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Fine-scale spatial heterogeneity of invertebrates within cryoconite holes

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Abstract Cryoconite holes (water-filled reservoirs) are considered ecologically simple ecosystems but represent biological hotspots of biodiversity on glaciers. In order to check for fine-scale spatial distribution of metazoans on the bottom of the holes, in this study, we analysed three groups of grazing invertebrates as a model: tardigrades, rotifers, and mites. We addressed differences within cryoconite holes comparing the distribution of invertebrates within and between separate holes and between glaciers at a worldwide scale. We divided each cryoconite hole into three sampling zones (established in relation to water flow on a glacier) and collected nine subsamples within cryoconite holes on glaciers in the Arctic (Longyearbreen), Norway (Blåisen), the Alps (Forni) and maritime Antarctic (Ecology Glacier). Generally, we found no consistent difference in sampling zones within cryoconite holes, which suggests homogeneity on the hole floors. However, we did find strong differences and high heterogeneity between subsamples, even within the same zone. Invertebrate densities ranged between 52 and 426 individuals per ml in subsamples collected from the same hole. We found from zero to four trdigrade species in the cryoconite hole on Longyearbreen. Our results show that benthic animals in cryoconite holes

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in various climatic zones have heterogeneous spatial distribution, even if no preference could be highlighted for upstream versus downstream areas with respect to water flow. The distribution of invertebrates may result from ecosystem disturbance by flushing water and animals' active movement. Cryoconite holes, usually considered to be simple ecosystems, seem to be complex habitats where hidden spatial heterogeneity may affect abundance and diversity of organisms.

Keywords Extreme habitats · Polar and alpine ecosystems · Random distribution · Rotifera · Supraglacial environments · Tardigrada

Introduction

Cryoconite is the dark mineral and organic dust on the surface of glaciers. It plays an important role in the functioning of supraglacial ecosystems by reducing ice albedo and, along with microbial communities, forming dark bioreactors—cryoconite granules (Cook et al. 2016a, b; Hodson et al. 2008; Takeuchi et al. 2001a, b; Wharton et al. 1985). In places where the albedo is diminished by the presence of cryoconite, water-filled reservoirs, i.e. cryoconite holes, form on the surface (Hodson et al. 2008; Wharton et al. 1985). Cryoconite holes are the most biologically active environments on glaciers and constitute dynamic extreme ecosystems that are affected by flushing water, high UV doses, and seasonal as well as daily freezing (e.g. Anesio and Laybourn-Parry 2012; Cook et al. 2015; Franzetti et al. 2016; Hodson et al. 2008; Zawierucha et al. 2019).

Cryoconite holes represent an entire ecosystem with a consistent occurrence of primary producers (algae, cyanobacteria) and secondary consumers, including grazers (unicellular eukaryotes and microscopic invertebrates such as tardigrades and rotifers), interacting during the creation and maintenance of cryoconite holes (Hodson et al. 2008; Zawierucha et al. 2015). Benthic microbial communities are known to control organic matter production (Stibal et al. 2010), and even to influence the morphological evolution of cryoconite holes (Cook et al. 2016a).

The ecology and biology of cryoconite holes as ecosystems have been the subject of intensive studies (Cook et al. 2015; Darcy et al. 2018; Pittino et al. 2018; Poniecka et al. 2018; Zawierucha et al. 2018). The majority of recent works focused on the diversity, physiology and metabolism of bacteria and algae (Yallop et al. 2012; Grzesiak et al. 2015; Gawor et al. 2016) in the processes forming cryoconite granules (Takeuchi et al. 2001b; Uetake et al. 2019), on interactions between surface ice structures, cryoconite holes, weathering crust and organisms, on the so-called biocryomorphology (Cook et al. 2016a), and on the contamination of supraglacial ecosystems (Ferrario et al. 2017). Similar studies analysed the entire cryosphere, i.e. the ecosystem of glaciers, studying spatial differences in prokaryote communities within and between glaciers (Edwards et al. 2011), as well as the dispersal and behaviour of microorganisms (Cook et al. 2016a; Musilova et al. 2015; Franzetti et al. 2017a; Perkins et al. 2017). Studies on the cryosphere ranged from those performed on a large scale, e.g. concerning glaciers (Smith et al. 2017; Darcy et al. 2018), to the ones conducted on a small-scale, e.g. investigating cryoconite granules (Takeuchi et al. 2001a, b; Uetake et al. 2019).

