

Dear prof. Middelburg, dear referees,

we are submitting a revised version of our manuscript. Thank you for all reviews, we believe that they helped to improve the quality of our manuscript. We tried to take them into account and incorporate all suggestions.

Here, we present all the changes and the answers to referees and scientific community.

Yours sincerely,

Tereza Jaroměřská

## Point-by-point response to the reviews

### Responses to prof. Bartels

**1. My chief concern is your discussion of the reason for the higher nitrogen content in rotifers than tardigrades and relating that to the potential contribution of bacteria to the cryoconite nitrogen. That seems a bit of a leap. I don't think you established that fully, so this is quite speculative. Right?**

Due to the absence of data about the exact composition of the diet of cryoconite consumers (it is the aim of our current research), we had to operate with various sources of empirical and experimental information about the feeding behaviour of studied groups. The suggestion of a higher  $\delta^{15}\text{N}$  in rotifers due to a feeding preference of bacteria comes partly from (1) the assumption of a  $^{14}\text{N}$  depletion in food for rotifers (bacteria, decomposers) compared to food for tardigrades (mostly algae) (e.g. Kling et al., 1992; Peterson and Fry, 1987; McCutchan et al., 2003) (2) the known higher content of  $^{15}\text{N}$  in cyanobacteria due to the fixation of atmospheric nitrogen (e.g. Gu and Alexander, 1993), (3) and from the commonly observed preference for bacterivory in rotifers. We also found a high representation of mostly herbivorous hypsibids which supports the suggestion that the majority of measured tardigrades had fed on algae (e.g. Bryndová et al., 2020). However, all knowledge about the consumers' food we have is based on laboratory experiments, studies of non-glacier species, or experiments with artificial particles. Moreover, as it is evident from the literature, there are many more factors influencing nitrogen stable isotopic ratios, in various ecosystems, including the fractionation within the body of consumer based on the isotopic composition of the food (Aberle and Malzahn, 2007), and thus further detailed analyses are needed in cryoconite holes.

Therefore, indeed, due to the lack of direct data on composition of the diet our causal explanation of the different isotopic body composition of rotifers remains speculative. We changed the text in order to make it explicit that the mechanism of heavy nitrogen increase in rotifers is an assumption based on indirect empirical data.

**2. Abstract (line 14): Explain dynamic nature: you mean the food webs themselves are dynamic?**

By "dynamic nature" we meant the dynamic nature of the formation and functioning of cryoconite holes. We changed the sentence to make this meaning more explicit.

**3. Discussion (line 244): Strange to say various causes then only give one example. Need to specify other causes.**

The sentence was modified and we added other examples.

**4. Discussion (line 265–270): Isn't it possible that some of the rotifers are carnivorous (or at least omnivorous)? That would also explain the pattern.**

The only known rotifer predators in cryoconite holes are representatives of the genus *Encentrum* (Monogonta) which are very rare there. We did not observe any in our study. The *Macrotrachella* species are as far as we know always microbivores/microfiltrators.

**5. Discussion (line 277): Unclear what you mean by representative. You mean bacteria is the predominant determinant of  $\delta^{13}\text{C}$ ?**

We do not know for sure if bacteria are the major food of rotifers causing this pattern. However, it was a logical conclusion based on the assumption and an indirect evidence of differences in carbon stable isotopic ratios related to the variability in the composition of organic matter between

habitats. Moreover, it makes sense in case of tardigrades' consumption of algae which uptake atmospheric CO<sub>2</sub> which is the same everywhere.

However, we modified the paragraph in order to make its meaning clear.

**6. Conclusions (line 334–335): This seems like a big logical jump. You don't really know that bacteria are the major contributor to C, so this becomes circular reasoning.**

We agree and we modified the conclusion and discussion to highlight that this suggestion is an indirect evidence which needs further investigation focused on the particular diet of cryoconite consumers.

### **Responses to Referee #2**

**1. Having said that, while I feel that the paper presents interesting and novel data, I also strongly feel that it lacks conclusiveness to warrant publication in a top journal like Biogeosciences. My main reason for this somewhat harsh conclusion is that the results presented on stable isotope signatures of tardigrades, rotifers and cryoconite of three gletsjer systems can only be described and compared, but that virtually all data needed to explain the observed patterns are lacking.**

We are aware that this manuscript is a pioneer descriptive study presenting the first view on stable isotopic composition of consumers and organic matter on the glacier surface with all limitations which it may pose on the generalization of our conclusions. Conclusions include parts where our results were overinterpreted and where our suggestions go beyond the safe ground provided by our data. We changed these parts accordingly.

**2. Isotopic signatures of nutrient and OM subsidies are missing, and so are isotopic signatures of primary producers vs detritus vs heterotrophic bacteria. I perfectly understand that these are not easy to obtain, but it was foreseeable that such data would be required to explain observed patterns, and methods exist for determining each of the above factors.**

We are aware that isotopic signatures of primary producers (ideally cyanobacteria and algae separately), heterotrophic bacteria and detritus in cryoconite holes are necessary to understand the trophic structure and processes leading to presented differences in isotopic signatures of cryoconite tardigrades and rotifers. However, the main purpose of our study was not to provide detailed description of trophic relationships and pathways in cryoconite holes but rather to obtain a general view on the stable isotopic composition of supraglacial tardigrades and rotifers and find out whether it is worth keeping this direction in further studies or not. We agree that there are other methods such as SIMS which may solve these problems. However, we did not choose such methods due to the quantity of material necessary for our purpose. We feel that we were not explicit in this subject and modified the text accordingly.

**3. I feel the authors should do an extra effort to make this manuscript better accessible and therefore more interesting for readers who are not familiar with the cryoconite holes. Aspects I wondered about, not knowing these specific systems, is what size and depth range such cryoconite holes have and how common they are, or better still, what % of a gletsjer's surface they make up.**

The diameter and depth of cryoconite holes are noticeably variable, but both usually range from a few centimetres to tens of centimetres (Gerdel and Drouet, 1960; Fountain et al., 2004; Zawierucha et al., 2019a).

Cryoconite holes usually cover around 7 % of the ablation zone of glaciers (the area with an ice loss exceeding its increase), which still remains a relevant portion of the planetary surface (Bøggild et al., 2010; Fountain et al., 2004; Stibal et al., 2012a; Zemp et al., 2008). Their distribution and structure (e.g. size and depth) depend on many factors such as the size of the ablation zone, the insolation on the glacier surface, optical properties of the ice, temperature, sediment input (mainly airborne) and output (mainly with meltwater), type of sediment, type of adjacent area (terrestrial, marine) and the topography of the glacier surface.

The fact that the supraglacial sediment has an active biotic component contributing to its distribution and physical and chemical properties, makes it an important factor that has to be considered when assessing the functioning of biogeochemical processes in glaciated catchments and the impact on e.g. understanding ecological succession in deglaciated areas and the change of marine ecosystems in the Arctic.

We modified the abstract and the introduction to make the study better accessible for readers less familiar with the topic.

#### **4. It is one element I would need to answer the “so what” question: what could be the quantitative importance of these ‘miniature’ ecosystems in the ecology and biogeochemistry of gletsjers?**

As reported by Cameron et al. (2012) and Hodson et al. (2008), cryoconite holes are the most nutrient-rich and biologically active habitats within the supraglacial environment. Therefore, their impact on glacier ecosystems nutrient pathways (e.g. carbon, nitrogen, and other microelements) and on downstream ecosystems is a key component to understanding the glacial ecosystems functioning (Anesio et al., 2010; Bagshaw et al., 2013; Telling et al., 2011). For example, the rate of photosynthesis in cryoconite holes is comparable with those in arctic polar lakes and consequently the rate of respiration and utilization of organic matter is very high (Sävström et al., 2002). Thus, cryoconite holes form an important net carbon sink or source in polar ecosystems which depend on the balance between autotrophic and heterotrophic production (Stibal et al., 2012b). Moreover, due to its high biological activity, cryoconite holes efficiently retain nutrients on the glacier surface (Bagshaw et al., 2013) and accumulated matter can consequently provide a source of important nutrients into adjacent areas where it is transported with the meltwater (Anesio et al., 2010; Porazinska et al., 2004).

