



Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing different position in supraglacial trophic network

5 Tereza Jaroměřská¹, Jakub Trubač², Krzysztof Zawierucha³, Lenka Vondrovicová², Miloslav Devetter^{4,5} and Jakub D. Žárský¹

¹Department of Ecology, Charles University, Prague, 128 44, Czech Republic

²Institute of Geochemistry, Mineralogy and Mineral Resources, Charles University, Prague, 128 43, Czech Republic

³Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, 61-614, Poland

10 ⁴Biology Centre, Institute of Soil Biology, Czech Academy of Sciences, České Budějovice, 370 05, Czech Republic

⁵Centre for Polar Ecology, University of South Bohemia, České Budějovice, 370 05, Czech Republic

Correspondence to: Tereza Jaroměřská (jaromert@natur.cuni.cz)

Abstract. Cryoconite holes are ecosystems on the glacier surface characterized by dynamic nature and truncated
15 food webs. It is acknowledged that cryoconite holes play an important role being biodiversity hot-spots and factories for organic matter on glaciers. The most common cryoconite apex consumers are the cosmopolitan invertebrates – tardigrades and rotifers. Several studies have highlighted the relevance of cryoconite tardigrades and rotifers to cryoconite holes' ecosystem functioning. However, due to the dominant occurrence of prokaryotes on glaciers, these consumers are usually out of the major scope of most studies aiming at biological
20 processes on glaciers. The aim of this study is to present data about isotopic composition of tardigrades, rotifers and cryoconite from three High Arctic glaciers in Svalbard and discuss their potential trophic relations. We found that tardigrades have lower $\delta^{15}\text{N}$ values than rotifers, which indicates different food requirements of both consumers. The $\delta^{13}\text{C}$ values revealed similarities among the consumers from the same glaciers and differences between consumers and cryoconite among glaciers. The resulted $\delta^{13}\text{C}$ values point to similar carbon requirement
25 of consumers within a glacier but differences in carbon input between glaciers. The results comprise the first observation of cryoconite holes' consumers through stable isotopic analyses using an improved method of cryoconite sample processing and pave the way for further studies of the supraglacial trophic network.

1 Introduction

Supraglacial habitat, the environment on the glacier surface, forms the most biologically active and productive
30 part of glaciers all around the world (Hodson et al., 2008). The flat parts of glacier surface accumulate sediment (so called cryoconite, Nordenskiöld (1875)) which – due to its dark colour – reduces albedo and creates water-filled depressions called cryoconite holes (Cook et al., 2016; Takeuchi et al., 2001). At the bottom of these holes, cryoconite forms aggregates composed of bacteria, organic and inorganic matter (Takeuchi et al., 2001). As biodiversity hot-spots of the glacier surface, cryoconite holes host a myriad of organisms ranging from bacteria,
35 algae and fungi to metazoans such as tardigrades (phylum Tardigrada) and rotifers (phylum Rotifera) (Cook et al., 2016; Kaczmarek et al., 2016; Zawierucha et al., 2015). Tardigrades and rotifers are cosmopolitan microscopic invertebrates contributing to multiple aquatic and terrestrial trophic levels as carnivorous, herbivorous, omnivorous and microbivorous species (Guidetti et al., 2012; Guil and Sanchez-Moreno, 2013; Hallas and Yeates, 1972; Kutikova, 2003). Due to their ability to survive various extreme conditions (Guidetti et al., 2011; Ricci, 2001),



40 these animals represent a large component of microfauna in polar and high mountain regions and are the dominant metazoans inhabiting cryoconite holes (Klekowski and Opałiński, 1986; Zawierucha et al., 2019a).

As the top consumers of Arctic cryoconite holes, tardigrades and rotifers presumably play an important role in controlling the populations of primary producers and thus contribute to the local community structure (Vonnahme et al., 2016; Zawierucha et al., 2015; Zawierucha et al., 2018). Previous research on cryoconite holes metazoans from Svalbard archipelago revealed that the size distribution of algae, particularly Zygnococcales and Chlorococcales, correlates with the community structure of consumers represented by tardigrades and rotifers (Vonnahme et al., 2016). Nevertheless, other studies from the margin of the Greenland ice sheet indicate a lack of quantitative relations between numbers of top consumers and potential food such as cyanobacteria and algae (Zawierucha et al., 2018), which demonstrates the variability influenced by multiple factors occurring on various glaciers (Porazinska et al., 2004). As described by Štrítecká and Devetter (2015), tardigrades and rotifers are efficient filtrators and especially rotifers reveal high filtration rates in cryoconite. The feeding behaviour and morphology of the feeding apparatus indicate that cryoconite species consume mostly algae, bacteria and detritus (Devetter, 2009; Iakovenko et al., 2015; Zawierucha et al., 2016). However, their diet in various environments differs interspecifically (De Smet and Van Rompu, 1994; Guidetti et al., 2012; Guil and Sanchez-Moreno, 2013; Hallas and Yeates, 1972; Kutikova, 2003; Mialet et al., 2013; Wallace and Snell, 2010; Zawierucha et al., 2016).

Analyses of stable isotopes are a well-developed tool which enables us to uncover the trophic interactions of organisms within various systems (McCutchan et al., 2003; O'Reilly et al., 2003; Wada, 2009; Yoshii et al., 1999). Because of the differences in isotopic fractionation, $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$) and $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$) isotopic ratios of organisms and their potential food can reflect their possible mutual relationships and position within the food web (Michener and Lajtha, 2008). Isotopic fractionation is caused by physical or biochemical processes which discriminate heavier or lighter isotopes (Michener and Lajtha, 2008). The $\delta^{13}\text{C}$ value reflects the difference between the organism and is similar or slightly higher within the animal compared to its food (Peterson and Fry, 1987). The slight increase between organismal $\delta^{13}\text{C}$ and the $\delta^{13}\text{C}$ values of its diet is caused by higher assimilation of ^{13}C supported by preferential ^{12}C depletion (of CO_2) during respiration (Blair et al., 1985; DeNiro and Epstein, 1978; Ekblad and Högberg, 2000; Wada, 2009). Therefore, the process of consumption and growth generally tends to increase the heavier isotope (^{13}C) value within the consumer's body compared to its diet. Larger variations in values are however balanced by a higher release of ^{13}C during excretion (DeNiro and Epstein, 1978). The $\delta^{15}\text{N}$ values reflect the nitrogen isotopic composition of the organism's diet and point to the position of organisms in a food chain (DeNiro and Epstein, 1981). The $\delta^{15}\text{N}$ value is usually higher in the animal body compared to its diet and increases with the trophic level (DeNiro and Epstein, 1981; Kling et al., 1992; Zah et al., 2001). This increase is mostly caused by a higher proportion of proteins within the diet and subsequent preferential excretion of $\delta^{14}\text{N}$ during protein metabolism (Kling et al., 1992; McCutchan et al., 2003). Furthermore, if the environment is limited by a nutrient (the biogenic element), the consumer's body fractionates isotopes differently than in case of no nutrient limitation (Michener and Lajtha, 2008; Šantrůček et al., 2018). Stable isotopes of carbon and nitrogen are the most common food web tracers used in ecological studies (Michener and Lajtha, 2008). In case of invertebrates, many studies focus on aquatic or soil food webs where producers and consumers can be easily collected and prepared, and their body size enables us to create a required number of analyses with a sufficient number of individuals (e.g. Ponsard and Arditi, 2000; Wada, 2009). Several studies have also focused on carbon and nitrogen



80 stable isotopes in polar systems. However, none of them on glaciers, which are an essential part of polar ecosystems.

