite microbiome analysis previously. Metaproteomics has a whole range of limitations, but it is of great interest because it gains information about the functional state of communities. We identified 475 protein groups, a third of which were found in both glaciers. Proteins from Cyanobacteria dominated in both sites, but we found a slight shift to heterotrophic bacteria in the Caucasus.

Keywords: cryoconite, metaproteomics, glacial, anthropogenic pollution, TimsToF Pro

Introduction

Glaciers are considered to be the benchmark of airborne anthropogenic pollution (Law and Stohl, 2007; Ji et al., 2019). Anthropogenic pollution may cause a darkening of the ice surface which increases solar energy absorption, and, therefore, snow and ice melting (Kang, Zhang, Qian and Wang, 2020). The early stage of glacial melting is the formation of cryoconites — small cylindrical holes in the ablation zone of the glacier surface.

In addition to small rocks and inorganic particles, cryoconites also contain specific microbial communities consisting of algae, bacteria, fungi, and rotifers (Zawierucha, Kolicka, Takeuchi and Kaczmarek, 2015). Cryoconite holes as a microbiological habitat were found in glaciers around the world, including polar (Arctic and Antarctic) and temperate (alpine) ice (Wharton, McKay, Simmons and Parker, 1985). Microbial growth increases the deposition of dark organic matter, causing additional snowmelt (Musilova et al., 2016). Anthropogenic pollution might cause some functional changes in the structure of cryoconite communities which enhance deposition of dark organic matter.

Metaproteomics is aimed to analyze proteins in the environmental sources (for example, soil, fresh and seawater, bottom sediments, human intestines, bioengineering systems, etc.). Studying the total protein composition of a community

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of organisms provides an understanding of the functional biodiversity of microbial communities and the functional interactions between microbes and their hosts or the environment (Wilmes, Heintz-Buschart and Bond, 2015). In some experimental designs metaproteomic analysis has a greater potential than metagenomics as it gains functional information. Nevertheless, it has many limitations, e. g. dependencies of the protein identification from the database, difficulties in discrimination of homological proteins, loss of information about less abundant proteins which is typical of the most informative ones, etc.

The aim of our study is a functional comparison of the cryoconite community in two glaciers with different pollution levels. Therefore, we applied metaproteomics approach to compare microbial cryoconite communities from Novaya Zemlya (Mushketova glacier with anthropogenic pollution; N 79°05'46", E 101°51'25.35") and in Kabardino-Balkaria (Garabashi less polluted glacier; N 43°18'18", E 42°27'49") alongside with geochemical analysis of heavy metals level in the sampling sites.

Material and methods

Sample Collection

The samples were scraped off from the cryoconite holes form the depth of 0–20 cm. The cryoconites consisted of skeletal fraction (up to 25 %) and fine earth (about 75 %) in both sampling sites. The pH was about 6.5–6.9, total organic carbon content was 0.05–0.25 %. Samples were frozen on-site and transported in frozen state to the laboratory in St Petersburg. Collection of all samples was performed in the September of 2020. We had five samples for Kabardino-Balkaria and six for Novaya Zemlya. Sample sites with coordinates are given in the supplementary materials 1.

Heavy metal measurement

To evaluate the level of anthropogenic pollution we measured the contents of heavy metals quantitatively determined by X-ray fluorescence (FR.1.31.2018.32143. Determination of elements and oxides of elements in soil and bottom sediment samples) using a Spectroscan Max-G spectrometer.

The samples were ground in a disk grinder to a particle size of ≤71 μm and air-dried (clauses 5.1–5.5, State Standard ISO 11464-2015). Then, they were pressed into a cup of boric acid. For this, we poured boric acid into a mold and formed a cup (at least 3 mm deep) with a figured punch. The sample was then poured into the cup and pressed with a smooth punch and a press.

The tablet was placed into the sample holder and then into the spectrometer. The samples were automatically analyzed to determine the total contents of 11 elements (oxides): strontium, lead, arsenic, zinc, nickel, cobalt, vanadium, chromium, iron oxide (III), manganese oxide, and titanium dioxide.