Another importance of cryoconite holes comes from the dark colour of cryoconite granules which alters the albedo of the glacier surface and can lead to an increase in the surface melt and eventually to the expansion of the ablation zone (Ryan et al., 2018; Takeuchi et al., 2001). Due to its accumulative nature, cryoconite holes are also used for the exploration and tracking of contamination (e.g. black carbon, radionuclides) in polar areas (e.g. Łokas et al., 2016).

Tardigrades and rotifers inhabiting cryoconite holes are most likely an important factor shaping the community of primary producers by grazing and nutrient recycling (Elser and Urabe, 1999; Vonnahme et al., 2016). Especially when considering their feeding rate (Střítecká and Devetter, 2015). The closest similar studies about stable isotopic composition of tardigrades and rotifers focused only on microbial mats and soil from Antarctica (Almela et al., 2019; Shaw et al., 2018; Velázquez et al., 2017). Due to the dominance of prokaryotic biomass which was in the main scope of most of previous studies from supraglacial habitats, the ecology of cryoconite tardigrades and rotifers and their influence on the supraglacial system functioning is currently unexplored.

We modified the introduction and added information about the importance of cryoconite holes.

**5. I would also have liked to read more clearly structured information about the different organic matter resources – both autochthonous and allochthonous – that tend to be present in cryoconite holes.**

Regarding allochthonous sources of organic matter, it is mostly dust from adjacent areas or snow which is transported to cryoconite holes with meltwater or wind. The components of allochthonous organic matter are mostly death organic matter, snow algae, remains of plants and organisms (Takeuchi et al., 2001) and bird guano (Vonnahme et al., 2016; Žárský et al., 2013).

The autochthonous sources of organic matter are mostly of microbial origin (from cryoconite bacteria, cyanobacteria, and algae) (e.g. Telling et al., 2012).

We added all above-mentioned into the introduction.

**6. Now, there are no very clear goals, questions or hypotheses about such expected patterns, and I am left with the question: since you sampled glaciers in three different settings, how did you expect their food webs and fuelling resources to differ?**

The heterogeneity of three chosen glaciers (size, adjacent areas, geological setting, distance from the sea) should demonstrate possible regional variability in stable isotopic composition of its cryoconite consumers and organic matter among various glaciers in central Svalbard. We expected that different geomorphological characteristics will be reflected in the input of organic matter (e.g. Svenbreen is surrounded by steep slopes) and thus in the composition of their consumers. Nevertheless, our selection should not be understood as a sufficient source of information for hypothesis-driven analysis of controls of the stable isotopic composition in our study.

Based on previous studies we knew that the composition of biota tends to differ between glaciers (e.g. Cameron et al., 2012; Edwards et al., 2013a; Edwards et al., 2013b). The main objective of our study was to use stable isotopic analyses as a tool which could reveal if the food source of cryoconite consumers differ and therefore give an indication whether tardigrades and rotifers can be reasonably expected to influence the cryoconite stoichiometry.

We modified the introduction and added above-mentioned into the text.

**7. In lines 44-47, you are mentioning a correlation between the size distribution of primary producers and the community composition of consumers. In what way, and what is the relevance here?**

Vonnahme et al. (2016) described that the abundance of rotifers correlates with larger and smaller microalgae (Chlorococcales and Zygnematales) concentrations and the abundance of tardigrades only with larger Zygnematales concentration. This study also revealed that the length of trichomes of Oscillatoriales (cyanobacteria) negatively correlates with the abundance of filtering rotifers. We felt that it is important to mention these findings because it indicates that grazing has likely an impact on the structure of primary producers in cryoconite holes and by that presumably contributes to cryoconite nutrient levels.

We modified the text to make the meaning more explicit.

**8. I was rather surprised to read the following final sentence of the conclusions section: “Another outcome of this study is the introduction of a modified technique of sample preparation avoiding procedures such as sugar centrifugation or oven drying.” While reading M&M, I did notice some minor differences in sample elutriation and sorting procedures compared to common practices,**

**but nowhere did I see a clear statement about an important novel methodological approach to treating samples.**

The commonly used methods were modified because cryoconite invertebrates live in a very specific environment and we wanted to avoid alteration of their chemical composition during the preparation for isotopic analyses. Therefore, we chose the lyophilization instead of oven drying and we wanted to avoid any added component which could potentially contaminate our samples.

We agree that we did not describe precisely why we modified the technique. We are also aware that our methodical approach did not present a fully novel method. We modified the text accordingly.

**9. Given the subtitle 'community structure', I had somehow hoped to read a species or genus-level analysis of consumer communities, yet in the end, rotifers were just treated as rotifers, and tardigrades were largely left without a name and assigned to feeding groups, which are well known to be of very limited relevance to describe the actual feeding behaviours of tardigrades, particularly with respect to their potential to feed on microalgae.**

We replaced the community structure by the cryoconite holes community composition and changed the text accordingly.

We are also aware that community structure description lacks detailed information about species composition. Regarding rotifers, the identification depends on the visibility of their coronal cilia which only actively filtering individuals display. We did not observe a sufficient number of filtering rotifers during the collection of rotifers for analyses and therefore other identification except few individuals identified as *Macrotrachella* sp. and *Adineta* sp. was impossible.

Regarding the species composition of tardigrades, the representative number of individuals was collected, mounted, and determined apart from individuals intended for analyses. We found *Pilatobius* sp., *Hypsibius* sp., *Hypsibius* cf. *dujardini*, *Isohypsibius* sp. and very rarely *Cryoconicus kaczmareki*. *Pilatobius* sp. was in the past identified as *P. recamieri* which inhabit Arctic tundra commonly. However, utilization of DNA barcoding revealed that suggested *P. recamieri* is a new species for science and it is not formally named yet. *Hypsibius* contains, according to DNA, few cryptic lines. *Grevenius* (previously identified as *Isohypsibius* sp.) is also new for science and not formally named. Moreover, because some species are cryptic and identified only based on DNA (morphologically similar), they most probably use the same food source. Due to the lack of information about the diet of particular species living in cryoconite holes, we chose the trophic group division based on already published knowledge about feeding behaviours and feeding apparatus morphology of related species and on the personal communication with specialists culturing relative species.

**10. So, there is no info on community composition, only some more anecdotal statements as to certain abundantly present Cyanobacteria and algae. This is probably also why there is no statistical analysis of differences in community composition of either consumers or primary producers.**

The data presenting the community structure of primary producers on each glacier were not incorporated into the manuscript because the preservation of the samples by freezing has usually a taxon-specific effect on the survival of the cells of phototrophs. Thus, it makes it impossible to provide reliable quantification comparable between taxa. For example, the cells of glacial algae withstand the repeated freeze-thaw cycles only with a low success, whereas cyanobacteria usually have no significant loss in observed cell numbers. Even though we agree that the information lacks

further data, we felt that it is important to mention at least all information we were able to get from our samples. Due to these reasons, we excluded the data on primary producers from statistical analyses.

The community composition of consumers could not be statistically analysed since we were not able to provide sufficient data about the species composition of rotifers among glaciers.

**11. Incidentally, I wondered why both a Spearman rank and a Pearson product-moment correlation were tested. I would expect that if the data fit the assumptions for parametric tests, one would choose the latter, and if not, the former.**

We had to use both tests because some data did not have a normal distribution. However, we were not explicit in the methods that the tests have not been used both on the same data. We modified the text to make the meaning clear.