Glaciers are one of the key indicators in observation of climate changes and more importantly, they are dynamic biogeochemical reactors capable of altering processes in downstream deglaciated areas as well as in coastal marine ecosystems (Bardgett et al., 2007; Foreman et al., 2007; Hodson et al., 2008; Hood et al., 2009; Williams and Ferrigno, 2012). The primary producers such as cyanobacteria and algae are an important factor reflecting differences in the nutrient input on the glacier surface contributing to the glacial ecosystem functioning (Hodson et al., 2008; Stibal et al., 2012; Vonnahme et al., 2016). Studies focusing on the role of top consumers in cryoconite holes are ~~however lacking~~, which may hinder our understanding of cryoconite holes' ecology and glacial ecosystem's functioning. This study is based on data from three High Arctic inland glaciers, all three located in a different geographical and geological context. We examined carbon and nitrogen stable isotopic composition of cryoconite, tardigrades and rotifers, and made a synthesis of their potential relationships in the cryoconite hole trophic food web.

2 Material and Methods

2.1 Study site and sampling

95 Samples of cryoconite were collected from three glaciers (Ebbabreen, Nordenskiöldbreen and Svenbreen; *breen* means glacier in Norwegian) located at Central Svalbard (78° N and 14–17° E) during July and August 2016. Svenbreen is a representative of small glaciers in the geologically older part of Billefjorden Fault Zone. Ebbabreen and Nordenskiöldbreen are larger valley glaciers within a geologically younger zone. On each glacier, representative cryoconite holes (varied in shape, size and depth) were sampled in the upper (close to the equilibrium line) and the lower part (closer to the glacier terminus) of the ablation zone around the main axis of the glacier. Sampling was conducted twice from each glacier (within the interval of approximately one week between each sampling) using a high-density polyethylene (HDPE) bottle with two siphons according to Mueller et al. (2001) with modifications after Vonnahme et al. (2016). Sampled cryoconite from each part of the ablation zone was poured together and put into sterile Whirl-Pak® (Nasco, Fort Atkinson, WI). Water pH was measured during the sampling by a Hanna Instrument (HI 98130). Data about the air temperature were provided by the meteorological station at Bertilbreen which is a glacier adjacent to the examined Svenbreen. After sampling, cryoconite was stored on ice in a field refrigerator (a plastic barrel entrenched into permafrost) and subsequently frozen at –20 °C and kept frozen until analysis.

2.2 Preparation of samples for isotopic analyses

110 For each replicate, a part of cryoconite (~ 2–4 cm³) was separately melted by dropping distilled water through the sample into a glass beaker, transferred into a falcon tube and stored in a cooling box. Animals were collected under a light microscope (Olympus CX31 and Leica DM750) using a glass Pasteur pipette. All work was performed in nitrile gloves to avoid carbon contamination. Every individual specimen was cleaned from alien particles and transferred at least once to a drop of clean distilled water before transferring into an Eppendorf tube. The Eppendorf tubes were also continuously cooled by a cooling pad. The collected individuals were stored in a freezer at –20 °C until lyophilization and further processing started. After at least 300 individuals of both taxa (tardigrades and



rotifers) were collected from each sample, the Eppendorf tubes were thawed and all individuals from each sample were transferred into a pre-weighed tin capsule (Costech 41077, 5 × 9 mm). If the water content in the capsule exceeded ½ of the volume, capsules were dried inside a desiccator with silica gel (0.5–2.5 h) until the water inside the capsules was reduced to 1/3 of the volume. The samples were consequently frozen at –20 °C and at least half
120 an hour before the lyophilization stored at –80 °C. The duration of the lyophilization was 4 hours. Thereafter, samples were weighed (Mettler Toledo Excellence Plus XP6, linearity = 0.0004 mg), the capsules were closed and wrapped, and analysed immediately or stored in a desiccator until the analyses were performed. The average weight of a dry sample of invertebrates was ~ 29.5 µg. Four replicates of tardigrades, rotifers and cryoconite from Svenbreen, five replicates of tardigrades, four replicates of rotifers and three replicates of cryoconite from
125 Nordenskiöldbreen, and three replicates of tardigrades, two replicates of rotifer and two replicates of cryoconite from Ebbabreen were collected for the isotopic analyses.

Cryoconite intended for the isotopic analyses was cleaned from tardigrades and rotifers, which were collected in parallel for isotopic analyses. After the collection, cryoconite was stored in Eppendorf tubes at –20 °C. When all samples were prepared, cryoconite was homogenised using an agate pestle and mortar and dried in a thin layer on
130 a Petri dish at 45 °C. The duration of drying was 8 hours.

For the analyses of δ¹⁵N in organic matter (OM), cryoconite was transferred without any other preparation into pre-weighed tin capsules (Costech 41077, 5 × 9 mm) and weighed. The average amount of cryoconite used for analyses was ~ 31 mg. For the analyses of δ¹³C in organic matter, 11–12 mg of cryoconite was transferred into pre-weighed silver capsules (Elemental Analyses, 8 × 5 mm, D2008) and carbonates (e.g. calcite, dolomite) were
135 dissolved using 10% HCl moistened with diH₂O. The acid was pipetted into the capsules followed by additions of 10, 20, 30, 50 and 100 mL with drying after each addition according to Brodie et al. (2011) with the modification after Vindušková et al. (2019). After the last acid addition, samples were left drying at 50 °C for 17 hours. After drying, silver capsules were inserted into tin capsules and put into a desiccator for 10–20 days.

2.3 Stable isotopes analyses

The δ¹³C and δ¹⁵N values in all samples were analysed using a Flash 2000 elemental analyser (ThermoFisher Scientific). Released gasses (NO_x, CO₂) separated in a GC column were transferred to an isotope-ratio mass spectrometer Delta V Advantage (ThermoFisher Scientific) through a capillary by Continuous Flow IV system (ThermoFisher Scientific). The stable isotope results are expressed in standard delta notation (δ) with samples measured relatively to Pee Dee Belemnite for carbon isotopes and atmospheric N₂ for nitrogen isotopes and
140 normalized to a calibration curve based on international standards IAEA-CH-6, IAEA-CH-3, IAEA 600 for carbon and IAEA-N-2, IAEA-N-1, IAEA-NO-3 for nitrogen. The calibration curve for analyses of cryoconite was based on the international standard ST-Soil Standard (Peaty) and ST-Soil Standard (SSclay). Analytical precision as a long reproducibility for standards was within ±0.03 ‰ for δ¹³C and ±0.02 ‰ for δ¹⁵N.

The isotopic values of nitrogen in OM as well as organic carbon (decarbonized cryoconite) in cryoconite were
150 used as reference to isotopic composition of potential food source for invertebrates.



2.4 X-Ray Diffraction

To reveal the differences in geological composition of sediment among the three glaciers, mineral phases of homogenized sediment were determined by an X-Ray diffraction analysis on the PANalytical X'PertPro (PW3040/60) with an X'Celerator detector. The measurements were conducted under following conditions:
155 radiation – CuK α , 40 kV, 30 mA, angular range –3–70° 2 θ , step 0.02°/150 s. The results were evaluated using a X'Pert HighScore Plus software 1.0d program with a JCPDS PDF-2 (ICDD, 2002) database.

2.5 Community structure

For the species identification, at least 10 cm³ of cryoconite was used from each sample. Tardigrades were collected using a glass Pasteur pipette and the first observation was made under a stereomicroscope (Olympus SZ 51).
160 Immediately after collecting, clean tardigrades were transferred on glass slides and mounted in a small drop of the Hoyer's medium (Anderson, 1954; Ramazzotti and Maucci, 1983). After one day of drying in 56 °C, tardigrades were identified under a light microscope with phase contrast (Olympus BX53) associated with a digital camera ARTCAM 500. Tardigrades were classified to the dominant feeding behaviour groups according to Guidetti et al. (2012), Guil and Sanchez-Moreno (2013), Hallas and Yeates (1972) and Kosztyła et al. (2016). Specimens of
165 bdelloid rotifers were identified using a compound light microscope when moving (identification is performed using the morphology of their cirri and trophi). Identification of feeding behaviour of rotifers was primarily conducted following the monography by Doner (1965). For the identification of eukaryotic primary producers, small drops of thawed and well-mixed cryoconite were placed on the mount. Afterwards, algae and cyanobacteria were identified using a light microscope Olympus BX51 equipped with Nomarski interference contrast and the
170 digital camera Olympus EOS 700D. Identification was based on publications by Starmach (1966), Ettl and Gärtner (2014) and Wehr et al. (2015).