Regarding spatial patterns of the occurrence of species, Edwards et al. (2011) showed that bacteria communities in cryoconite holes differ in taxonomic composition between glaciers. While exploring potential processes driving taxonomic differences, Pittino et al. (2018) demonstrated that prokaryote communities change throughout time, between years and seasons. Musilova et al. (2015) showed that cryoconite bacterial composition remained stable in the Greenland Ice Sheet during ablation periods, but that rapid turnovers in bacterial composition happened at the beginning of summer during snow melting. Mieczan et al. (2013) demonstrated differential vertical distribution of ciliates in Antarctic cryoconite holes. Sommers et al. (2019) investigated differences in bacteria and algae species richness and biomass between sediments and water in Antarctic closed cryoconite holes. The authors found evidence that holes with greater richness of taxa and biomass showed less partitioning of taxa by the sediments versus the water and suggested the probability of sediment microbes being mixed into the water is higher from thicker sediments. However, the study was conducted only in closed (not dynamic) cryoconite holes in a single geographical locality (the Dry Valleys of Antarctica), and, as the authors stressed, future work should include more sampling of cryoconite holes at a finer spatial scale, and characterise the communities of the sediments (Sommers et al. 2019). Indeed, until now there have been no studies on organism distribution within a cryoconite hole floor.

The distribution of biota in cryoconite holes has been suggested to be driven by external environmental factors such as ice melting or rain, which influence mixing, redistribution and erosion of sediments (Zawierucha et al. 2018, 2019). Understanding the factors shaping the diversity and densities of organisms in an ecosystem is key to determining the power of our inference on such an ecosystem (Kang and Mills 2006; Stec et al. 2016). Owing to a relevant role of cryoconite holes in the functioning of the cryosphere, including their impact on carbon flow or glacier surface melting (Hodson et al. 2007; Fountain et al. 2004), highlighting the commonalities in the patterns of occurrence of organisms across different cryoconite holes, different glaciers, and different geographical areas is a crucial step towards understanding the mechanisms sustaining cryoconite holes as ecosystems.

The distribution of organisms in ecosystems is determined by a number of variables. For example, in other terrestrial environments such as soils, where microscopic organisms interact at a similar spatial scale as in cryoconite holes, both the size of soil particles and the combination of aggregates result in a diversified environment with heterogeneity readily displayed at different scales (Franklin et al. 2002; Franklin and Mills 2003; Green et al. 2004; Grundmann and Debouzie 2000). Hidden heterogeneity of a habitat may mask significant ecological processes. In soil ecosystems, for instance, a considerable number of niches can be contained within tiny spaces (Curtis et al. 2002; Kang and Mills 2006), and cryoconite holes may constitute their justifiable analogues. Cryoconite holes contain the most complex microbial assemblies in a glacial biome (e.g. Hodson et al. 2008) with microscopic invertebrates as top consumers (Zawierucha et al. 2015). In addition, even within a single cryoconite hole, different ecological niches may form for instance close to the hole walls or in the centre of the floor, which is influenced by light (Cook et al. 2016a).

The aim of our study was to find generalities in the distribution of the biota within cryoconite holes. To do so, we addressed the differences within each hole comparing the distribution of microscopic invertebrates within and between separate holes and between glaciers at a worldwide scale, including Arctic, Subarctic, Alpine, and Antarctic glaciers, and sampling holes of different size and with varying levels of water. Our expectations were that invertebrate densities and diversity, regardless of differences in the geography and ecology of a cryoconite hole, vary on the floor of the hole, and that such distribution does not differ randomly but may be structured by ecological processes, particulary through a gradient with higher densities in the part of cryoconite holes oriented towards a glacier terminus due to surface water gently flushing towards the glacier end and passively transporting organisms. We focused on microscopic invertebrates – our model organisms, which are frequent and abundant top consumers in cryoconite holes of polar and alpine regions (De Smet and Van Rompu 1994; Porazińska et al. 2004; Zawierucha et al. 2015, 2016a), and which are characterised by potentially more stable and traceable densities than prokaryotes or unicellular eukaryotes.