**12. I would have preferred to see absolute abundances of tardigrades and rotifers in table 1 instead of a general dominance-indication. That dominance indication is not very scientific, and it does not provide any relevant info on potential differences in abundance of consumer groups among gletsjer systems. Actually, since you sampled sufficient replicates in 2 out of 3 gletsjers, you could statistically underpin whether tardigrades were more or less abundant than rotifers in a specific gletsjer system.**

We have changed the table to present frequency of tardigrades and rotifers on each glacier related to the total amount of collected individuals.

**13. Results (Fig. 2): Figure only shows the correlation between cryoconite  $\delta^{13}\text{C}$  and rotifer  $\delta^{13}\text{C}$ . That of tardigrades is not shown because it was not statistically significant. I would then at least like to see the  $r$  and actual  $p$ -value for the tardigrade correlation, as well as an estimate of the slope of the regression for both rotifers and tardigrades.**

The  $r$  in the correlation between  $\delta^{13}\text{C}$  of decarbonized cryoconite and  $\delta^{13}\text{C}$  of tardigrades is 0.67 and the  $p$ -value is 0.07. We added the table and the value to the manuscript.

**14. I would have started the results section with what is now 3.2. Cryoconite composition.**

We transferred the section 3.2 to the beginning of the section Results.

**15. Section 3.3: as mentioned above, table 1 should provide absolute abundances of consumers, and it should also provide info on the proportion of dormant consumers!**

The proportion of dormant consumers is not possible to count during the sample preparation procedure because we use melted material and dormant tardigrades and rotifers are waking up during the whole time of the collecting for isotopic analyses.

**16. I would be interested to read in one or two sentences to what extent other meiofauna or small invertebrates than tardigrades and rotifers were present (any Nematoda, Copepoda, Ostracoda, . . .?).**

Based on the current knowledge, Arctic cryoconite holes are inhabited exclusively by tardigrades and rotifers (e.g. Zawierucha et al., 2018; Zawierucha et al., 2019b). Currently, we have data from 20 glaciers from Svalbard which indicate that no other animals inhabit cryoconite holes in this region (Zawierucha et al. 2020, in review). For our investigation, we used material from many seasons which was analysed by students and experienced researchers and no other metazoans have been found. Moreover, only a few groups of other animals inhabit cryoconite holes worldwide and most

of these species are endemic. Crustaceans (Copepoda) were found only in Himalaya, insects (Chironomidae and Plecoptera) have been found in the Himalaya and Patagonia, recently mites have been found on one Antarctic glacier. Our unpublished meta-analysis suggests that tardigrades and rotifers are the most common cryoconite animals. We corrected the introduction and underlined that exclusively rotifers and tardigrades play roles as apex consumer of cryoconite holes in the Arctic.

**17. At the end of the first discussion §, you finally provide some ‘expectation’, but it is not clear what this expectation is based upon. In the relatively lengthy and speculative discussion, I read virtually nothing about temperature effects on isotopic fractionation between consumers and resources.**

The temperature in cryoconite holes is very stable (around 0 °C) and the fluctuation usually do not exceed tenths of °C (e.g. Säwström et al., 2002; Zawierucha et al., 2019a). Any increase in the temperature of the sediment is efficiently buffered by ice at 0 °C because any added heat will lead to melting instead. Publications focusing on the temperature effect on the isotopic fractionation usually describe that the changes in fractionation begin with differences higher than 2 °C (e.g. Bosley et al., 2002; Degens et al., 1968; Hinga et al., 1994; Olive et al., 2003).

We added the information about the temperature effect into the discussion and modified the discussion to make its meaning clearer and more explicit.

**18. In section 4.2., dissolved organic matter suddenly becomes an important candidate food for these consumers. That may well be, but what results is this based upon here?**

This suggestion is based on the description and explanation of observed distribution of  $\delta^{13}\text{C}$  in consumers and DOC from cyanobacterial exudates in the study of Velázquez et al. (2017). This study focused, apart from microbial trophic interaction, on isotopic composition of tardigrades and rotifers from Antarctica as well.

We added the reference into the text.

**19. 1) Line 71: “preferential excretion of  $\delta^{14}\text{N}$ ” should be “preferential excretion of (light)  $^{14}\text{N}$ ”. 2) Line 250: “rotifers revealed higher values of  $^{15}\text{N}$  isotope” should be “rotifers had higher  $\delta^{15}\text{N}$ ” or “rotifers had heavier stable nitrogen isotopic signatures’. 3) Line 251: “potential differences in  $\delta^{15}\text{N}$  composition” should be “potential differences in N isotope ratios/signatures”.**

We incorporated all above suggested changes and corrections.

### **Responses to Mr. Rozwalak**

**1. I would like to get your attention to more precisely distinguish occurrences of primary producers on different glaciers. Currently, it is not clear whether e.g. *Ancylonema* sp. exist on the all glaciers or only in the Ebbabreen or Svenbreen.**

The data presenting the community structure of primary producers on each glacier were not incorporated into the manuscript because the preservation of the samples by freezing has usually a taxon-specific effect on the survival of the cells of phototrophs. Thus, it makes it impossible to provide quantification. For example, the cells of glacial algae withstand the repeated freeze-thaw cycles only with a low success, whereas cyanobacteria usually have no significant loss in observed cell numbers.

Even though we do have data about the presence or absence of particular species of primary producers on each glacier, we were not able to make a reliable quantitative image of the community structure of primary producers which could help us to elucidate the differences in the stable isotopic composition of cryoconite and invertebrates among glaciers and the differences in trophic groups of tardigrades among glaciers.

Regarding the presence/absence data, all determined species were present on all glaciers except for *Leptolyngbya* sp., which occurred only on Svenbreen and Ebbabreen.

### **Responses to Mr. Buda**

**1. You should add a short description or reference how you assessed trophic groups of tardigrades. As a pioneer study, this information may be helpful for future research.**

The description of used methodical approach during the division of tardigrades into trophic groups together with the references is described in methods (part 2.5). We considered using of the term dominant feeding groups and trophic groups as a comparable in case of our study. However, due to the improvement of the comprehensibility of the text, we unified these terms.

**2. Based on visual observation of boxplots in groups, variance looks unequal between groups, it may or may not indicate heteroscedasticity. Variance in Svenn seems to be completely different in comparison to the rest of glaciers. In very low sample size it can highly bias result so it can cause Type I error using ANOVA.**

We are aware that due to the limited number of analysed samples, the visualization of results by boxplots is favouring a bias result. However, we considered boxplots with the visible values as a convenient and clear presentation of our results. Since our work presents a pioneer study, we took into consideration variation in results in the discussion and highlighted the need of further investigation. We mentioned the risk of used visualization in the legend of the Figure 3.

**3. You should consider changing this value to a precise number p and r. Value 0.05 as a threshold is arbitrary, thus it can be important in interpreting results, it can be 0.90 or 0.06.**

The r in the correlation between  $\delta^{13}\text{C}$  of decarbonized cryoconite and  $\delta^{13}\text{C}$  of tardigrades is 0.67. We added this value into the results.

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## **List of all relevant changes made in the manuscript**

### **Abstract**

We modified the abstract in order to make it better accessible for general scientific audience and to introduce the modified version of our manuscript properly.

### **Introduction**

We added more information about the supraglacial system functioning and the role of cryoconite holes on glaciers. We also added goals and questions of our study.

### **Methods**

We added information about the modification of used methods, we also modified the section 2.5 and added information about the taxon-specific effect on the survival of cells of the phototrophs. We added information about correlation coefficients which were used in section 2.6.

### **Results**

We transferred the section 3.2 to the beginning of the section results. We also modified Table 1 and added the frequency of consumers on each glacier. The  $p$ -value of the correlation between  $\delta^{13}\text{C}$  values of decarbonized cryoconite and the  $\delta^{13}\text{C}$  of tardigrades was added together with the figure. We changed the name of the section 3.3 and we added more information about the cryoconite community composition.