2.6 Statistical Analysis

All statistical analyses were conducted in R version 3.5.3 (R Development Core Team, 2018). To test the differences between $\delta^{15}\text{N}$ isotopic values of tardigrades and rotifers, Kruskal–Wallis rank sum test was used.
175 Before the correlation coefficient tests were applied, Shapiro–Wilk test was used to test the normal distribution of the data. Therefore, Spearman's rank correlation coefficient and Pearson's product-moment correlation coefficient were compared between isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of cryoconite and isotopic values of tardigrades and rotifers. Correlation coefficients using Shannon–Wiener Index of Diversity were used to reveal differences between species composition and isotopic values ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) of tardigrades. To compare isotopic values of tardigrades, rotifers and cryoconite from each sampling site, One-Way ANOVA and Tukey multiple comparisons of means were
180 applied. For the purpose of statistical analyses, all replicates from the same sampling campaigns were averaged.

3 Results

3.1 Isotopic signatures

The isotopic signature of nitrogen showed significant differences in $\delta^{15}\text{N}$ between tardigrades and rotifers in all
185 samples (Kruskal–Wallis chi-squared = 12.685, $df = 1$, $n = 22$, p -value = 0.00037). All measured $\delta^{15}\text{N}$ values of tardigrades revealed lower $\delta^{15}\text{N}$ values than rotifers as shown in Fig. 1 and Table 1. Furthermore, we measured



$\delta^{15}\text{N}$ values of nitrogen in organic matter from cryoconite, but there was no significant relation with $\delta^{15}\text{N}$ values of tardigrades and rotifers found.

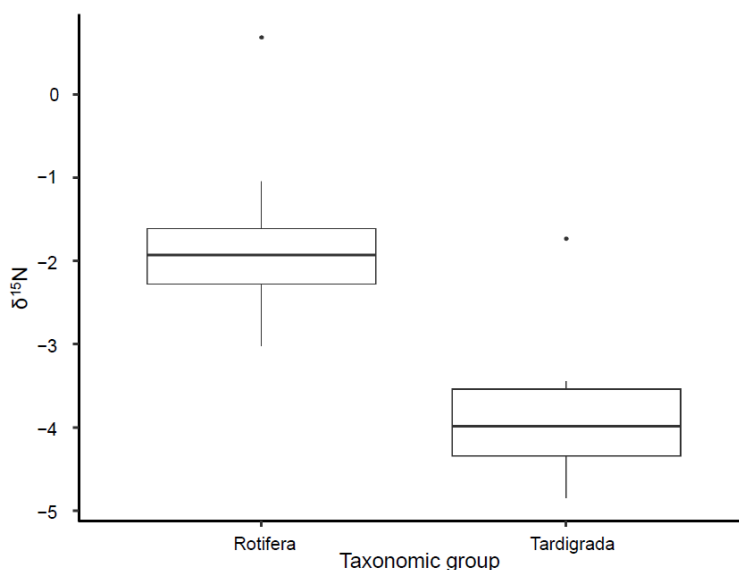


Figure 1. Differences in $\delta^{15}\text{N}$ between tardigrades and rotifers analysed by Kruskal–Wallis rank sum test. The diagram displays medians and distribution of measured $\delta^{15}\text{N}$ values. The whiskers represent the lowest and highest measured values. The outliers represent $\delta^{15}\text{N}$ values from one replicate from Ebbabreen.

Table 1. Description of samples and isotopic values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of tardigrades, rotifers and cryoconite. Isotopic values are presented as ‰ and related to the international standards Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen. The $\delta^{13}\text{C}^*$ are values of cryoconite after carbonate removal. The T and R letters in Dominance column signify: T (dominance of tardigrades), R (dominance of rotifers), T = R (equal proportion of tardigrades and rotifers) in particular samples collected during sampling campaigns.

Glacier	Tardigrades		Rotifers		Cryoconite		Dominances phylum
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}^*$	
Sven	-3.55	-23.75	-1.57	-27.09	-2.20	-22.65	T
	-4.04	-26.36	-1.81	-26.69	-2.17	-22.08	R
	-3.52	-26.33	-2.04	-31.16	-2.24	-24.09	R
	-4.39	-26.45	-1.04	-28.53	-1.64	-23.25	R
Nordenskiöld	-4.39	-22.91	-2.20	-23.36	-3.58	-21.56	T = R
	-3.45	-23.30	-1.72	-23.25	-2.30	-20.56	T = R
	-3.76	-22.58	-2.30	-23.02	-2.98	-22.52	T = R
	-4.15	-24.78	-3.02	-25.19			
	-3.93	-25.11					
Ebba	-1.73	-24.19	0.69	-26.32	-2.30	-22.24	R
	-4.85	-25.22	-2.38	-25.13	-4.29	-23.31	T
	-4.33	-25.15					



Regarding the isotopic signatures of carbon, we found a positive correlation of the $\delta^{13}\text{C}$ values of decarbonized cryoconite and the $\delta^{13}\text{C}$ of rotifers ($r = 0.83$, $n = 19$, p -value = 0.006) (Fig. 2). The respective relationship among
200 tardigrades was not significant ($n = 21$, p -value > 0.05).

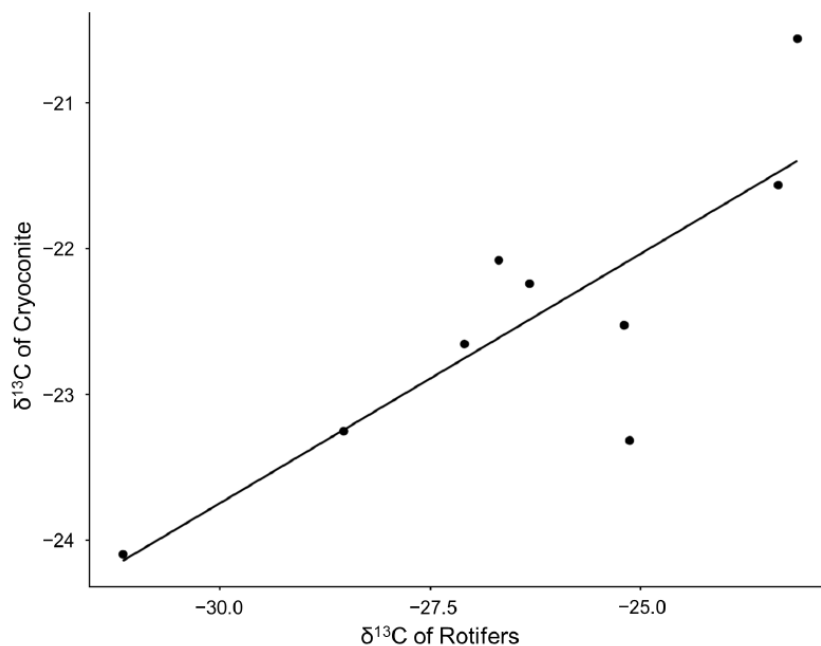


Figure 2. Correlation between $\delta^{13}\text{C}$ of rotifers and cryoconite with the linear regression line.

In all samples, differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tardigrades and rotifers among glaciers were tested using ANOVA test with the mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the Tukey multiple comparisons of means. These analyses showed a significant difference in $\delta^{13}\text{C}$ values of rotifers between glaciers (p -value = 0.029) (Fig. 3a), mostly between
205 Nordenskiöldbreen and Svenbreen (p -value = 0.025). All other tests did not reveal any significant pattern (Fig. 3).