Methods

Study locations

Glaciers were chosen to represent different areas of the world (Fig. 1). The four selected glaciers were from: the Arctic (Longyearbreen, in Svalbard), the Subarctic (Blåisen, in Norway), the temperate mountains (Forni, in the European Alps) and Antarctica (Ecology Glacier, in maritime Antarctica, King George Island). Longyearbreen (WGS84 coordinates: 78°10′49″N, 15°30′21″E) is a land-terminating, cold glacier located near the town of Longyearbyen, in the catchment of Adventfjorden, western Spitsbergen. The northeast-facing Longyearbreen descends from over 1000 m to approximately 250 m a.s.l. The area of Longyearbreen is 2.7 km^2 (Etzelmüller et al. 2000). Blåisen (60°33′74″N, 7°29′49″E) is a northern outlet valley glacier from Hardangerjøkulen (Central Southern Norway). The glacier covers 4.9 km^2, and its elevation ranges between 1370 and 1850 m a.s.l. (Dahl and Nesje 1994). The Forni Glacier (46°12′30″N, 10°13′50″E) is an Italian temperate valley glacier belonging to Ortles-Cevedale Group in the Alps. The area of Forni is 10.83 km^2 (Azzoni et al. 2017). Its

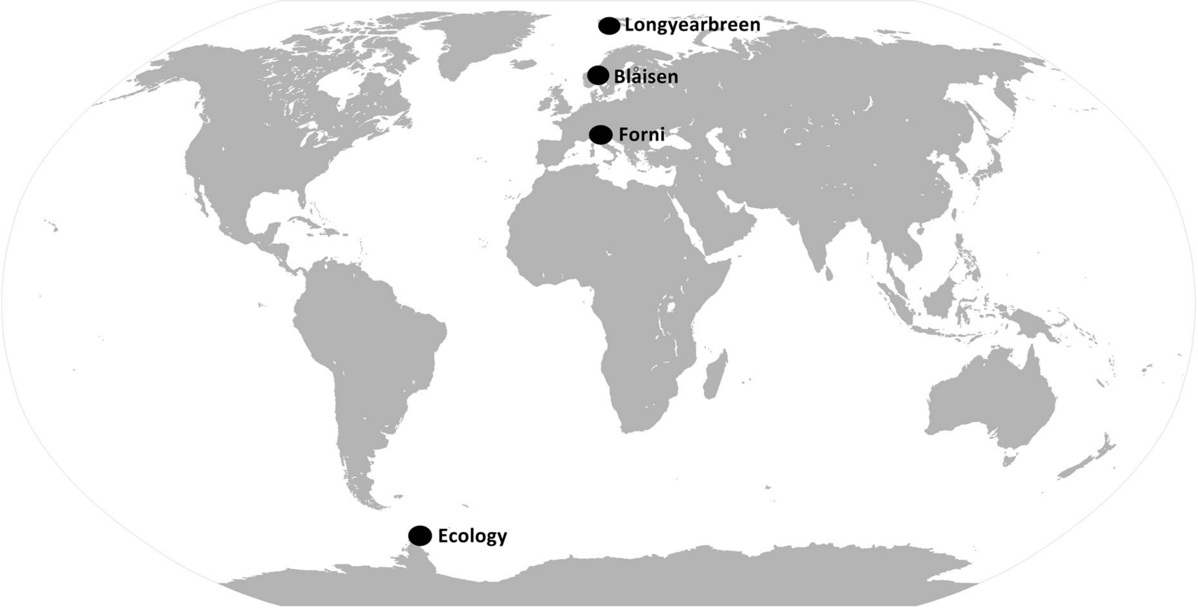

Fig. 1 Map of the world with sampling sites

elevation ranges between 2600 and 3670 m a.s.l. The Ecology Glacier (62°11′92″S, 58°30′78″W) is located on King George Island in the South Shetland Archipelago. The Ecology Glacier is a valley-tidewater glacier located on the western shore of Admiralty Bay. The area of the glacier is 5 km^2 (Pętlicki et al. 2017).

The four glaciers represent different geographical regions (three glaciers are from Europe and one is from Antarctica), and different environments [two glaciers are from areas where light and temperature follow a seasonal cycle (Longyearbreen, Ecology Glacier) and two from where they follow both a seasonal and a daily cycle (Blåisen, Forni)]. Thus, any commonalities in invertebrate distribution notwithstanding such extreme geographical and ecological differences will represent an intrinsic feature of the ecosystem of cryoconite holes.

Sampling strategy

A total of 14 cryoconite holes was analysed. Samples were collected from: four cryoconite holes on Longyearbreen at 360–380 m a.s.l. in August 2016, three cryoconite holes on Blåisen at 1300–1400 m a.s.l. in August 2018, three cryoconite holes on Forni at 2600–2700 m a.s.l. in July 2018, and four cryoconite holes on Ecology Glacier at 69-265 m a.s.l. in February 2018. The holes were of size between 12 and 25 cm of the longer chord, and with water content ranging between 3 and 15 cm of vertical depth.