The obtained data were analyzed in GraphPad Prizm software. The level of heavy metals was compared in two sites by T-test with correction for multiple comparisons. As a result, we revealed that the Caucasus glacier is much less polluted with heavy metals compared to the Novaya Zemlya glacier (Fig. 1a).

Protein extraction

The scheme of protein extraction procedures is represented in Fig. 1b. 1g of each sample was transferred to protein LoBind Eppendorf tube (2 ml) and mixed with 1 ml of 1 % SDS solution. The samples were homogenized in a mixer mill Retsch MM 400 for 25 min at 30 Hz. Then the samples were incubated in an ultrasonic bath for 10 min at 80 °C and were centrifugated at 15,000 g for 15 min. The samples were then transferred to a new tube and centrifuged again until there was no visible pellet.

The protein was precipitated from the SDS solution by four volumes of cold acetone followed by incubation in –20 °C for one hour and centrifugation at 15000 g for 15 min with 4 °C. Protein pellet was washed by cold acetone and air dried for 5–10 min. Then it was resuspended in approximate six volumes of resuspension buffer (8M urea / 50 mM ammonium bicarbonate; Sigma). The protein concentration was measured by Qubit 4 fluorometer (Invitrogen) by QuDye Protein Quantification Kit (Lumiprobe). 10 ug of each sample was used for further tryptic digestion.

In-solution digestion

Tryptic digestion was performed by a standard "in-solution" procedure. The samples were incubated for 1 hour at 37 °C with 5 mM DTT (Sigma Aldrich) with subsequent incubation in 15 mM iodoacetamide for 30 minutes in the dark at RT (Sigma Aldrich). Next, the samples were diluted with seven volumes of 50 mM ammonium bicarbonate and incubated for 16 hours at 37 °C with 200 ng of Trypsin Gold (ratio 1:50; Promega, Madison, WI, USA). Then the samples were mixed with formic acid (Sigma Aldrich) to 1 % final concentration, evaporated in Labconco Centrivap Centrifugal Concentrator, desalted with C18 ZipTip (Millipore) according to manufacturer recommendations, and evaporated in Labconco Centrivap Centrifugal Concentrator. Desalted peptides were dissolved in 15 ul of water / 0.1 % formic acid for further LC-MS/MS analysis.

LC-MS/MS analysis

Shotgun proteomics analysis was performed in nano LC-MS/MS with trapped ion mobility spectrometry on Bruker TimsToF Pro instrument.