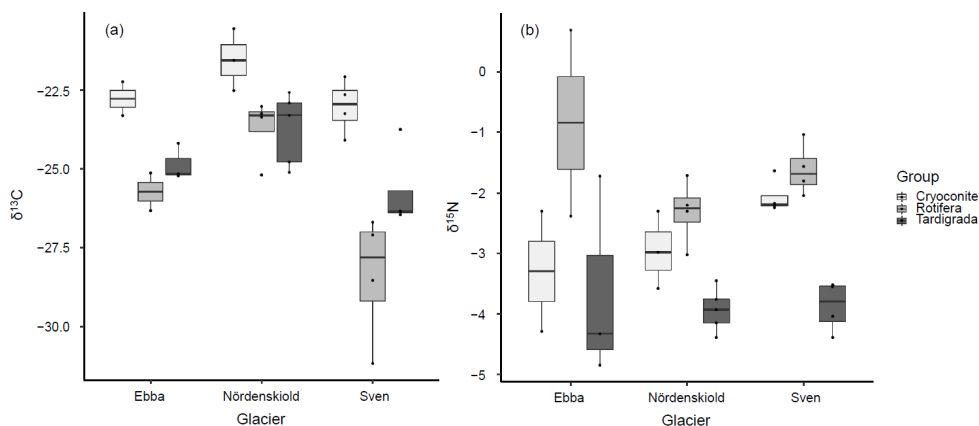


Figure 3. Distribution of $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) isotopic values in tardigrades ($n = 12$), rotifers ($n = 10$) and cryoconite ($n = 9$) among glaciers.



3.2 Composition of cryoconite

210 X-Ray diffraction of cryoconite showed that the glaciers differ in mineral composition. Svenbreen has a low amount of dolomite and amphibole which are dominantly found within the metamorphic basement rocks around Ebbabreen and Nordenskiöldbreen. The distribution of minerals within each glacier is shown in Table A1 (Appendices). The ANOVA analysis applied on the mean $\delta^{13}\text{C}$ values of OC in cryoconite did not reveal any significant difference between glaciers. Due to the logistical issues, pH in cryoconite holes was measured only on Svenbreen and Nordenskiöldbreen with values $\text{pH} < 7$.

215 3.3 Community structure

During the collection of animals for isotopic analyses, we also counted the proportion in abundances of tardigrades and rotifers within all replicates. A difference in relative abundance lower than 5 % was considered an equal proportion (Table 1).

220 Regarding the species composition of primary producers, we identified representatives of algae and cyanobacteria from all samples. In case of algae, we observed mostly Zygnematales (*Ancylonema* sp., *Mesotaenium* sp.). Oscillatoriales (*Phormidium* sp.), Nostocales (*Nostoc*) and Synechococcales (*Leptolyngbya* sp.) were observed as the most abundant cyanobacteria. Quantification of primary producers was not done due to the preservation by freezing which presumably had a taxon-specific effect on the survival of cells of the phototrophs.

225 During the division of consumers into trophic groups, only tardigrades were identified in a sufficient number for analyses. Species composition of rotifers was not analysed because of the majority of individuals occurring in a dormant stage, which made it impossible to identify the required number for analyses. Rotifers found within the samples were identified as *Macrotrachella* sp. and *Adineta* sp. Regarding tardigrades, we identified 1117 individuals which were divided into three trophic groups: *Pilatobius* sp. as microbivorous (41.24 %), hypsibids as herbivorous (53.33 %) and isohypsibids as omnivorous (5.43 %). As shown in Fig. 4, the composition of tardigrade trophic groups is not equal among glaciers.

Correlations between trophic groups of tardigrades and isotopic values ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) of tardigrades and decarbonized cryoconite did not reveal any significant relationship.

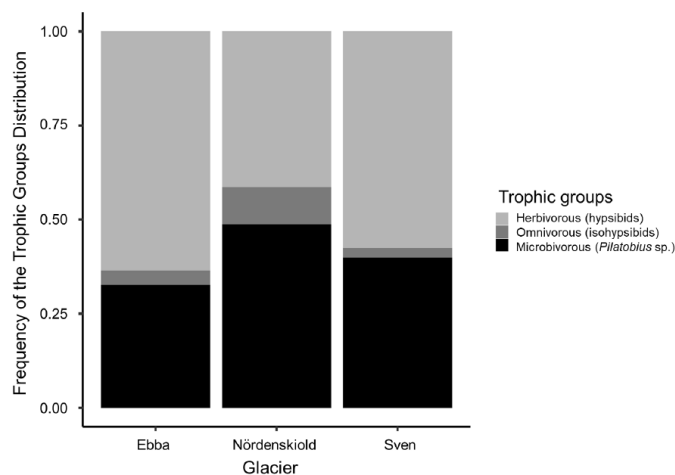




Figure 4. Bar plot visualization of tardigrades' trophic groups relative distribution among glaciers.

4 Discussion

235 4.1 Isotopic signatures and trophic relations of cryoconite consumers

There are only three studies focusing on the isotopic composition of polar tardigrades and rotifers, all conducted in Antarctica: the research of Almela et al. (2019) and Velázquez et al. (2017), who focused on microbial mats in the non-glaciated part of Byers Peninsula and the research of Shaw et al. (2018), who focused on the non-glaciated part of the Taylor Valley. Isotopic composition of tardigrades and rotifers from Taylor Valley was nearly the same reflecting similarities in composition of their diet. On the contrary, in the study of Almela et al. (2019), tardigrades reflected lower values of $\delta^{15}\text{N}$ and lower values of $\delta^{13}\text{C}$ compared to rotifers, and in the study of Velázquez et al. (2017) tardigrades reflected lower $\delta^{15}\text{N}$ and higher values of $\delta^{13}\text{C}$ than rotifers. Moreover, in both studies from Byers Peninsula, the $\delta^{15}\text{N}$ values of proposed food was always higher than the $\delta^{15}\text{N}$ values of consumers. It is known that the variability in absolute isotopic composition among systems has various reasons, for example differences in the isotopic composition of the nutrient pool (Montoya et al., 1990). The isotopic values of consumers can also vary based on the seasonal variability in isotopic values of their food (Zah et al., 2001). In cryoconite holes, the input of nutrients as well as changes in the community structure of microbes vary during the season (Säwström et al., 2002; Stibal et al., 2008). Therefore, the variability in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values as well as variation in the distribution of isotopic composition as described in Antarctic studies was expected.

250 Our results showed that rotifers revealed higher values of ^{15}N isotope compared to tardigrades suggesting potential differences in $\delta^{15}\text{N}$ composition of their diet. Predominantly, higher values of $\delta^{15}\text{N}$ usually indicate a higher trophic level (Kling et al., 1992; Wada, 2009). However, based on the observed food preferences of tardigrades and rotifers from cryoconite (Střítecká and Devetter, 2015; Zawierucha et al., 2016) we cannot assume a strict trophic division of their food. Primarily, tardigrades found in samples were identified as probably

255 microbivorous (*Pilatobius* sp.), herbivorous (hypsibids) and omnivorous (isohypsibids) species. Nevertheless, we cannot exclude that *Pilatobius* sp. with its ventrally located mouth does consume algae during scratching biofilms from the surface of granules. The same applies to isohypsibids, which have a relatively wide buccal tube and can utilise more food sources including algae, protozoans and other small invertebrates. Moreover, in the laboratory cultures, all studied groups of tardigrades feed on algae (Kosztyła et al., 2016). Rotifers were

260 mostly identified as filter feeders (*Macrotrachella* sp.) or scrapers (*Adineta* sp.) (Herzig et al., 2006). However, *Adineta* sp. never exceed 10 % of their total amount and the living specimens can be well distinguished from *Macrotrachella* sp. during collecting. The differences in $\delta^{15}\text{N}$ between both consumers could suggest $\delta^{15}\text{N}$ enrichment in food for rotifers caused by consumption of bacteria or DOM enriched in $\delta^{15}\text{N}$. This assumption is supported by Nagarkar et al. (2004) and Kohler et al. (2018), who described that cyanobacteria have higher

265 content of proteins and a higher $\delta^{15}\text{N}$ values typical for nitrogen fixing organisms. The other potential explanation of the observed pattern is tardigrades' consumption of algae, which can vary in $\delta^{15}\text{N}$ depending on their C:N ratio (Adams and Sterner, 2000). In cryoconite holes, consumers are probably highly limited by the lack of nutrients and the small size of food. Therefore, the ingested food composition may shift from its optimum.