The samples were collected in order to analyse the spatial distribution of invertebrates on the bottom of the holes. Nine subsamples were collected from each cryoconite hole: three for each of three rows. When cryoconite holes were elliptical, subsamples were collected along the longer axis of each cryoconite hole (Fig. 2). From the cryoconite hole perspective, the first

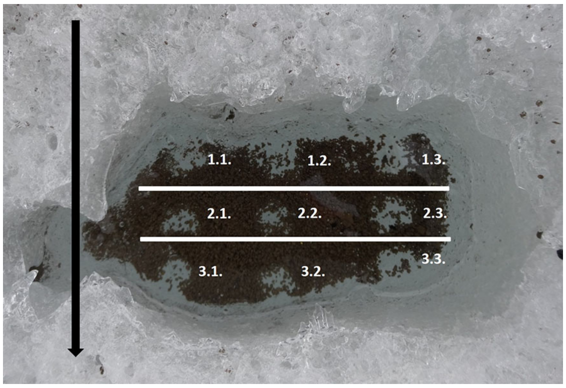

Fig. 2 Example of sampling design in a cryoconite hole from Longyearbreen. The arrow indicates glacier terminus

row was always located towards the snowline, the second row in the middle, and the third row located towards the glacier terminus. Cryoconite holes on each glacier were similar in size but with different shape. On Longyearbreen, they were circular, elliptical and irregular, on Blåisen and Forni circular or irregular. On Ecology Glacier, being irregular and shallow, the holes resembled puddles. Nine subsamples were collected from each cryoconite hole, except for one hole on Lyongearbreen where only eight subsamples were collected, with one missing subsample in the first row. Thus, a total of 125 subsamples were investigated. The area of the collected sediments in one subsample ranged between 1 and 1.5 cm^2 (depending on the size of the hole). Each subsample was collected with a separate disposable plastic Pasteur pipette and transferred into a 15-ml plastic test tube. The samples were collected to harvest the sediment from the bottom of the cryoconite holes, to search for invertebrates. Following the collection, the samples were preserved in 96% ethanol.

In laboratory, the whole cryoconite material was poured onto plastic Petri dishes. In order to aid dish scanning, parallel lines were engraved with a pencil on the outer bottom of each dish, so that at the magnification of 40 times, the space between the lines fell into the range encompassed by the visual field of a stereomicroscope. Such an approach was previously used and described by Dastych (1980) and Stec et al. (2015).

All the animals found in each of the subsamples were identified and grouped into three categories: Acari, Rotifera and Tardigrada. For rotifers, no further taxonomic identification was possible, whereas for mites and tardigrades, the identification was performed to the most detailed taxonomic level of morphological species, both for already known species and for potentially new or unidentified species (Zawierucha et al. 2016a).

Invertebrate counts from each sample were converted into density of individuals per 1 ml of cryoconite.

Data analysis

Using generalised linear mixed models (GLMMs) implemented via the glmmTMB package (Brooks et al. 2017), in R Development (R Core Team 2017), we tested whether the density of the taxonomic groups differs among the rows within each cryoconite hole. Due to different motility, feeding behaviour, size and shape of invertebrates found in the samples, which might have influenced their dispersal within and between the holes, for each group we built a separate, negative binomial model (using a log-link function) with the group count as response variable, cryoconite hole ID as random effect, and a row ID (indicating row position within a hole, i.e. whether it was the top, middle or bottom row) as a fixed effect. The analysis was repeated for each glacier we sampled (i.e. four times). However, to explore generalities on all the glaciers, and on all groups of animals, we built a negative binomial model (using a log-link function) with the animal count as the response variable, glacier ID and cryoconite hole ID nested into glacier ID as random effects, and two fixed effects: a row ID, animal group (i.e. mites, rotifers or tardigrades) along with their interaction as a fixed effects. In all the models, the volume of the sample was used as an offset. We tested for statistical significance of the fixed factors with the likelihood ratio test.

The framework described above involves several consecutive statistical tests on the same data. Thus, we used conservative Bonferroni-corrected alpha-values to maintain the correct experiment-wise error rate (Forstmeier et al. 2017). We ran five tests for each group, so that the level of significance for each test was adjusted to alpha = 0.05/5 = 0.01.

Rotifers from the collected subsamples were preserved in alcohol, consequently their identification to species level using methods of classical taxonomy was impossible. Tardigrades on Forni, and mites on the Ecology Glacier were represented by one species only. Therefore, a comparison of species frequency (expressed in %) between sampling points was done only for tardigrades found on Longyearbreen.