### **Discussion**

The whole discussion was modified in order to put the results better into context and avoid the overinterpretation of the results.

### **Conclusions**

Conclusions were slightly modified to summarize the revised manuscript properly.

# Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing ~~different position~~ divergent roles in a supraglacial trophic network

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**Abstract.** Cryoconite holes ~~are ecosystems~~ represent highly biologically active aquatic habitats on the ~~glaciers~~ glacier surface characterized by dynamic nature of their formation and ~~truncated food webs~~. ~~It is acknowledged that cryoconite holes play an important role being biodiversity hot spots and factories for organic matter on glaciers functioning.~~ The most common cryoconite apex consumers are the cosmopolitan invertebrates – tardigrades and rotifers. Several studies have highlighted the potential relevance of ~~eryoconite~~ 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 processes on glaciers. The aim of this study is to present pioneering data ~~about~~ on isotopic composition of tardigrades, rotifers and cryoconite from three High Arctic glaciers in Svalbard and discuss their potential role in cryoconite hole trophic relations network. We found that tardigrades have lower  $\delta^{15}\text{N}$  values than rotifers, which indicates different food requirements or different isotopic fractionation of both consumers. The  $\delta^{13}\text{C}$  values revealed similarities among the consumers from the same glaciers and differences between consumers and organic matter in cryoconite among glaciers. ~~The resulted  $\delta^{13}\text{C}$  values point to similar carbon requirement~~ However, the mechanistic explanation of consumers within a glacier but differences in carbon input between glaciers. The results comprise these variations requires further investigation focused on the particular diet of cryoconite consumers and their isotopic ratio. Our study introduces the first observation of ~~eryoconite holes' consumers~~ through carbon and nitrogen stable isotopic ~~analyses using~~ composition of top consumers in cryoconite holes analysed by an improved method ~~of~~ for cryoconite sample processing and ~~pave~~ paving the way for further studies of the supraglacial trophic network.

## 1 Introduction

Supraglacial habitat, the environment on the glacier surface, is exhibited to a continuous as well as episodic input of allochthonous and autochthonous material and forms a biogeochemical reactor involving a variety of biotic and abiotic processes. Most of the most biologically active and productive part of glaciers all around biotic activity is usually connected to ablation zones (areas with an ice loss exceeding its increase) which have a global tendency to extend due to the world disbalance between the glacier extent and the climate (Hodson et al., 2008; Stibal et al., 2012a). Moreover, the export of the biological communities and their metabolic production has a potential to influence the downstream deglaciated areas and the coastal marine ecosystems (Bardgett et al., 2007; Foreman et al., 2007; Hodson et al., 2008; Hood et al., 2009; Williams and Ferrigno, 2012).

The ~~flat parts of accumulated fine material on the~~ glacier surface ~~accumulate sediment~~ (so called cryoconite, Nordenskiöld (1875)) ~~which~~ due to its dark colour – reduces albedo ~~of the glacier ice~~ and creates water-filled depressions called cryoconite holes (Cook et al., 2016; Takeuchi et al., 2001). The diameter and the depth of cryoconite holes usually range from a few centimetres to tens of centimetres (Gerdel and Drouet, 1960; Fountain et al., 2004; Zawierucha et al., 2018a; Zawierucha et al., 2019a). At the bottom of ~~thesethe~~ 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~~ which provide a suitable environment to various organisms (Zawierucha et al., 2015). The supply of organic and inorganic matter into cryoconite holes is restricted to allochthonous input from atmospheric deposition, weathering of mineral dust, aeolian deposition, and locally from bird guano deposition (Anesio et al., 2009; Benassai et al., 2005; Edwards et al., 2014; Hodson et al., 2005; Stibal et al., 2008; Telling et al., 2011; Vonnahme et al., 2016; Xu et al., 2010; Žárský et al., 2013). The autochthonous input of matter is generally restricted to microbial activity and recycling (Telling et al., 2011; Telling et al., 2012). Moreover, adjacent areas of glaciers can vary a lot in terms of topography, geology, vegetation and stage of soil development. Therefore, the allochthonous matter brought to the glacial surface can influence the composition of its surface material and biota (Grzesiak et al., 2015; Marshall and Chalmers, 1997; Stibal et al., 2008).

Cryoconite holes cover about 7 % of the surface of the ablation zone (Bøggild et al., 2010; Fountain et al., 2004; Stibal et al., 2012b) and form the most nutrient-rich and biologically active habitats within the supraglacial environment (Cameron et al., 2012; Hodson et al., 2008). As mentioned by Sävström et al. (2002), the rate of photosynthesis in cryoconite holes is comparable with rates of arctic polar lakes and consequently the rate of respiration and utilization of organic matter is very high (Hodson et al., 2008). Thus, cryoconite holes form an important net carbon sink or source in polar ecosystems which depends on the balance between autotrophic and heterotrophic production (Stibal et al., 2012a). Moreover, due to their high biological activity, cryoconite holes efficiently retain nutrients (Bagshaw et al., 2013) and the accumulated matter can consequently provide a source of important nutrients into adjacent areas (Anesio et al., 2010; Porazinska et al., 2004). Therefore, the impact of cryoconite holes on glacier ecosystems nutrient pathways (e.g. carbon, nitrogen, and other microelements) and on downstream ecosystems is a key component for an understanding of the glacial ecosystems functioning (Anesio et al., 2010; Bagshaw et al., 2013; Stibal et al., 2012a; Telling et al., 2011).

Organisms inhabiting cryoconite holes range from bacteria, 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), these animals represent a large component of microfauna in polar and high mountain regions; and are the ~~dominant~~ exclusive metazoans inhabiting cryoconite holes in the Arctic (Klekowski and Opałiński, 1986; Zawierucha et al., ~~2019a~~ 2018a; Zawierucha et al., 2019b).

As the top consumers of Arctic cryoconite holes, tardigrades and rotifers ~~presumably play~~ may represent an important ~~role in controlling~~ driver of the ~~populations~~ community of primary producers by grazing and nutrient recycling, thus ~~contributes~~ setting stoichiometric constrains to the local community ~~structure~~ (Elsler and Urabe,