270 Generally, the $\delta^{13}\text{C}$ values of tardigrades and rotifers in our study were lower than $\delta^{13}\text{C}$ values in decarbonized
cryoconite. This difference is similar as described by Almela et al. (2019) and Velázquez et al. (2017) in Antarctic
microbial mats. Nevertheless, it contrasts with the fundamental literature (Peterson and Fry, 1987; Wada, 2009)
as well as with the study of Shaw et al. (2018) focusing on Antarctic soil microbial mats, which presented $\delta^{13}\text{C}$
275 values of tardigrades and rotifers similar or slightly higher than $\delta^{13}\text{C}$ values of their potential diet. The differences
between these results could point to differences in carbon fractionation on glacier surface or within tardigrades
and rotifers in comparison with freshwater zooplankton and soil microfauna. The observed correlation between
cryoconite and rotifers in $\delta^{13}\text{C}$ could also suggest that rotifer food (suspended bacteria) is the representative
for much of the cryoconite organic carbon. On the other hand, such correlation in tardigrades is not significant, because
280 they feed on primary producers (algae and cyanobacteria) related mostly to air CO_2 , which has the same $\delta^{13}\text{C}$
everywhere. It may therefore serve as indirect evidence for the bacterivory of rotifers and the algalivory of
tardigrades in cryoconite holes.

4.2 Variations in isotopic signatures among glaciers

As shown in the results, the isotopic composition of tardigrades, rotifers and cryoconite between the replicates and
between the glaciers differ. Furthermore, we observed variations in the proportional representation of tardigrades
285 and rotifers, and in the community structure of tardigrades among the glaciers and the replicates as well.

The variability in $\delta^{13}\text{C}$ values could indicate specific nutrient requirements of primary producers affected by the
variability in spatial characteristics of the glacier surroundings and consequent variations in the nutrient input onto
glacier surface (Bagshaw et al., 2013; Hagen et al., 1993). As presented by Post (2002), who focused on freshwater
food webs, larger studied lakes evinced higher $\delta^{13}\text{C}$ values than small lakes suggesting higher occurrence of
290 autochthonous carbon input favouring heavier ^{13}C isotope signature of the food web. It is highly possible that due
to its smaller size, Svenbreen presumably has a higher allochthonous input of nutrients in the form of organic
matter from adjacent habitats, which causes depletion of ^{13}C in isotopic signature because of a longer chain of
fractionations favouring lighter ^{12}C typical for allochthonous source of carbon (Peterson and Fry, 1987; Post,
2002). Consequently, the depletion in ^{13}C of consumers from Svenbreen could signify consumption of DOM from
295 the primary production or detritus (Abelson and Hoering, 1961; Iakovenko et al., 2015; Macko and Estep, 1984).
Oppositely, tardigrades and rotifers from Ebbabreen and Nordenskiöldbreen had higher $\delta^{13}\text{C}$ than consumers from
Svenbreen. We assume that this increase was caused by a larger size of these glaciers and a potential larger
component of autochthonous production (Stibal et al., 2010) which uses “heavier” carbon from atmospheric CO_2
(Post, 2002) and has a shorter chain of transformations and discriminations against $\delta^{13}\text{C}$ during the assimilation of
300 inorganic matter (Michener and Lajtha, 2008). However, the observed variations in $\delta^{13}\text{C}$ among glaciers could also
reflect a different proportional representation of herbivorous and other consumers (DeNiro and Epstein, 1978;
Michener and Lajtha, 2008), or a dynamical character of sudden processes occurring on the glacial surface
including changes in the input of organic and inorganic matter (Chandler et al., 2015; Telling et al., 2012;
Wagenbach et al., 1996; Zah et al., 2001). Therefore, further investigations are essential.

305 Considering the close $\delta^{13}\text{C}$ isotopic values of consumers from Ebbabreen and Nordenskiöldbreen, the observed
pattern could signify an overlap in food preferences of both consumers (Almela et al., 2019; DeNiro and Epstein,
1978; Mialet et al., 2013; Ricci, 1984; Velázquez et al., 2017). The non-strict division of consumed food between



310 tardigrades and rotifers was apparent in various feeding experiments (Mialet et al., 2013; Ricci, 1984; Strítecká and Devetter, 2015) even though Almela et al. (2019) demonstrated that tardigrades were related to larger particles (e.g. algae) and rotifers mostly to smaller ones (e.g. POM). Similarities in distribution of $\delta^{13}\text{C}$ in comparison with differences in $\delta^{15}\text{N}$ between tardigrades and rotifers could also indicate that rotifers consume DOC originating from extracellular exudates of algae or cyanobacteria, but the source of nitrogen (e.g. bacteria, cyanobacteria and organic detritus) is different.

315 Regarding differences in $\delta^{15}\text{N}$, some samples evinced high presence of cyanobacteria *Leptolyngbya* sp., and the high $\delta^{15}\text{N}$ could refer to a higher content of ^{15}N in cyanobacteria populations (Darby and Neher, 2012). However, the observed variation could also be a result of different mineral composition of cryoconite among glaciers located in different parts of Billefjorden Fault Zone. Especially in samples from Svenbreen, we detected a very low amount of amphibole and dolomite which were common on Ebbabreen and Nordenskiöldbreen. Considering higher potential solubility of minerals due to acidic pH of cryoconite holes (4.48–5.9) and differences in mineral 320 composition of cryoconite aggregates among glaciers, the differences in the community structure of microbial communities and consequent isotopic signatures could also be related to the variability in composition of available minerals released by biogeochemical weathering (Barker and Banfield, 1998; Carson et al., 2007; Roberts et al., 2004; Zawierucha et al., 2019b). Moreover, upper parts of Svenbreen were covered by snow during sampling, whereas before and during sampling of Ebbabreen, the air temperature increased to 8.8 °C (according to the meteorological station at Bertilbreen). Therefore, the higher content of $\delta^{15}\text{N}$ in these samples could also be caused 325 by presence of NO_3^- in the meltwater (Hodson et al., 2005).

5 Conclusions

This study presents the first description of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic composition of cryoconite consumers and their potential food. Despite the variability in distribution of isotopic values, we showed that $\delta^{15}\text{N}$ differs among 330 tardigrades and rotifers. The $\delta^{13}\text{C}$ values reveal variability in their distribution among the taxa as well as glaciers. In particular, the $\delta^{15}\text{N}$ values present evidence of differences in feeding behaviour between both groups. The $\delta^{13}\text{C}$ values provide evidence that the input and source of carbon among glaciers may differ and these differences can influence the isotopic composition of $\delta^{13}\text{C}$ in cryoconite as well as in animals. We also revealed a significant correlation between organic carbon from cryoconite and rotifers, which indicates that rotifers may be related 335 more to cryoconite carbon from bacteria than tardigrades, which are considered to be more herbivorous. Nevertheless, further research is required to elucidate the cryoconite trophic levels, the entire diet of the consumers and their contribution to supraglacial nutrient pathways. Another outcome of this study is the introduction of a modified technique of sample preparation avoiding procedures such as sugar centrifugation or oven drying.

340



6 Appendices

Table A1. Mineral composition in particular samples analysed by X-Ray diffraction. X letter means presence of the mineral, XX means high presence of the mineral. The sign (–) means that the mineral was not detected.

Sample	Quartz	Plagioclase	K-Feldspar	Amphibole	Dolomite	Muscovite/Illite	Chlorite
SL1	XX	X	X	–	–	XX	X
SU1	XX	X	X	–	–	X	X
SL2	XX	X	X	–	–	XX	XX
SU2	XX	X	X	X	X	XX	XX
NL1	XX	X	X	X	X	XX	XX
NU1	XX	X	X	X	X	XX	XX
NL2	XX	X	X	X	X	XX	XX
EL2	X	X	X	X	XX	XX	XX
EU2	XX	X	X	X	X	XX	XX

345 *Code availability.* All codes related to figures and analyses were made in R (version 3.5.1) and are available upon request of the corresponding author.