Results

Contrary to our expectations, we did not find any common differences in invertebrate densities between the rows of subsamples I-III (gradient in relation to water flow with expectedly higher densities of animals in the part of cryoconite holes oriented towards the glacier terminus). Likewise, additional model considering all the glaciers and animals together, showed no

Table 1 Summary of the negative binomial GLMMs tested for the differences in abundance among rows (see "Methods" for details)

Glacier	Tardigrada	Rotifera	Acari	Total
Lyongearbreen	χ^2 = 2.25, p = 0.32	χ^2 = 0.77, p = 0.68	NA	χ^2 = 1.23, p = 0.54
Blåisen	NA	χ^2 = 7.29, p = 0.03	NA	NA
Forni	χ^2 = 0.18, p = 0.91	NA	NA	NA
Ecology	NA	χ^2 = 4.15, p = 0.12	χ^2 = 1.09, p = 0.58	χ^2 = 2.91, p = 0.23

Degrees of freedom equal five in all models. NA indicates that the particular group was not present in the samples at the glacier (or present in very limited abundance that precluded modelling, i.e. tardigrades on Blåisen). No significant effects at a Bonferroni-corrected α = 0.01 were detected

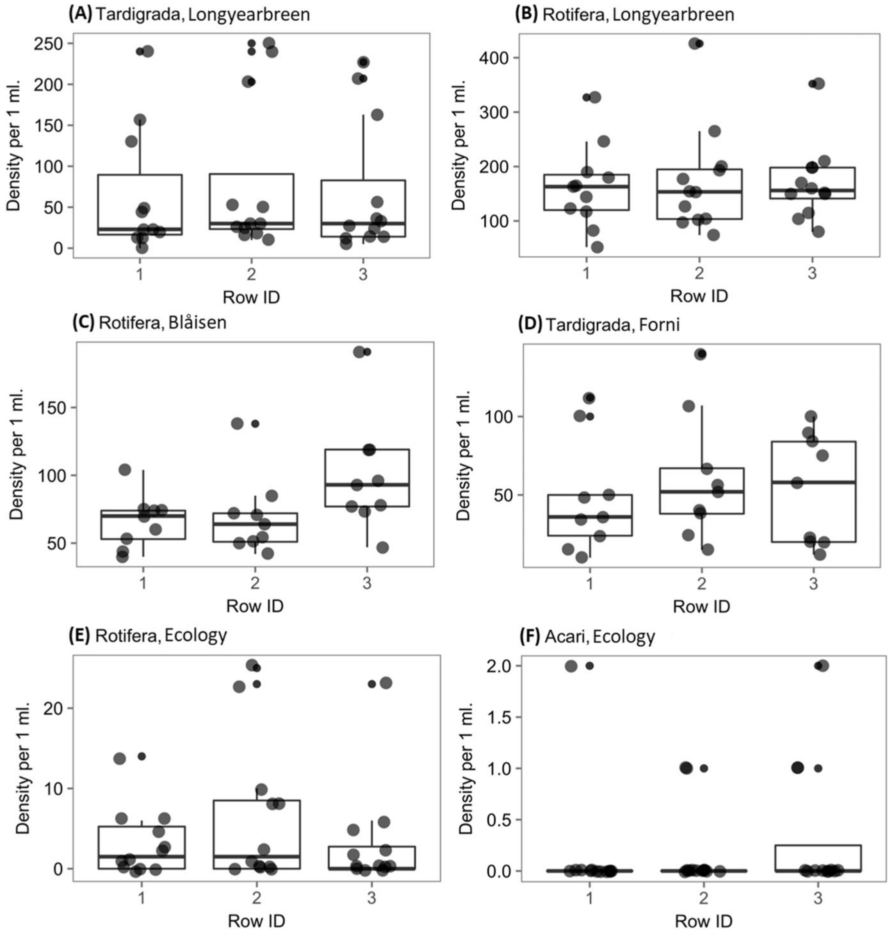

Fig. 3 The density of invertebrates in three parallel rows on the cryoconite hole floor: **a**, **b** Tardigrada and Rotifera on Longyearbreen, **c** Rotifera on Blåisen, **d** Tardigrada on Forni, **e**, **f** Rotifera and Acari on Ecology. Row ID indicate row position within each hole: 1 top row, 2 middle row, 3 bootom row (toward the glacier terminus). The boxes denote the 25th, 50th, and 75th percentiles; the whiskers indicate the furthest data points within a 1.5 interquartile range; the grey circles refers to sampling points within rows; and the dark circles refer to data points beyond a 1.5 interquartile range

relation between animal densities and row position (Table 1, Fig. 3a–f, Figures S2-S5).