1999; Vonnahme et al., 2016; Zawierucha et al., 2015; Zawierucha et al., ~~2018~~2018a). Previous research on biota from cryoconite holes' metazoans from holes on Svalbard archipelago revealed that the size distribution and concentration of algae, particularly Zygnematales and Chlorococcales, correlates with the community structure of consumers represented by tardigrades and rotifers (Vonnahme et al., ~~2016~~2016). Presented correlations indicate that grazing likely has an impact on the structure of primary producers in cryoconite holes and presumably contributes to available nutrient quantities and ratios in cryoconite. Nevertheless, other studies from the margin of the Greenland ice sheet ~~indicate~~revealed a lack of quantitative relations between the numbers of top consumers and potential food such as cyanobacteria and algae (Zawierucha et al., ~~2018~~), ~~which demonstrates~~2018a) and demonstrated the variability of supraglacial systems which is influenced by multiple factors occurring on various glaciers (Porazinska et al., 2004). As described by Strítecká and Devetter (2015), tardigrades and rotifers are efficient filtrators and especially rotifers reveal high filtration rates in cryoconite holes. 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/values 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 prefer or discriminate heavier or lighter isotopes (Michener and Lajtha, 2008). The  $\delta^{13}\text{C}$  value reflects the diet of 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 a higher assimilation of  $^{13}\text{C}$  supported by preferential  $^{12}\text{C}$  depletion (of  $\text{CO}_2$ ) 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 ( $^{13}\text{C}$ ) value within the consumer's body compared to its diet. ~~Large~~However, larger variations in values are ~~however~~ balanced by a higher release of  $^{13}\text{C}$  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  $\delta^{14}\text{N}/^{14}\text{N}$  during protein metabolism (Kling et al., 1992; McCutchan et al., 2003). Furthermore, if the environment is limited by a specific 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~~2018). For example, Adams and Sterner (2000) described that if the diet had a high C:N, the  $\delta^{15}\text{N}$  of consumers' body increased. Another study presented, that if the diet is limited by a nutrient, the consumers' body increased or decreased the uptake of isotopes to keep its isotopic signatures almost constant (Aberle and Malzahn, 2007). 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 Ardit, 2000; Wada, 2009). Several studies have also focused on carbon and nitrogen stable isotopes in polar ~~systems areas~~ (Almela et al., 2019; Shaw et al., 2018; Velázquez et al., 2017). 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~~ biotic component reflecting differences in the nutrient input on the glacier surface and contributing to the glacial ecosystem functioning (Hodson et al., 2008; Stibal et al., ~~2012~~2012b; Vonnahme et al., 2016). Studies focusing on the role of top consumers in cryoconite holes are ~~however~~ lacking, however, which may hinder our understanding of cryoconite holes' ~~ecology~~ and glacial ~~ecosystem's functioning~~ ecosystems' ecology. This study is based on data from three High Arctic inland glaciers, all three located in a different ~~geographical~~ geomorphological and geological context. We ~~examined carbon and nitrogen stable isotopic~~ expected that different geomorphological characteristics will be reflected in the input of organic matter and thus in the composition of ~~eryoconite, their consumers~~ (Cameron et al., 2012; Edwards et al., 2013a; Edwards et al., 2013b). The current state of knowledge about abundances and feeding rates of glacier invertebrates suggests that they possess a substantial capacity to influence the biotic fluxes of nutrients and energy on the glacier surface. Here we employ the stable isotope analysis to test whether the top consumers – tardigrades and rotifers, and made a synthesis of their potential relationships – significantly differ in their food sources in the ~~eryoconite hole trophic food web~~ glacial ecosystem.

## 140 2 Material and Methods

### 2.1 Study site and sampling

Samples of cryoconite were collected from three glaciers (Ebbabreen, Nordenskiöldbreen and Svenbreen; *breen* means glacier in Norwegian) located at ~~the~~ 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 the 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 ~~were~~ 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

For each replicate, a part of cryoconite ( $\sim 2\text{--}4\text{ cm}^3$ ) 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  $-20\text{ }^\circ\text{C}$  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,  $5 \times 9\text{ mm}$ ). If the water content in the capsule exceeded  $\frac{1}{2}$  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  $\frac{1}{3}$  of the volume. The samples were consequently frozen at  $-20\text{ }^\circ\text{C}$  and at least half an hour before the lyophilization stored at  $-80\text{ }^\circ\text{C}$ . 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 [dry weight of a dry sample of invertebrates in the capsule](#) was  $\sim 29.5\text{ }\mu\text{g}$ . [Also, since the identification of species requires specific preparation \(see the section 2.5\), samples for isotopic analyses were pooled samples of all species occurring in used cryoconite.](#) Four replicates of tardigrades, rotifers and cryoconite from Svenbreen, five replicates of tardigrades, four replicates of rotifers and three replicates of cryoconite from Nordenskiöldbreen, and three replicates of tardigrades, two replicates of rotifer and two replicates of cryoconite from Ebbabreen were collected for the isotopic analyses. [Due to the adaptation of cryoconite consumers to specific conditions occurring on the glacier surface \(e.g. low temperature, low content of available nutrients\), we modified commonly used methods to avoid alteration of their chemical composition during the preparation for isotopic analyses. Therefore, we chose the lyophilization instead of oven drying and we wanted to avoid any added component which could potentially contaminate samples.](#)

Cryoconite intended for the isotopic analyses was cleaned from tardigrades and rotifers, which were collected in parallel for isotopic analyses- [described above](#). After the collection, cryoconite was stored in Eppendorf tubes at  $-20\text{ }^\circ\text{C}$ . When all samples were prepared, cryoconite was homogenised using an agate pestle and mortar and dried in a thin layer on a Petri dish at  $45\text{ }^\circ\text{C}$ . The duration of drying was 8 hours.

For the analyses of  $\delta^{15}\text{N}$  in organic matter (OM), cryoconite was transferred without any other preparation into pre-weighed tin capsules (Costech 41077,  $5 \times 9\text{ mm}$ ) and weighed. The average amount of cryoconite used for analyses was  $\sim 31\text{ mg}$ . For the analyses of  $\delta^{13}\text{C}$  in organic matter, 11–12 mg of cryoconite was transferred into pre-weighed silver capsules (Elemental Analyses,  $8 \times 5\text{ mm}$ , D2008) and carbonates (e.g. calcite, dolomite) were dissolved using 10% HCl moistened with  $\text{dH}_2\text{O}$ . 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  $50\text{ }^\circ\text{C}$  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

195 The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in all samples were analysed using a Flash 2000 elemental analyser (ThermoFisher Scientific). Released gasses ( $\text{NO}_x$ ,  $\text{CO}_2$ ) 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 ( $\delta$ ) with samples measured relatively to Pee Dee Belemnite for carbon isotopes and atmospheric  $\text{N}_2$  for nitrogen isotopes and 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  $\pm 0.03$  ‰ for  $\delta^{13}\text{C}$  and  $\pm 0.02$  ‰ for  $\delta^{15}\text{N}$ .

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

### 205 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 [the](#) following conditions: radiation –  $\text{CuK}\alpha$ , 40 kV, 30 mA, angular range  $-3-70^\circ 2\theta$ , step  $0.02^\circ/150$  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~~

#### 2.5 Cryoconite holes community composition

215 For the species identification, at least  $10\text{ cm}^3$  of cryoconite was used from each sample. Tardigrades were collected using a glass Pasteur pipette and the first observation was made under a ~~stereo microscope~~[stereomicroscope](#) (Olympus SZ 51). 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  $56^\circ\text{C}$ , tardigrades were identified under a light microscope with phase contrast (Olympus BX53) associated with a digital camera ARTCAM 500. ~~Tardigrades~~[Due to the ambiguities associated with the identification of cryoconite species \(species complexes and hidden molecular lines \(Zawierucha et al., in review\), tardigrades](#) were classified to the [trophic groups based on the](#) dominant feeding behaviour ~~groups and feeding apparatus morphology~~ according to Guidetti et al. (2012), Guil and Sanchez-Moreno (2013), Hallas and Yeates (1972) and Kosztyła et al. (2016). Specimens of 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 ~~were~~[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 digital camera Olympus EOS 700D. Identification was based on publications by Starmach (1966), Ettl and Gärtner (2014) and Wehr et al. (2015). [Quantification of primary producers was omitted due to the preservation of samples by freezing which presumably has a taxon-specific effect on the survival of cells of the](#)

230 phototrophs. This presumption is based on observed low survival rate of glacial algal cells (*Mesotaenium*, *Ancylonema*) in freeze-thaw cycles (Jakub D. Žárský, personal communication, 2020). The proportional representation of consumers in each sample was calculated during the collecting of tardigrades and rotifers for isotopic analyses and it is presented as frequency (in %) towards the total amount of collected animals on each glacier. A difference in relative abundance lower than 5 % was considered an equal proportion.

## 235 **2.6 Statistical ~~Analysis~~Analyses**

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, a Kruskal–Wallis rank sum test was used. Before the correlation coefficient tests were applied, Shapiro–Wilk test was used to test the normal distribution of the data. Therefore, ~~Spearman’s the Pearson’s~~ rank correlation coefficient ~~and Pearson’s product moment correlation coefficient were counted~~was calculated for all the correlations between isotopic values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of cryoconite and isotopic values of tardigrades and rotifers— excluding  $\delta^{15}\text{N}$  of tardigrades and frequency of isohypsibids among glaciers which were non-normally distributed, leading to the usage of the Spearman’s product-moment correlation coefficient. 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 applied. For the purpose of statistical analyses, all replicates from the same sampling campaigns were averaged.