Data availability. All data about isotopic composition, trophic groups composition and mineral composition are available upon request to the corresponding author. Meteorological data from Bertilbreen were kindly provided by Associate Professor Kamil Láška and all requests must be sent to him.

350 *Author contributions.* JDŽ, TJ, JT and KZ developed the study design. The field sampling was conducted by TJ and JDŽ. The stable isotopes analyses were conducted by TJ, JT and LV. The identification of trophic groups of tardigrades were conducted by TJ and KZ. The identification of rotifers was conducted by MD. TJ compiled and processed all presented data and prepared the manuscript contributing revisions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

355 *Acknowledgements.* We thank the Research Centre for Radiogenic and Stable Isotopes at the Faculty of Science, Charles University and the Laboratory of X-ray Diffraction of the Institute of Geochemistry, Mineralogy and Mineral Resources of Faculty of Science, Charles University (operator Petr Drahota, Ph.D.) for all support with cryoconite analyses. Furthermore, we would like to thank to Associate Professor Pavel Škaloud, Jakub Štenc, Pavel Pipek, Petra Seifertová, Helena Hubáčková, Jan Soumar, Dan Vondrák and David Novotný for their
360 specialized help with the determination of primary producers, statistics, lyophilization and manuscript preparation. We also want to thank to Associate Professor Kamil Láška who kindly provided meteorological data from Bertilbreen. Special thanks go to the Cryosphere Ecology Group (cryoeco.eu) and the Department of Ecology at Charles University.

Financial support. This research was supported by the Centre for Polar Ecology and Czech Arctic Polar
365 Infrastructure of University of South Bohemia – Josef Svoboda station at Svalbard (project CzechPolar LM2015078 supported by Ministry of Education, Youth and Sports); Foundation Nadání Josefa, Marie a Zdenky Hlávkových; Mobility Funds of Charles University; Internationalization Funding of Charles University; Centre for Geosphere Dynamics (UNCE/SCI/006) and the Operational Programme Prague – Competitiveness (Project



370 CZ.2.16/3.1.00/21516). Studies on organic matter, role of invertebrates and productivity of glacial ecosystems
was supported via grant NCN 2018/31/B/NZ8/00198 awarded to KZ.

References

- Abelson, P. H. and Hoering, T. C.: Carbon isotope fractionation in formation of amino acids by photosynthetic organisms, *P. Natl. Acad. Sci. USA*, 47, 623, doi:10.1073/pnas.47.5.623, 1961.
- 375 Adams, T. S. and Sterner, R. W.: The effect of dietary nitrogen content on trophic level ¹⁵N enrichment, *Limnol. Oceanogr.*, 45, 601–607, doi:10.4319/lo.2000.45.3.0601, 2000.
- Almela, P., Velázquez, D., Rico, E., Justel, A., and Quesada, A.: Carbon pathways through the food web of a microbial mat from Byers Peninsula, Antarctica, *Front. Microbiol.*, 10, 628, doi:10.3389/fmicb.2019.00628, 2019.
- 380 Anderson, L. E.: Hoyer's solution as a rapid permanent mounting medium for bryophytes, *The Bryologist*, 57, 242–244, 1954.
- Bagshaw, E. A., Tranter, M., Fountain, A. G., Welch, K., Basagic, H. J., and Lyons, W. B.: Do cryoconite holes have the potential to be significant sources of C, N, and P to downstream depauperate ecosystems of Taylor Valley, Antarctica?, *Arct. Antarct. Alp. Res.*, 45, 440–454, doi:10.1657/1938-4246-45.4.440, 2013.
- 385 Bardgett, R. D., Richter, A., Bol, R., Garnett, M. H., Bäuml, R., Xu, X., Lopez-Capel, E., Manning, D. A., Hobbs, P. J., Hartley, I. R., and Wanek, W.: Heterotrophic microbial communities use ancient carbon following glacial retreat, *Biol. Letters*, 3, 487–490, doi:10.1098/rsbl.2007.0242, 2007.
- Barker, W. W. and Banfield, J. F.: Zones of chemical and physical interaction at interfaces between microbial communities and minerals: a model, *Geomicrobiol. J.*, 15, 223–244, doi:10.1080/01490459809378078, 1998.
- 390 Blair, N., Leu, A., Muñoz, E., Olsen, J., Kwong, E., and Des Marais, D.: Carbon isotopic fractionation in heterotrophic microbial metabolism, *Appl. Environ. Microb.*, 50, 996–1001, 1985.
- Brodie, C. R., Leng, M. J., Casford, J. S., Kendrick, C. P., Lloyd, J. M., Yongqiang, Z., and Bird, M. I.: Evidence for bias in C and N concentrations and $\delta^{13}\text{C}$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods, *Chem. Geol.*, 282, 67–83, doi:10.1016/j.chemgeo.2011.01.007, 2011.
- 395 Carson, J. K., Rooney, D., Gleeson, D. B., and Clipson, N.: Altering the mineral composition of soil causes a shift in microbial community structure, *FEMS Microbiol. Ecol.*, 61, 414–423, doi:10.1111/j.1574-6941.2007.00361.x, 2007.
- 400 Chandler, D. M., Alcock, J. D., Wadham, J. L., Mackie, S. L., and Telling, J.: Seasonal changes of ice surface characteristics and productivity in the ablation zone of the Greenland Ice Sheet, *The Cryosphere*, 9, 487–504, doi:10.5194/tc-9-487-2015, 2015.
- Cook, J., Edwards, A., Takeuchi, N., and Irvine-Fynn, T.: Cryoconite: the dark biological secret of the cryosphere, *Prog. Phys. Geog.*, 40, 66–111, doi:10.1177/0309133315616574, 2016.
- 405 Darby, B. J. and Neher, D. A.: Stable isotope composition of microfauna supports the occurrence of biologically fixed nitrogen from cyanobacteria in desert soil food webs, *J. Arid Environ.*, 85, 76–78, doi:10.1016/j.jaridenv.2012.06.006, 2012.