Invertebrate densities, regardless of variations in geography and the type of glacier, differ on the floor of cryoconite holes. The density of animals varied considerably among the subsamples taken within the same hole (Fig. 3, Figures S2-S5). For the Arctic Longyearbreen glacier, in the case of rotifers, the largest absolute difference in the density between two subsamples taken within the same hole was 374 ind./ml (maximum: 426 ind./ml vs. minimum: 52 ind./ml) and for tardigrades it was 110 ind./ml (maximum: 230

ind./ml vs. minimum: 130 ind./ml). For the Scandinavian Blåisen glacier, the greatest absolute difference in the density of rotifers between two subsamples taken within the same hole was 151 ind./ml (maximum: 191 ind./ml vs. minimum: 40 ind./ml). On the alpine Forni Glacier, the difference in the density of tardigrades between two subsamples was 74 ind./ml (maximum: 84 ind./ml vs. minimum: 10 ind./ml). For the Antarctic Ecology glacier, in the case of rotifers, the most prominent absolute difference in density was 22 ind./ml (maximum: 25 ind./ml vs. minimum: 3 ind./ml), and for mites it was 1 ind./ml (maximum: 2 ind./ml vs. minimum: 1 ind./ml).

We did not find commonality in the diversity and dominance of invertebrates in cryoconite holes. On Longyearbreen, tardigrades (*Pilatobius* sp., *Isohypsibius* sp., *Hypsibius* sp., *Hypsibius* cf. *dujardini*) and unidentified rotifers were found. On Blåisen, mostly bdelloid rotifers were located with few tardigrades (*Pilatobius* sp., excluded from the main analysis owing to its presence in just two subsamples). Cryoconite holes on Forni were characterised by exclusive predominance of tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959. On the Ecology Glacier, only bdelloid rotifers and mites were found (species designation will be described elsewhere). Tardigrades found in the study belong to typical grazers, microbivorous, and herbivorous/omnivorous species. Rotifers are most probably filter feeders, and, in turn, mites are suspension feeders.

Tardigrades were identified to morphospecies level, and four species were found on Longyearbreen. A microbivorous grazer, *Pilatobius* sp. was the dominant species in cryoconite holes, followed by an herbivorous species, *Hypsibius* sp. (Fig. 4a–d). Species richness in subsamples within the same cryoconite hole ranged from zero to four (Fig. 4a). In cryoconite hole no. 3 the abundance of *Pilatobius* sp. in subsamples always exceeded 50% reaching even 100% (Fig. 4c). We could not identify any general pattern of differences in species occurrence or abundance between the rows of subsamples.

Discussion

Our results show that animals in cryoconite holes have heterogeneous fine-scale spatial distribution on a hole bottom, with each subsample being different from the nearby ones, even if no preference could be highlighted for upstream versus downstream areas in relation to water flow. The results demonstrate that invertebrate densities varied considerably among the subsamples taken within the same cryoconite hole. These findings imply that our power of inference on biota abundance and diversity in cryoconite holes depends on the number of subsamples taken within a hole or the amount of collected cryoconite, especially in larger cryoconite holes (> 12 cm in diameter, like the ones we analysed), which offer more space for animals to move around actively or passively. Furthermore, the occurrence and abundance of animals on glaciers do not differ among the rows of subsamples collected along the longer axis of the holes, implying that invertebrate distribution in the holes does not show a general pattern.

In environmental studies, important interactions between organisms and their environment are often hard to detect, especially in highly complex environments such as forests, rivers or ponds (Bogdziewicz et al. 2017; Peacor and Werner 1997; Schmitz and Suttle 2001). Even though cryoconite holes are often considered to be ecologically simple ecosystems (Koshima 1987; Hodson et al. 2008), they are characterised by complex microbial communities with high biodiversity (e.g. Gokul et al. 2016). Cryoconite holes are complex not only at the level of functional diversity (Gokul et al. 2016; Pittino et al. 2018) but also, as we have shown herein, in the distribution of organisms on a hole floor. The heterogeneous distribution on glaciers of grazers, which may feed on other biota, may mask our inference of interactions between organisms within the hole, and our general understanding of supraglacial ecosystem functioning. The distribution of invertebrates on a hole bottom probably mirrors heterogeneity of this ecosystem. For example, on Longyearbreen we observed a clear difference in the occurrence of tardigrade species representing various feeding modes. In cryoconite hole no. 3 microbivorous species was dominant in all subsamples but in cryoconite hole no. 2 herbivorous *Hypsibius* sp. was more abundant in four subsamples. On Forni (the Alps), 24 (per ml) tardigrades were determined in a sample taken from the middle of the cryoconite hole. Yet, another subsample from the same hole contained 84 tardigrades. The same phenomena exist in cryoconite holes where more than one group of animals coexists. When a subsample was taken from the