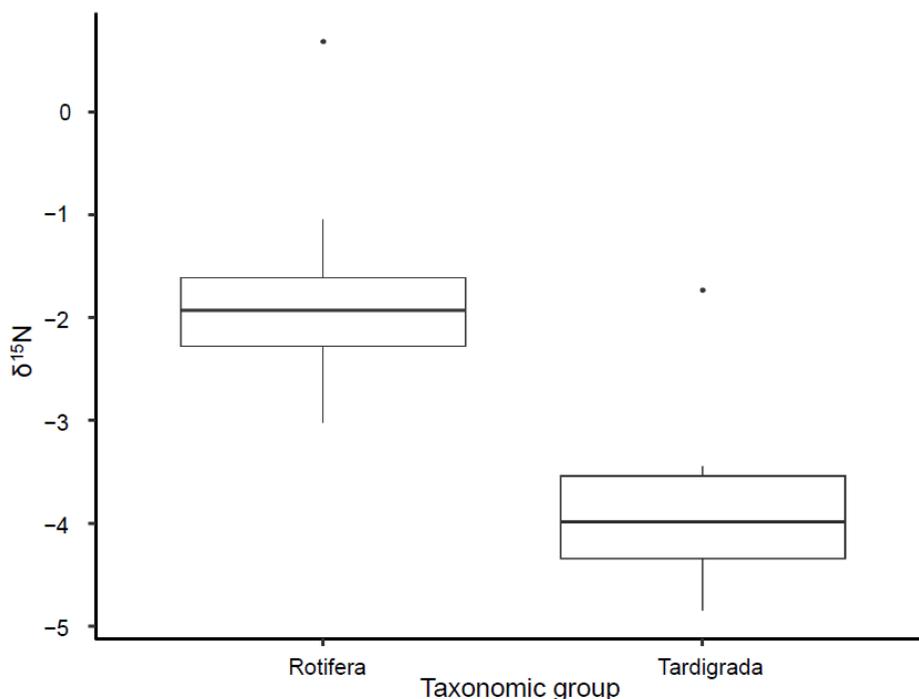
## 245 **3 Results**

### 3.1 Mineral composition and characteristics of cryoconite

250 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  $\text{pH} < 7$ .

### 3.13.2 Isotopic signatures

The isotopic signature of nitrogen showed significant differences in  $\delta^{15}\text{N}$  between tardigrades and rotifers in all samples (Kruskal–Wallis chi-squared = 12.685,  $df = 1$ ,  $n = 22$ ,  $p\text{-value} = 0.00037$ ). 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 Both outliers represent  $\delta^{15}\text{N}$  values from of one replicate from Ebbabreen.

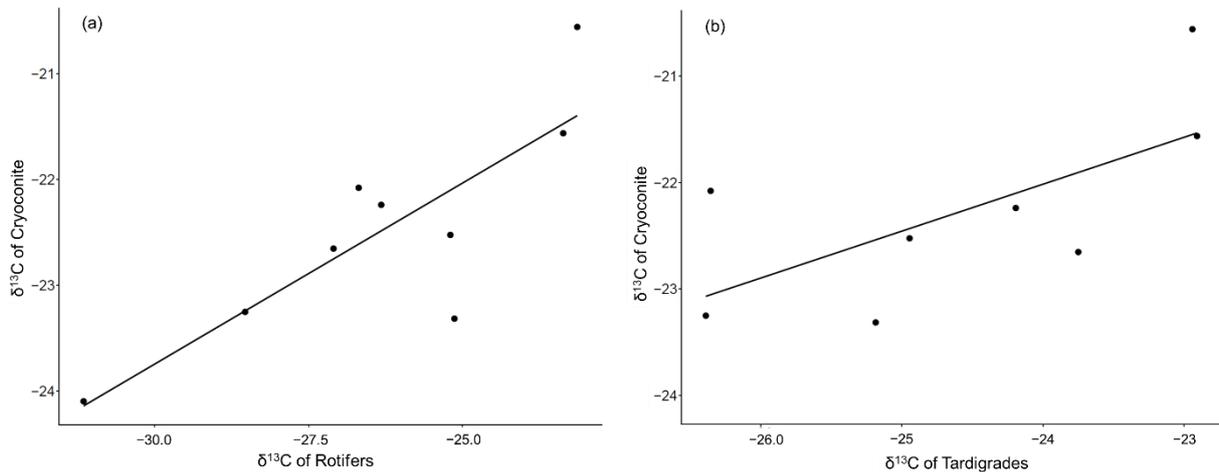
**Table 1.** Description of samples and isotopic values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of tardigrades, rotifers and cryoconite. Isotopic values are presented as ‰ and related to the international standards Pee Dee Belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen. The  $\delta^{13}\text{C}^*$  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. The frequency of consumers on each glacier is expressed as % relative to the total amount of collected consumers for isotopic analyses. T signifies tardigrades and R signifies rotifers.

Glacier	Tardigrades		Rotifers		Cryoconite		Frequency	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}^*$	T	R
Sven	-3.55	-23.75	-1.57	-27.09	-2.20	-22.65	39	61
	-4.04	-26.36	-1.81	-26.69	-2.17	-22.08		
	-3.52	-26.33	-2.04	-31.16	-2.24	-24.09		
	-4.39	-26.45	-1.04	-28.53	-1.64	-23.25		
Nordenskiöld	-4.39	-22.91	-2.20	-23.36	-3.58	-21.56	49	51
	-3.45	-23.30	-1.72	-23.25	-2.30	-20.56		
	-3.76	-22.58	-2.30	-23.02	-2.98	-22.52		
	-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	58	42

-4.85 -25.22 -2.38 -25.13 -4.29 -23.31  
-4.33 -25.15

275

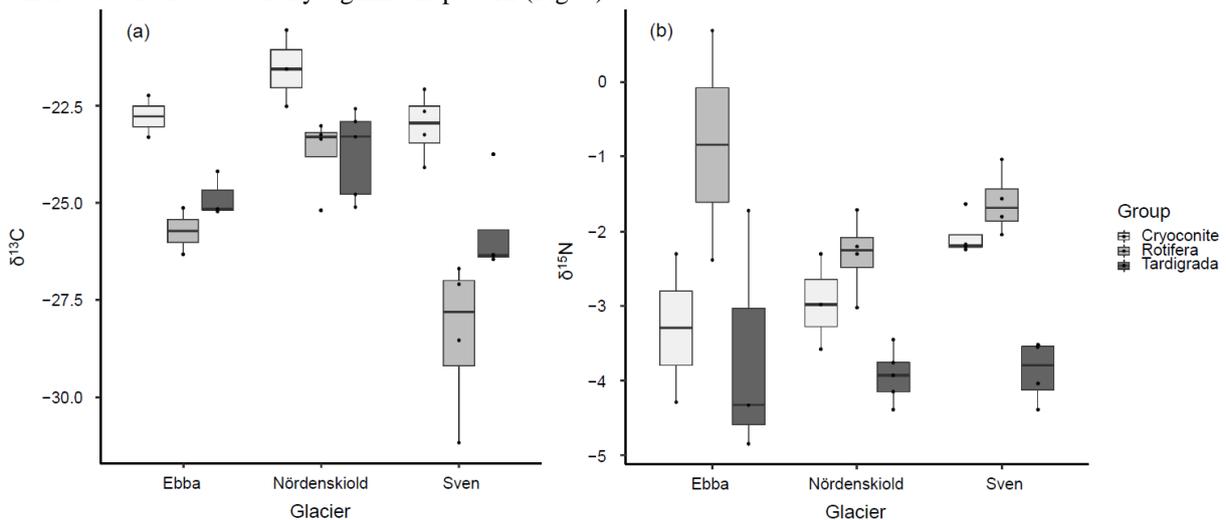
Regarding the isotopic signatures of carbon, we found a positive correlation of the  $\delta^{13}\text{C}$  values of decarbonized cryoconite and the  $\delta^{13}\text{C}$  of rotifers ([Pearson's product-moment correlation](#):  $r = 0.83$ ,  $n = 19$ ,  $p\text{-value} = 0.006$ ) (Fig. 2a). The [analogical/respective](#) relationship among tardigrades was not significant ([Pearson's product-moment correlation](#):  $r = 0.67$ ,  $n = 21$ ,  $p\text{-value} \geq 0.05$ ) (Fig. 2b).