- DeNiro, M. J. and Epstein, S.: Influence of diet on the distribution of carbon isotopes in animals, *Geochim. Cosmochim. Ac.*, 42, 495–506, doi:10.1016/0016-7037(78)90199-0, 1978.
- DeNiro, M. J. and Epstein, S.: Influence of diet on the distribution of nitrogen isotopes in animals, *Geochim. Cosmochim. Ac.*, 45, 341–351, doi.org/10.1016/0016-7037(81)90244-1, 1981.
- 410 De Smet, W. H. and Van Rompu, E. A.: Rotifera and Tardigrada from some cryoconite holes on a Spitsbergen (Svalbard) glacier, *Belg. J. Zool.*, 124, 27–27, 1994.
- Devetter, M.: Clearance rates of the bdelloid rotifer, *Habrotrocha thienemanni*, a tree-hole inhabitant, *Aquat. Ecol.*, 43, 85–89, doi:10.1007/s10452-007-9160-9, 2009.
- Donner, J.: *Ordnung Bdelloidea (Rotatoria, Rädertiere)*, Akademie-Verlag, Berlin, Germany, 297, 1965.
- 415 Ekblad, A. and Högberg, P.: Analysis of $\delta^{13}\text{C}$ of CO_2 distinguishes between microbial respiration of added C4-sucrose and other soil respiration in a C3-ecosystem, *Plant Soil*, 219, 197–209, doi:10.1023/A:1004732430929, 2000.
- Ettl, H. and Gärtner, G.: *Syllabus der Boden-, Luft- und Flechtenalgen*, Springer Spektrum, Berlin, Germany, 2014.
- 420 Foreman, C. M., Sattler, B., Mikucki, J. A., Porazinska, D. L., and Priscu, J. C.: Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica, *J. Geophys. Res-Bioge.*, 112, G04S32, doi:10.1029/2006JG000358, 2007.
- Guidetti, R., Altiero, T., and Rebecchi, L.: On dormancy strategies in tardigrades, *J. Insect Physiol.*, 57, 567–576, doi:10.1016/j.jinsphys.2011.03.003, 2011.
- 425 Guidetti, R., Altiero, T., Marchioro, T., Amade, L. S., Avdonina, A. M., Bertolani, R., and Rebecchi, L.: Form and function of the feeding apparatus in Eutardigrada (Tardigrada), *Zoomorphology*, 131, 127–148, doi:10.1007/s00435-012-0149-0, 2012.
- Guil, N. and Sanchez-Moreno, S.: Fine-scale patterns in micrometazoans: tardigrade diversity, community composition and trophic dynamics in leaf litter, *Syst. Biodivers.*, 11, 181–193, doi:10.1080/14772000.2013.798370, 2013.
- 430 Hagen, J., Liestøl, O., Roland, K., and Jørgensen, T.: *Glacier Atlas of Svalbard and Jan Mayen*, Norsk Polarinstitutt, Oslo, Norway, 129, 1993.
- Hallas, T. E. and Yeates, G. W.: Tardigrada of the soil and litter of a Danish beech forest, *Pedobiologia*, 12, 287–304, 1972.
- 435 Herzig, A., Gulati, R. D., Jersabek, C. D., and May, L. (Eds.): *Rotifera X: Rotifer Research: Trends, New Tools and Recent Advances*, 181, Springer Science & Business Media, Berlin, Germany, 2006.
- Hodson, A. J., Mumford, P. N., Kohler, J., and Wynn, P. M.: The High Arctic glacial ecosystem: new insights from nutrient budgets, *Biogeochemistry*, 72, 233–256, doi:10.1007/s10533-004-0362-0, 2005.
- 440 Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J., and Sattler, B.: Glacial ecosystems, *Ecol. Monogr.*, 78, 41–67, doi:10.1890/07-0187.1, 2008.
- Hood, E., Fellman, J., Spencer, R. G., Hernes, P. J., Edwards, R., D'Amore, D., and Scott, D.: Glaciers as a source of ancient and labile organic matter to the marine environment, *Nature*, 462, 1044–1047, doi:10.1038/nature08580, 2009.



- 445 Iakovenko, N. S., Smykla, J., Convey, P., Kašparová, E., Kozeretka, I. A., Trokhymets, V., Dykyy, I., Plewka, M., Devetter, M., Duriš, Z., and Janko, K.: Antarctic bdelloid rotifers: diversity, endemism and evolution, *Hydrobiologia*, 761, 5–43, doi:10.1007/s10750-015-2463-2, 2015.
- International Centre for Diffraction Data, JCPDS PDF-2 Database, ICDD, Newtown Square, Pennsylvania, USA, 2002.
- 450 Kaczmarek, L., Jakubowska, N., Celewicz-Góldyn, S., and Zawierucha, K.: The microorganisms of cryoconite holes (algae, Archaea, bacteria, cyanobacteria, fungi, and Protista): a review, *Polar Rec.*, 52, 176–203, doi:10.1017/S0032247415000637, 2016.
- Klekowski, R. Z. and Opaliński, K. W.: Matter and energy flow in Spitsbergen ornithogenic tundra, *Polar Res.*, 4, 187–197, doi:10.3402/polar.v4i2.6931, 1986.
- 455 Kling, G. W., Fry, B., and O'Brien, W. J.: Stable isotopes and planktonic trophic structure in arctic lakes, *Ecology*, 73, 561–566, doi:10.2307/1940762, 1992.
- Kohler, T. J., Stanish, L. F., Liptzin, D., Barrett, J. E., and McKnight, D. M.: Catch and release: Hyporheic retention and mineralization of N-fixing *Nostoc* sustains downstream microbial mat biomass in two polar desert streams, *Limnology and Oceanography Letters*, 3, 357–364, doi:10.1002/lol2.10087, 2018.
- 460 Kosztyła, P., Stec, D., Morek, W., Gąsiorek, P., Zawierucha, K., Michno, K., Ufir, K., Małek, D., Hlebowicz, K., Laska, A., Dudziak, M., Frohme, M., Prokop, Z. M., Kaczmarek, L., Michalczyk, Ł.: Experimental taxonomy confirms the environmental stability of morphometric traits in a taxonomically challenging group of microinvertebrates, *Zool. J. Linn. Soc.*, 178, 765–775, doi:10.1111/zoj.12409, 2016.
- Kutikova, L. A.: Bdelloid rotifers (Rotifera, Bdelloidea) as a component of soil and land biocenoses, *Biology Bulletin of the Russian Academy of Sciences*, 30, 271–274, doi:10.1023/A:1023811929889, 2003.
- 465 Macko, S. A. and Estep, M. L.: Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter, *Org. Geochem.*, 6, 787–790, doi:10.1016/0146-6380(84)90100-1, 1984.
- McCutchan, J. H., Lewis, W. M., Kendall, C., and McGrath, C. C.: Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur, *Oikos*, 102, 378–390, doi:10.1034/j.1600-0706.2003.12098.x, 2003.
- 470 Mialet, B., Majdi, N., Tackx, M., Azémar, F., and Buffan-Dubau, E.: Selective feeding of bdelloid rotifers in river biofilms, *PLOS One*, 8, e75352, doi:10.1371/journal.pone.0075352, 2013.
- Michener, R. and Lajtha, K. (Eds.): *Stable isotopes in ecology and environmental science*, John Wiley & Sons, New Jersey, USA, 594, 2008.
- Montoya, J. P., Horrigan, S. G., and McCarthy, J. J.: Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Chesapeake Bay, *Mar. Ecol-Prog. Ser.*, 65, 35–61, 1990.
- 475 Mueller, D. R., Vincent, W. F., Pollard, W. H., and Fritsen, C. H.: Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats, *Nova Hedwigia Beiheft*, 123, 173–198, 2001.
- Nagarkar, S., Williams, G. A., Subramanian, G., and Saha, S. K.: Cyanobacteria-dominated biofilms: a high quality food resource for intertidal grazers, *Asian Pacific Phycology in the 21st Century: Prospects and Challenges*, 89–95, Springer, Dordrecht, Germany, doi:10.1007/978-94-007-0944-7_12, 2004.
- 480 Nordenskiöld, A. E.: Cryoconite found 1870, July 19th–25th, on the inland ice, east of Auleitsivik Fjord, Disco Bay, Greenland, *Geol. Mag.*, Decade 2, 2, 157–162, 1875.