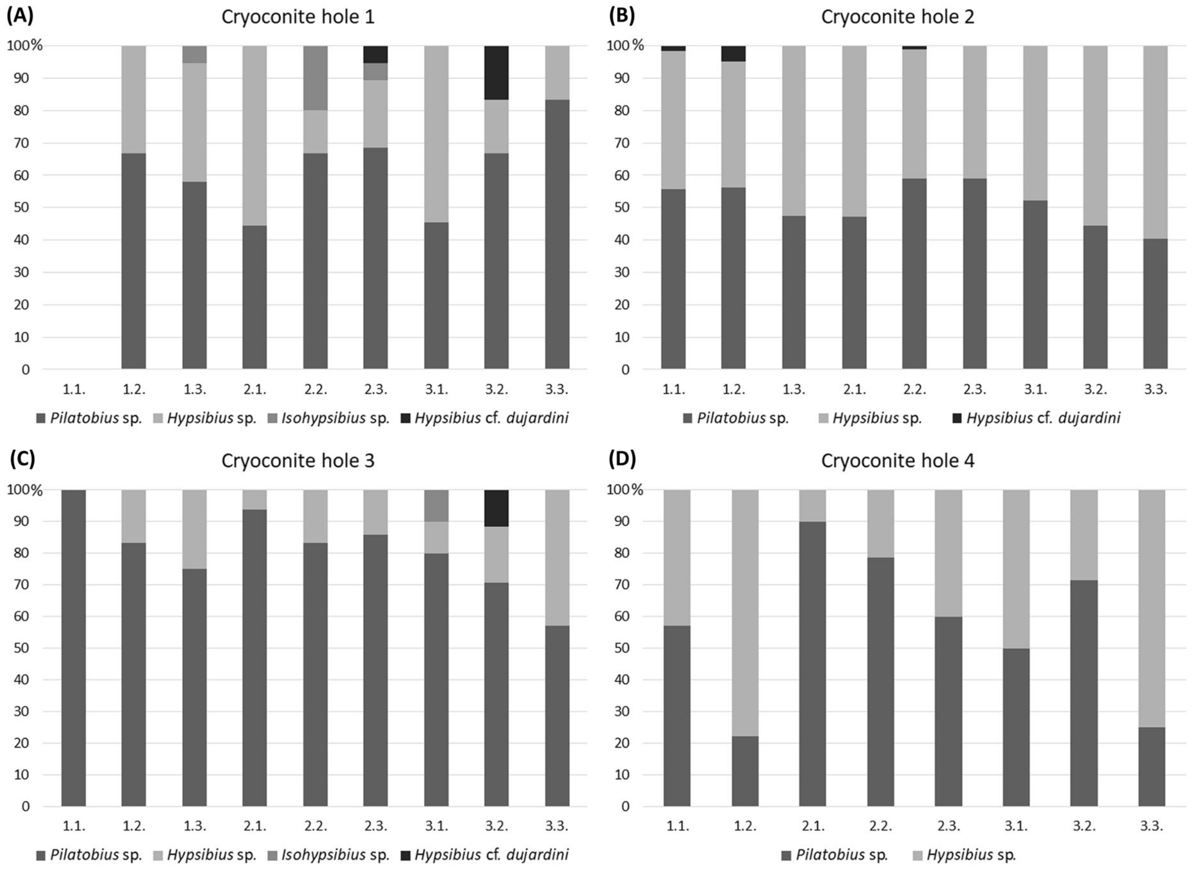

Fig. 4 The frequency of tardigrade species found in cryoconite holes on Longyearbreen. Numbers from 1.1. to 3.3. represent the ID of each subsample taken within each hole (see Fig. 2)

middle of a cryoconite hole on Longyearbreen (the Arctic), 30 tardigrades and 154 rotifers were found, but in the same hole the next subsample taken closer to the wall contained 52 tardigrades and 426 rotifers.