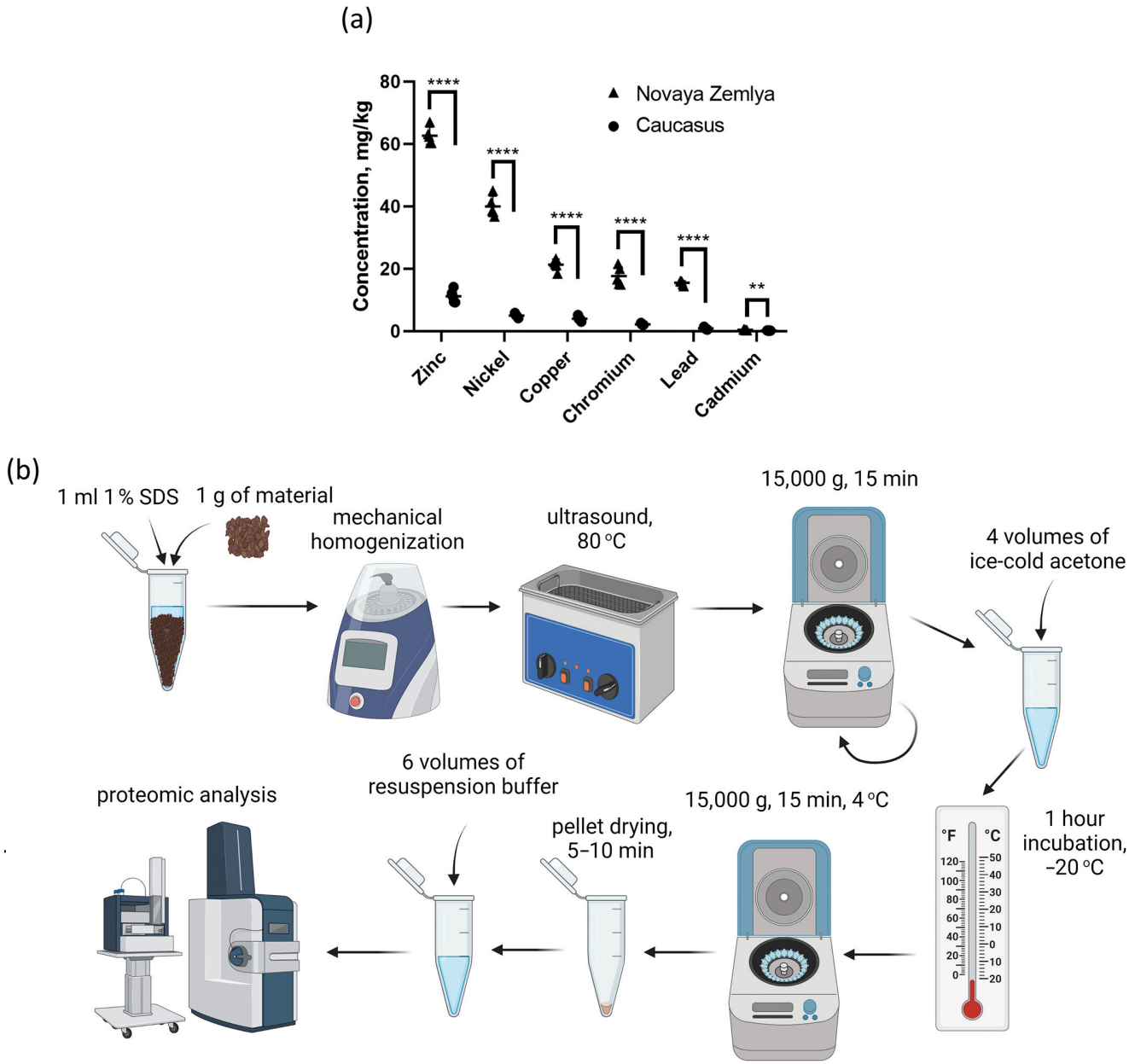

Fig. 1. (a) Comparison of heavy metal level in Caucasus (Kabardino-Balkaria) and Novaya Zemlya glaciers. ** P < 0.01, **** P < 0.0001. (b) Schematic representation of protein extraction procedures. See the description in the text. Figure created with BioRender.com

HPLC was performed in one-column separation mode with Bruker Ten separation column (C18 ReproSil AQ, 100x0.75 mm, 1.9 μm, 120 A; Bruker Daltonics) in gradient mode with 500 nl/min flow rate. Phase A was water / 0.1 % formic acid; phase B was acetonitrile / 0.1 % formic acid. The gradient was from 5 % to 30 % phase B for 17.8 minutes, then to 95 % of phase B to 18.3 minute with subsequent wash with 95 % phase B up to 20.7 minute. The column was equilibrated with 4 column volumes before each sample.

CaptiveSpray ion source was used for electrospray ionization with 1600 V of capillary voltage, 3 l/min N_2 flow and 180 °C source temperature. The mass spectrometry acquisition was performed in automatic DDA PASEF mode with 0.5 s cycle in positive polarity with the fragmentation of ions with at least two charges in m/z range from 100 to 1700 and ion mobility range from 0.85 to 1.30 1/K0.