- O'Reilly, C. M., Verburg, P., Hecky, R. E., Plisnier, P. D., and Cohen, A. S.: Food web dynamics in stable isotope ecology: time integration of different trophic levels, *Handbook of Scaling Methods in Aquatic Ecology*, CRC Press, USA, 145–154, 2003.
- 485 Peterson, B. J. and Fry, B.: Stable isotopes in ecosystem studies, *Annu. Rev. Ecol. Syst.*, 18, 293–320, 1987.
- Ponsard, S. and Ardití, R.: What can stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) tell about the food web of soil macro-invertebrates?, *Ecology*, 81, 852–864, doi:10.1890/0012-9658(2000)081[0852:WCSINA]2.0.CO;2, 2000.
- Porazinska, D. L., Fountain, A. G., Nylen, T. H., Tranter, M., Virginia, R. A., and Wall D. H.: The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica, *Arct. Antarct. Alp. Res.*, 36, 84–91, doi:10.1657/1523-0430(2004)036[0084:TBABOC]2.0.CO;2, 2004.
- 490 Post, D. M.: Using stable isotopes to estimate trophic position: models, methods, and assumptions, *Ecology*, 83, 703–718, doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2, 2002.
- Ramazzotti, G. and Maucci, W.: *II Phylum Tardigradam*, Terza edizione riveduta e corretta, Memorie dell'Istituto Italiano di Idrobiologia Dott. Marco Marchi, Pallanza, Italy, 41, 1–1012, 1983.
- 495 R Development Core Team: R: a language and environment for statistical computing, Vienna, Austria, <http://www.R-project.org>, 2018.
- Ricci, C.: Culturing of some bdelloid rotifers, *Hydrobiologia*, 112, 45–51, doi:10.1007/BF00007665, 1984.
- Ricci, C.: Dormancy patterns in rotifers, *Hydrobiologia*, 446, 1–11, doi:10.1023/A:1017548418201, 2001.
- Roberts, J. A., Hughes, B. T., and Fowle, D. A.: Micro-scale mineralogic controls on microbial attachment to silicate surfaces: iron and phosphate mineral inclusions, Wanty, R.B. and Seal, R.R., eds. *Water-Rock Interaction*, in: *Proceedings of the Eleventh International Symposium on Water-Rock Interaction WRI-11*, Saratoga Springs, NY, USA, 27 June – 2 July 2004, 2, 1149–1153, 2004.
- 500 Šantrůček, J., Šantrůčková, H., Kaštovská, E., Květoň, J., Tahovská, K., Vrábl, D., and Vráblová, M.: *Stabilní isotopy biogeních prvků: použití v biologii a ekologii*, Academia, Czech Republic, 2018.
- 505 Sävström, C., Mumford, P., Marshall, W., Hodson, A., and Laybourn-Parry, J.: The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79 N), *Polar Biol.*, 25, 591–596, doi:10.1007/s00300-002-0388-5, 2002.
- Shaw, E. A., Adams, B. J., Barrett, J. E., Lyons, W. B., Virginia, R. A., and Wall, D. H.: Stable C and N isotope ratios reveal soil food web structure and identify the nematode *Eudorylaimus antarcticus* as an omnivore–predator in Taylor Valley, Antarctica, *Polar Biol.*, 41, 1013–1018, doi:10.1007/s00300-017-2243-8, 2018.
- 510 Starmach, K.: *Cyanophyta—sinice (Cyanophyta—blue–green algae)*, *Flora słodkowodna Polski*, 2, PAN – Państw. Wyd. Nauk., Warszawa, Poland, 753, 1966.
- Stibal, M., Tranter, M., Benning, L. G., and Řehák, J.: Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input, *Environ. Microbiol.*, 10, 2172–2178, doi:10.1111/j.1462-2920.2008.01620.x, 2008.
- 515 Stibal, M., Lawson, E. C., Lis, G. P., Mak, K. M., Wadham, J. L., and Anesio, A. M.: Organic matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet, *Ann. Glaciol.*, 51, 1–8, doi:10.3189/172756411795931958, 2010.



- 520 Stibal, M., Telling, J., Cook, J., Mak, K. M., Hodson, A., and Anesio, A. M.: Environmental controls on microbial abundance and activity on the Greenland ice sheet: a multivariate analysis approach, *Microbial Ecol.*, 63, 74–84, doi:10.1007/s00248-011-9935-3, 2012.
- Střítecká, M. and Devetter, M.: Sledování filtrační aktivity vířníků v kryokonitech, Senior high school thesis, Česko-anglické gymnasium, České Budějovice, Czech Republic, 2015.
- 525 Takeuchi, N., Kohshima, S., and Seko, K.: Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier, *Arct. Antarct. Alp. Res.*, 33, 115–122, doi:10.1080/15230430.2001.12003413, 2001.
- Telling, J., Anesio, A. M., Tranter, M., Stibal, M., Hawkings, J., Irvine-Fynn, T., Hodson, A., Butler, C., Yallop, M., and Wadham, J.: Controls on the autochthonous production and respiration of organic matter in cryoconite holes on high Arctic glaciers, *J. Geophys. Res-Bioge.*, 117, G01017, doi:10.1029/2011JG001828, 2012.
- 530 Velázquez, D., Jungblut, A. D., Rochera, C., Rico, E., Camacho, A., and Quesada, A.: Trophic interactions in microbial mats on Byers Peninsula, maritime Antarctica, *Polar Biol.*, 40, 1115–1126, doi:10.1007/s00300-016-2039-2, 2017.
- Vindušková, O., Jandová, K., and Frouz, J.: Improved method for removing siderite by *in situ* acidification before elemental and isotope analysis of soil organic carbon, *J. Plant Nutr. Soil Sc.*, 182, 82–91, doi:10.1002/jpln.201800164, 2019.
- 535 Vonnahme, T. R., Devetter, M., Žárský, J. D., Šabacká, M., and Elster, J.: Controls on microalgal community structures in cryoconite holes upon high-Arctic glaciers, Svalbard, *Biogeosciences*, 13, 659–674, doi:10.5194/bg-13-659-2016, 2016.
- Wada, E.: Stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios in aquatic ecosystems, *Proceedings of the Japan Academy, Series B*, 85, 98–107, doi:10.2183/pjab.85.98, 2009.
- 540 Wagenbach, D., Preunkert, S., Schäfer, J., Jung, W., and Tomadin, L.: Northward transport of Saharan dust recorded in a deep Alpine ice core, The impact of desert dust across the Mediterranean, Springer, Dordrecht, Germany, 291–300, doi:10.1007/978-94-017-3354-0_29, 1996.
- Wallace, R. L. and Snell, T. W.: Rotifera, Ecology and classification of North American Freshwater Invertebrates, Academic Press, USA, 173–235, 2010.
- 545 Wehr, J. D., Sheath, R. G., and Kociolek, J. P. (Eds.): Freshwater algae of North America: ecology and classification, Elsevier, USA, 2015.
- Williams, R. S. Jr. and Ferrigno, J. G.: State of the Earth’s cryosphere at the beginning of the 21st century: Glaciers, global snow cover, floating ice, and permafrost and periglacial environments, *Director*, 508, 344–6840, 2012.
- 550 Yoshii, K., Melnik, N. G., Timoshkin, O. A., Bondarenko, N. A., Anoshko, P. N., Yoshioka, T., and Wada, E.: Stable isotope analyses of the pelagic food web in Lake Baikal, *Limnol. Oceanogr.*, 44, 502–511, doi:10.4319/lo.1999.44.3.0502, 1999.
- 555 Zah, R., Burgherr, P., Bernasconi, S. M., and Uehlinger, U.: Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream, *Freshwater Biol.*, 46, 871–882, doi:10.1046/j.1365-2427.2001.00720.x, 2001.



- Zawierucha, K., Koliccka, M., Takeuchi, N., and Kaczmarek, Ł.: What animals can live in cryoconite holes? A faunal review, *J. Zool.*, 295, 159–169, doi:10.1111/jzo.12195, 2015.
- 560 Zawierucha, K., Ostrowska, M., Vonnahme, T. R., Devetter, M., Nawrot, A. P., Lehmann, S., and Koliccka, M.: Diversity and distribution of Tardigrada in Arctic cryoconite holes, *J. Limnol.*, 75, 545–559, doi:10.4081/jlimnol.2016.1453, 2016.
- Zawierucha, K., Buda, J., Pietryka, M., Richter, D., Łokas, E., Lehmann-Konera, S., Makowska, N., and Bogdziewicz, M.: Snapshot of micro-animals and associated biotic and abiotic environmental variables on the edge of the south-west Greenland ice sheet, *Limnology*, 19, 141–150, doi:10.1007/s10201-017-0528-9, 2018.
- 565 Zawierucha, K., Buda, J., Fontaneto, D., Ambrosini, R., Franzetti, A., Wierzgoń, M., and Bogdziewicz, M.: Fine-scale spatial heterogeneity of invertebrates within cryoconite holes, *Aquat. Ecol.*, 53, 179–190, doi:10.1007/s10452-019-09681-9, 2019a.
- Zawierucha, K., Baccolo, G., Di Mauro, B., Nawrot, A., Szczuciński, W., and Kalińska, E.: Micromorphological features of mineral matter from cryoconite holes on Arctic (Svalbard) and alpine (the Alps, the Caucasus) glaciers, *Polar Sci.*, 22, 100482, doi:10.1016/j.polar.2019.100482, 2019b.
- 570