**Figure 2.** Correlation between  $\delta^{13}\text{C}$  of rotifers (a) and tardigrades (b) and decarbonized cryoconite with the linear regression line.

280

In all samples, differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of tardigrades and rotifers and differences in  $\delta^{13}\text{C}$  of decarbonized cryoconite 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 ( $p\text{-value} = 0.029$ ) (Fig. 3a), mostly between Nordenskiöldbreen and Svenbreen ( $p\text{-value} = 0.025$ ). 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. [The differences in variances among glaciers are the result of the low number of samples.](#)

285

### 3.2 — Composition of cryoconite

~~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  $\text{pH} < 7$ .~~

### 3.3 — Community structure

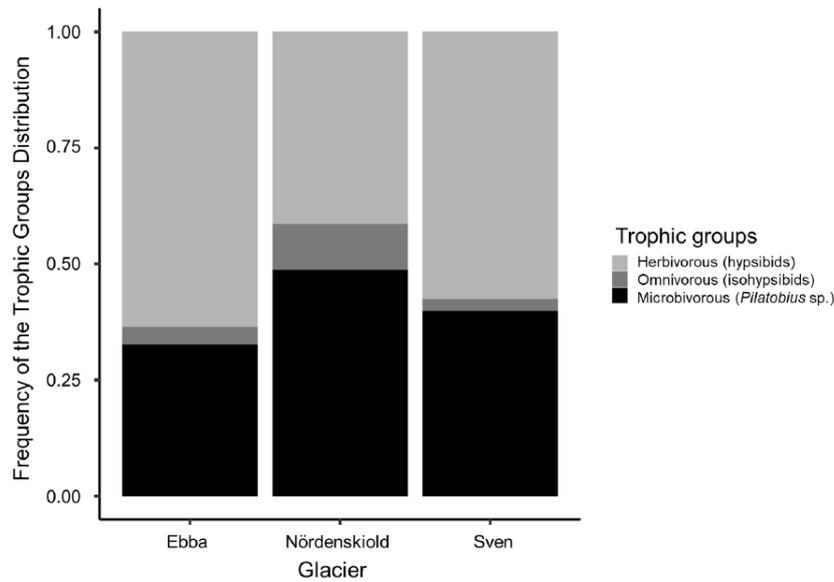
#### 3.3 Cryoconite holes community composition

During the collection of animals for isotopic analyses, we ~~also~~ counted the ~~proportion in abundances~~ frequency of tardigrades and rotifers within all replicates. ~~A difference in relative abundance lower than 5 % was considered an~~ (Table 1). Svenbreen revealed dominance of rotifers from the total number of 7375 collected individuals, Ebbabreen was dominated by tardigrades from the total number of 5163 collected individuals and Nordenskiöldbreen revealed equal proportion (Table 1) of tardigrades and rotifers from the total number of 6401 collected individuals.

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.). In case of cyanobacteria, we observed 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.~~

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. However, they could not be divided and analysed due to the majority of individuals occurring in a dormant stage, which made it impossible to observe the morphology of their cirri and trophi (jaws) necessary for their identification. 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 %). We also found few individuals of *Cryoconicus kaczmareki* Zawierucha et al., 2018b on Ebbabreen but they were not included into statistics due to their very rare occurrence. 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

### 4.1 Isotopic signatures and ~~trophic relations~~ the role of consumers in cryoconite consumer trophic network

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 almost similar 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 (Sävström 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.

Our results showed that rotifers revealed higher values of  $^{15}\text{N}$  isotope compared to tardigrades suggesting potential differences in  $^{15}\text{N}$  composition of their diet. The  $\delta^{15}\text{N}$  isotopic signatures of cryoconite consumers revealed higher values of  $^{15}\text{N}$  isotope in rotifers compared to tardigrades suggesting differences in  $\delta^{15}\text{N}$  of their diet or differences in the isotopic fractionation between both consumers. 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)

345 we cannot assume a strict trophic division of their food. Primarily, tardigrades found in samples were identified  
as probably ~~microbivorous (*Pilatobius* sp.), herbivorous (hypsibids) and omnivorous (isohypsibids) species.~~  
Nevertheless, we cannot exclude that *Pilatobius* sp. with its ventrally located mouth does not consume algae  
350 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 mostly identified as filter feeders (*Macrotrachella* sp.) or scrapers (*Adineta* sp.) (Herzig  
et al., 2006). However, whereas *Adineta* sp. never did not exceed 10% of their *Macrotrachella* sp. in the total  
amount and of collected rotifers (the living specimens can be well distinguished from *Macrotrachella* sp. during  
355 collecting. The differences in  $\delta^{15}\text{N}$  between both consumers could suggest  $\delta^{15}\text{N}$  enrichment). Tardigrades found  
in samples were identified as microbivorous (*Pilatobius* sp.), herbivorous (hypsibids) and omnivorous  
(isohypsibids) species. food for rotifers caused by consumption of bacteria or DOM enriched in  $\delta^{15}\text{N}$ . This  
assumption is supported by Nagarkar et al. Nevertheless, based on the knowledge of the (2004) and Kohler et al.  
(2018), who described that cyanobacteria have higher content of proteins and a higher  $\delta^{15}\text{N}$  values typical for  
360 nitrogen fixing organisms. The other potential explanation of the observed pattern is tardigrades' consumption  
of feeding apparatus morphology. *Pilatobius* sp. with its ventrally located mouth is likely able to consume algae  
during scratching biofilms from the surface of granules and isohypsibids, which have a relatively wide buccal  
tube, 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 utilise various food sources such as algae, protozoans  
365 and other small size of food. Therefore, the ingested food composition may shift from its optimum invertebrates.  
Moreover, all studied groups of tardigrades feed on algae in the laboratory cultures (Bryndová et al., 2020;  
Kosztyła et al., 2016).

370 Generally Therefore, the differences in  $\delta^{15}\text{N}$  between both consumers may suggest the  $\delta^{15}\text{N}$  enrichment in food  
for rotifers caused by preferential consumption of bacteria or DOM and consequent  $^{14}\text{N}$  depletion compared to  
food for tardigrades (Altabet and Small, 1990; Kling et al., 1992; Mariotti et al., 1980; McCutchan et al., 2003;  
Peterson and Fry, 1987). The results of Nagarkar et al. (2004) and Kohler et al. (2018) who described that  
cyanobacteria have higher content of proteins and higher  $\delta^{15}\text{N}$  values typical for nitrogen fixing organisms  
serves as indirect empirical indication supporting this assumption. On the other hand, lower  $\delta^{15}\text{N}$  of tardigrades  
375 may suggest the variation in  $\delta^{15}\text{N}$  of algae which can vary depending on their C:N ratio and  $\delta^{15}\text{N}$  ratio of their  
nitrogen source (Adams and Sterner, 2000; Gu and Alexander, 1993). The differences between consumers may  
also signify different nitrogen isotopic fractionation depending on the C:N ratio of their food (Aberle and  
Malzahn, 2007; Adams and Sterner, 2000). Moreover, we cannot exclude the fact that consumers in cryoconite  
holes are probably highly limited by the lack of nutrients and the small size of food, thus the ingested food  
composition may shift from its optimum compared to related species from other habitats.