Protein data analysis

Proteins were identified in the Peaks X Pro software (license on St Petersburg State University) against the UniProtKB (SwisProt + TrEMBL) database (uploaded 01.05.2021, number of sequences 214967504) and common Repository of Adventitious Proteins (cRAP; ver-

sion 2012.01.01) as a contamination database. Only protein groups with at least two unique peptides and FDR < 1 % were included for further data analysis in R. For Novaya Zemlya and Caucasus comparison we used proteins which were represented in at least two replicates of each glacier. Then we performed analysis of phyla distribution and functional annotation by Gene Ontology database. Due to high database redundancy, each protein group included many indistinguishable proteins. Therefore, in our analysis we operated only by protein families and large phyla to minimize artefacts of database redundancy.

Results and discussion

As a result, we identified 475 protein groups (Fig. 2; supplementary materials 2). About a third of the identified protein groups were found in both glaciers. Such similarity despite significant geographic distance is in good accordance with the previous observations: cryoconites from glaciers all over the world have similar invertebrate (Zawierucha, Kolicka, Takeuchi and Kaczmarek, 2015) and microbial (Edwards et al., 2014) fauna.

Analysis of the phylum enrichment (Fig. 2b) revealed that most identified protein groups were from the Cyanobacteria. Proteins from other phyla were much less represented (Fig. 2b). Contrary to this, in some Alpine glaciers the dominating component of cryoconite communities is heterotrophic Proteobacteria (Edwards et al., 2013; Edwards et al., 2014). This may be due to seasonal dynamics in the Alps, with autotrophic cyanobacteria dominating after snowmelt and heterotrophic bacteria becoming dominant towards the end of summer (Franzetti et al., 2017; Margesin and Collins, 2019). Protein groups from the mainly heterotrophic Actinobacteria, Proteobacteria, Bacteroidetes and Acidobacteria were also abundant (Fig. 2b). Interestingly, 24 Bacteroidetes protein groups were unique for the Caucasus.