Differences in the distribution of invertebrates within cryoconite holes may depend on the characteristics of a specific animal group. Tardigrades, rotifers and mites found in this study differ in feeding modes, motility, size and shape (Nelson et al. 2015; Fontaneto and De Smet 2015; Marshall and Pugh 1996), potentially having a crucial role in locomotion as well as passive dispersal in the holes. Past studies on small-scale spatial distribution of invertebrates have yielded various, sometimes contradictory, results. The distribution of tardigrades in freshwater ecosystems is poorly understood. However, this issue has been studied in bryophytes (e.g. Wright 1991; Nelson and Adkins 2001; Degma et al. 2011; Young et al. 2018). Wright (1991) indicated that some (yet not all) tardigrade species actively migrate between moss layers because of moisture (Wright 1991). Other studies point to the fact that, in general, tardigrade distribution may be the result of migration of animals within the moss, not being influenced by moisture (Nelson and Adkins 2001). This implies that the distribution of invertebrates in moss cushions is likely to be influenced by the biology and behaviour of a particular species. However, the presence of tardigrades in samples may be random and their distribution may have a patchy character established by the founders of a colony as was also suggested in the literature (Degma et al. 2011; Meyer 2006). Taking into account the literature we consulted, the ability of tardigrades to actively migrate in moss cushions, and the fact that aquatic and limno-terrestrial tardigrades in laboratory cultures actively crawl on Petri dishes (personal observation), we may clearly state they may also actively move on a cryoconite hole bottom.

Regarding rotifers, their distribution in mosses seems to be selective, and bdelloids appear to show spatial patterns (Fontaneto and Ricci 2006). Nevertheless, in terms of aquatic habitats, it is most probably random (Schmid-Araya 1993), with only scarce data for benthic species (Fontaneto and De Smet 2015). However, rotifers are also highly active in aquatic habitats. Locomotion in bdelloids is a typical leech-like creeping motion, and swimming is rather common (Ricci et al. 2001). The distribution of mites is more complex and depends on the type of environment, life history and feeding behaviour (Nielsen et al. 2012; Stoffels et al. 2003). Even though there are no data on the spatial distribution of mites in freshwater ecosystems, their active locomotion in both natural environments and laboratory cultures was observed (Miko 2016).

Random distribution may apply to invertebrates inhabiting cryoconite holes. For example, Zawierucha et al. (2018) provided evidence that invertebrates on the edge of the Greenland Ice Sheet are more common in cryoconite granules than in fine sediments. Observation on the erosion of granules by water flushing to mud suggests that fauna (tardigrades and rotifers) may be mechanically removed from the holes without any specific ecological relations with biotic variables (Zawierucha et al. 2018). Thus, invertebrate frequency and densities in, and between, cryoconite holes depend on typical random factors on glaciers such as melting, flushing and rain (Zawierucha et al. 2019), resulting in the disruption of these habitats and heterogeneity on a cryoconite hole floor, even in stable holes located in the upper part of glaciers tongues. Even though the invertebrates cannot be removed during rain or high ablation, their heterogeneous distribution on the hole bottom may result from inter-hole mixing. The effects of high meltwater production and open cryoconite holes in the Arctic and mountains (especially on the margin of the Greenland Ice Sheet and small glaciers) may influence the redistribution of cryoconite communities because of the so-called inter-hole water–sediment mixing (Mueller et al. 2001; Mueller and Pollard 2004; Zawierucha et al. 2016b, 2019). The abovementioned explanation suggests that fine-scale distribution of invertebrates may result from stochastic events such as ecosystem disturbance by flushing water (Zawierucha et al. 2019) and animals' active movements.

Cook et al. (2015) suggested integrating different approaches for describing processes in supraglacial environments. As a result, a new term—biocryomorphology—was introduced. It refers to ice–organism interactions (Cook et al. 2015). The distribution and behaviour of organisms in stable cryoconite holes may influence their evolution as an effect of melting owing to heat from biological reactions (Cook et al. 2016a, b; Perkins et al. 2017). Therefore, heterogeneous spatial distribution of grazing invertebrates may exert an impact on ecological relations in holes and, consequently, primary production, organic matter distribution, and hole morphological evolution.

Studies on biota are often based on holes sampled once and may constitute snapshots, which could lead to certain general outcomes (Edwards et al. 2011; Franzetti et al. 2017b; Zawierucha et al. 2018) but hinder our understanding of cryoconite hole ecology. As we showed in this study, sampling a small amount of sediment could give biased estimates of small invertebrate abundance and diversity and may constitute a weak point of sampling protocols for wide cryoconite holes. Thus, future studies on biota would benefit from increasing the number of replicated samples taken from each cryoconite hole. As cryoconite holes differ within and between glaciers and geographical zones (Mueller and Pollard 2004; Stibal et al. 2010; Cook et al. 2015), more studies are needed to elaborate the most efficient sampling strategy and understanding probably hidden interactions and processes in cryoconite holes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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