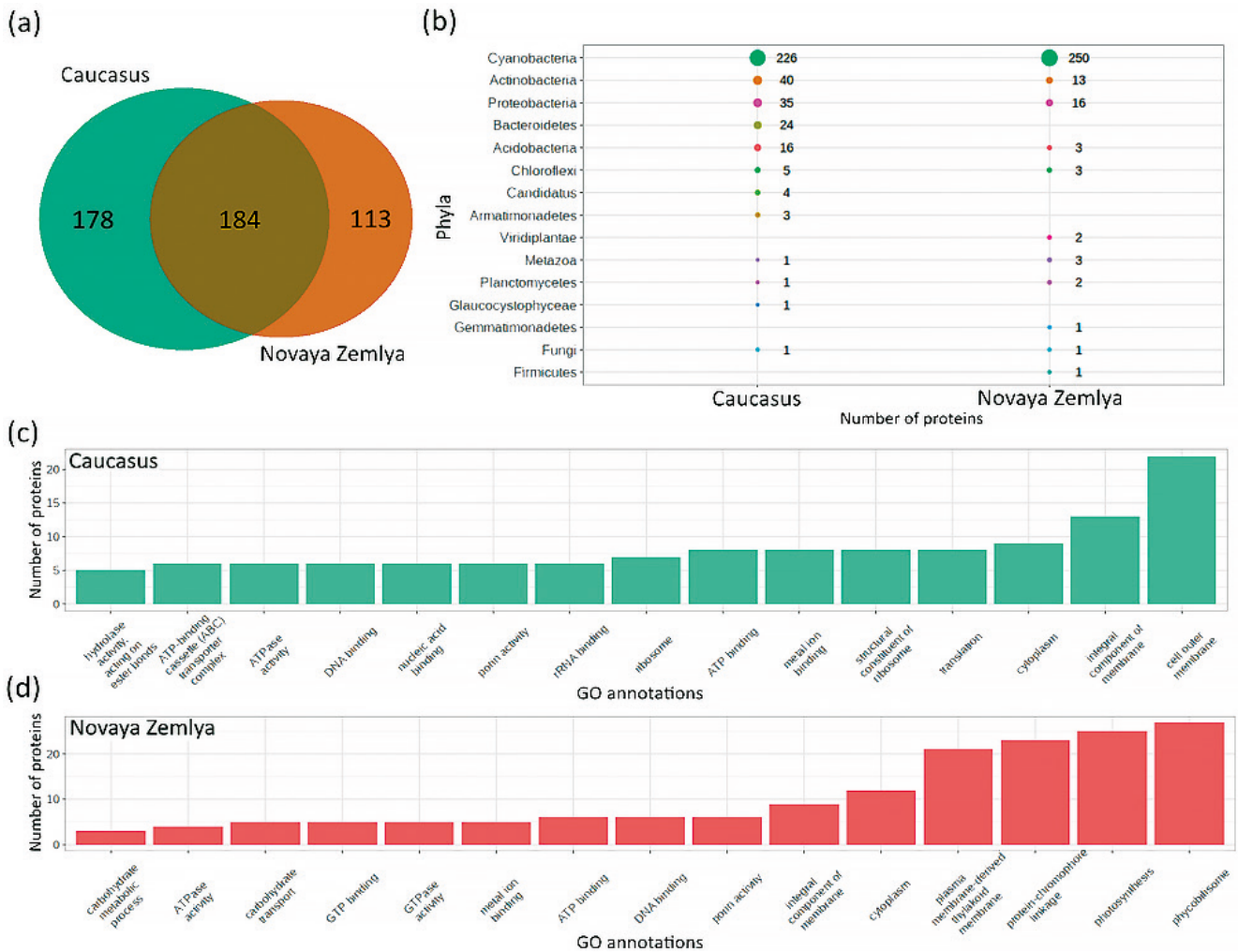

Fig. 2. Metaproteomic comparison of cryoconite communities for the Caucasian (Kabardino-Balkaria) and Novaya Zemlya glaciers. (a) Venn diagram of protein groups identified in the Caucasian and Novaya Zemlya glaciers. (b) Comparison of phyla where proteins identified in the Caucasian and Novaya Zemlya glaciers belong. (c, d) Top-15 of gene ontology enrichment annotations of protein groups unique for cryoconites from the Caucasian (c) or Novaya Zemlya (d) glaciers.

Seven of them are members of SusC/RagA family TonB-linked outer membrane protein. These outer membrane proteins form transporter complexes which import degradation products of proteins or carbohydrates. Acidobacterial protein groups were also identified mostly in the Caucasus (16 versus 3 protein groups; Fig. 2b).

In the Caucasus sample we found less Cyanobacterial protein groups than in the Novaya Zemlya sample (226 and 250 protein groups respectively) and more Actino- /Proteobacterial proteins (40/35 and 13/16 protein groups respectively).

Comparison of GO annotations of protein groups specific for the Caucasus and Novaya Zemlya cryoconites also revealed shifts from autotrophic bacteria dominating in Novaya Zemlya to heterotrophic organisms in the Caucasus (Fig. 2, c, d). While in Novaya Zemlya the most enriched GO is associated with phycobilisomes, photosynthesis, protein-chromatophore linkage and thylakoid membrane; in the Caucasus the most enriched GO is associated with outer and cell membrane and may be regarded as transport proteins of heterotrophic bacteria.

While we collected the samples in the end of the summer, a shift to heterotrophic bacteria observed in the Caucasus was expected (Franzetti et al., 2017; Margesin and Collins, 2019). The absence of this shift in Novaya Zemlya might be seen as the result of disturbances of the normal dynamics of cryoconite communities. We assume that it might be associated with much higher level of heavy metal pollution in the Novaya Zemlya, but our observations are too preliminary to draw clear conclusions.

One of the main limitations of our study is that we used Uniprot database for protein identification instead of metagenomics data from our sampling sites. Therefore, we were unable to resolve protein groups to species or order level and compelled to operate with phyla and GO terms. Still, even with such a rough analysis we could find some differences between the Caucasus and Novaya Zemlya glaciers. Re-analysis of the obtained LC-MS data with a specific database, e. g. from metagenomes of cryoconites from glaciers investigated here, will increase sensitivity and accuracy of our data.

In summary, we successfully performed metaproteomics analysis of cryoconite microbial communities by the novel methodology of shotgun proteomics with ion mobility in PASEF mode (Parallel Accumulation Serial Fragmentation). We compared metaproteomes of communities from cryoconites of the Caucasus (less anthropogenic pollution) and Novaya Zemlya (high anthropogenic pollution) glaciers and revealed a slight shift from the dominance of phototrophic Cyanobacteria in Novaya Zemlya to heterotrophic bacteria in the Caucasus. However, the absence of specific library for the LC-MS data interpretation significantly reduced the efficiency and sensitivity of our analysis. The observed differences in cryoconite communities might also be associated with the differences in seasonal dynamics of these two glaciers — we need to repeat our analysis to compare seasonal dynamics of communities from two glaciers across the year.

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References

Edwards, A., Mur, L. A., Girdwood, S. E., Anesio, A. M., Stibal, M., Rassner, S. M., and Sattler, B. 2014. Coupled cryoconite ecosystem structure-function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiology Ecology* 89:222–237. https://doi.org/10.1111/1574-6941.12283

Edwards, A., Pachebat, J. A., Swain, M., Hegarty, M., Hodson, A. J., Irvine-Fynn, T. D., and Sattler, B. 2013. A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environmental Research Letters* 8(3):035003. https://doi.org/10.1088/1748-9326/8/3/035003

Franzetti, A., Navarra, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Minora, U., and Ambrosini, R. 2017. Potential sources of bacteria colonizing the cryoconite of an Alpine glacier. *PLoS One* 12:e0174786. https://doi.org/10.1371/journal.pone.0174786

Ji, X., Abakumov, E., Antcibor, I., Tomashunas, V., Knoblauch, C., Zubzycki, S., and Pfeiffer, E. M. 2019. Influence of anthropogenic activities on metals in Arctic permafrost: a characterization of benchmark soils on the Yamal and Gydan peninsulas in Russia. *Archives of Environmental Contamination and Toxicology* 76:540–553. https://doi.org/10.1007/s00244-019-00607-y

Kang, S., Zhang, Y., Qian, Y., and Wang, H. 2020. A review of black carbon in snow and ice and its impact on the cryosphere. *Earth-Science Reviews* 210:103346. https://doi.org/10.1016/j.earscirev.2020.103346

Law, K. S. and Stohl, A. 2007. Arctic air pollution: origins and impacts. *Science* 315:1537–1540. https://doi.org/10.1126/science.1137695

Margesin, R., and Collins, T. 2019. Microbial ecology of the cryosphere (glacial and permafrost habitats): current knowledge. *Applied Microbiology and Biotechnology* 103:2537–2549. https://doi.org/10.1007/s00253-019-09631-3

Musilova, M., Tranter, M., Bamber, J. L., Takeuchi, N., and Anesio, A. M. 2016. Experimental evidence that microbial activity lowers the albedo of glaciers. *Geochemical Perspectives Letters* 2:106–116. https://doi.org/10.7185/geochemlet.1611

Wharton Jr, R. A., McKay, C. P., Simmons Jr, G. M., and Parker, B. C. 1985. Cryoconite holes on glaciers. *BioScience* 35:499–503. https://doi.org/10.2307/1309818

Zawierucha, K., Kolicka, M., Takeuchi, N., and Kaczmarek, Ł. 2015. What animals can live in cryoconite holes? A faunal review. *Journal of Zoology* 295:159–169. https://doi.org/10.1111/jzo.12195

Wilmes, P., Heintz-Buschart, A., and Bond, P. L. 2015. A decade of metaproteomics: where we stand and what the future holds. *Proteomics* 15(20):3409–3417. https://doi.org/10.1002/pmic.201500183