380 Regarding the  $\delta^{13}\text{C}$  values of, tardigrades and rotifers in our study were revealed lower than  $\delta^{13}\text{C}$  values in than  
decarbonized cryoconite. This ~~difference~~ is similar ~~to the results~~ described by Almela et al. (2019) and  
Velázquez et al. (2017) ~~in who focused on tardigrades and rotifers from the~~ Antarctic microbial mats.  
Nevertheless, ~~but~~ it contrasts with the fundamental literature (Peterson and Fry, 1987; Wada, 2009) as well as

385 with the study of Shaw et al. (2018) ~~foeusingwho focused~~ on Antarctic soil microbial mats, which presented  $\delta^{13}\text{C}$   
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 in the non-glaciated part of the Taylor Valley (Antarctica). These  
variations may point to differences in carbon fractionation on glacier ~~surface or surfaces~~, differences within  
tardigrades and rotifers in comparison with freshwater zooplankton and soil microfauna. ~~The observed or to the~~  
390 ~~variations in isotopic signatures of various species which differed in their frequencies among glaciers. The~~  
correlation between  $\delta^{13}\text{C}$  of cryoconite and rotifers ~~in  $\delta^{13}\text{C}$  could may~~ also ~~suggests indicate~~ that rotifer food  
(~~suspended bacteria~~) ~~is the representative for represents~~ much of the cryoconite organic carbon. ~~On the other~~  
~~hand, such~~ Such correlation in tardigrades ~~is was~~ not significant, ~~because they feed on primary producers (which~~  
~~could be likely caused by their potential consumption of~~ algae and cyanobacteria) related mostly to air  $\text{CO}_2$ ,  
395 which has the same  $\delta^{13}\text{C}$  everywhere. ~~It may therefore serve as indirect evidence for the bacterivory of rotifers~~  
~~and the algivory of tardigrades in cryoconite holes~~ The results presenting the distribution of  $\delta^{13}\text{C}$  in comparison  
with differences in  $\delta^{15}\text{N}$  between tardigrades and rotifers may indicate that rotifers consume DOC originating  
from extracellular exudates of algae or cyanobacteria (Velázquez et al., 2017), but the source of nitrogen (e.g.  
bacteria, cyanobacteria and organic detritus) is likely different.

400 ~~In comparison with studies focusing on the isotopic composition of consumers from soil and microbial mats in~~  
Antarctica (Almela et al., 2019; Shaw et al., 2018; Velázquez et al., 2017), isotopic composition of tardigrades  
and rotifers from Arctic cryoconite holes reveals differences in  $\delta^{15}\text{N}$  as well as in  $\delta^{13}\text{C}$ . Nevertheless, even  
though studies from Antarctica present different isotopic values, they include important information about the  
diet of these polar invertebrates. For example, in Almela et al. (2019), tardigrades were related mostly to a larger  
405 fraction of particulate organic matter (POM < 30  $\mu\text{m}$ ) composed generally of green algae, instead of rotifers  
which were related to a smaller fraction of POM (0.5–5  $\mu\text{m}$ ) composed generally of bacteria and detritus. In the  
study of Velázquez et al. (2017), tardigrades were related to cyanobacteria and POM (< 30  $\mu\text{m}$ ) and rotifers  
mostly to cyanobacteria and diatoms. Regarding the isotopic composition, the closest values were observed in  
tardigrades and rotifers from soil in Taylor Valley (Shaw et al., 2018) in which these consumers were considered  
410 mat grazers.

415 ~~It is known that the absolute isotopic composition varies among systems based on various causes, such as~~  
differences in the isotopic composition of the nutrient pool (Montoya et al., 1990), seasonal changes in the  
community structure (Cifuentes et al., 1988), seasonal variability in isotopic values of the food (Zah et al., 2001)  
or due to the effect of temperature on the isotopic fractionation (Bosley et al., 2002; Degens et al., 1968; Hinga  
et al., 1994; Olive et al., 2003). Thus, our results from cryoconite holes, in which the input of nutrients as well as  
415 changes in the community structure of microbes vary during the season (Sävström et al., 2002; Stibal et al.,  
2008), ~~require further investigation focused on isotopic composition of the gut content in tardigrades and rotifers,~~  
their isotopic fractionation and elemental ratio to fully reveal the causes of their different isotopic signatures.

#### 4.2 Variations in isotopic signatures among glaciers

420 As shown in the results, the isotopic ~~composition signatures among glaciers~~ revealed differences in  $\delta^{13}\text{C}$  of  
tardigrades, rotifers ~~and cryoconite~~ primarily between the replicates Nordenskiöldbreen and Svenbreen. The  
frequency of consumers on these two glaciers showed higher abundance of rotifers at Svenbreen and between the

425 ~~glaciers differ. Furthermore, we observed variations in the proportional representation an equal abundance of~~  
~~tardigrades and rotifers, and in the community structure of tardigrades among the glaciers and the replicates as~~  
~~well at Nordenskiöldbreen. Nordenskiöldbreen also revealed higher amount of microbivorous tardigrades~~  
~~compared to Svenbreen where herbivorous species dominated.~~

430 The ~~variability differences~~ in  $\delta^{13}\text{C}$  values ~~could may~~ 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  
435 higher occurrence of autochthonous carbon input favouring heavier  $^{13}\text{C}$  isotope signature of the food web. ~~It is~~  
~~highly possible~~ ~~Based on these findings, we assume~~ that due to its smaller size, Svenbreen ~~presumably has may~~  
~~have~~ a higher allochthonous input of nutrients in the form of organic matter from adjacent habitats, which  
~~causes could cause~~ depletion of  $^{13}\text{C}$  in isotopic signature because of a longer chain of fractionations favouring  
440 lighter  $^{12}\text{C}$  typical for allochthonous source of carbon (Peterson and Fry, 1987; Post, 2002). Consequently, the  
depletion in  $^{13}\text{C}$  of consumers ~~from on~~ Svenbreen could signify preferential consumption of DOM from the primary  
production or detritus (Abelson and Hoering, 1961; Iakovenko et al., 2015; Macko and Estep, 1984). Oppositely,  
~~tardigrades and rotifers consumers~~ from ~~Ebbabreen and~~ Nordenskiöldbreen ~~had and Ebbabreen revealed~~ higher  $\delta^{13}\text{C}$   
~~than consumers from Svenbreen. We assume that this increase was caused by which could be a result of~~ a larger  
445 size of these glaciers and a potential larger component of autochthonous production (Stibal et al., 2010) which  
uses “heavier” carbon from atmospheric  $\text{CO}_2$  (Post, 2002) and has a shorter chain of transformations and  
discriminations against  $\delta^{13}\text{C}$  during the assimilation of inorganic matter (Michener and Lajtha, 2008).  
~~However~~ ~~Nevertheless~~, 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  
450 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 focused on carbon isotopic ratios and fractionation in cryoconite holes are  
essential.

450 ~~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; Rieci, 1984; Velázquez et al., 2017). The non-strict division of consumed food between~~  
~~tardigrades and rotifers was apparent in various feeding experiments (Mialet et al., 2013; Rieci, 1984; Střitecká~~  
~~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~~  
455 Regarding the 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.~~

Regarding differences in  $\delta^{15}\text{N}$  among glaciers, some samples evinced high presence of cyanobacteria *Leptolyngbya*  
sp., ~~and the high  $\delta^{15}\text{N}$  could, which may~~ refer to  $\delta^{15}\text{N}$  variations between glaciers due to a higher content of  $^{15}\text{N}$  in  
455 ~~cyanobacteria the~~ populations of cyanobacteria (Darby and Neher, 2012). However, ~~the observed variation could~~

460 ~~also~~ as described in methods, we were not able to quantify primary producers, thus, our observation may be a result of different ~~influenced by inaccuracies caused by the preservation of samples by freezing.~~

465 ~~During the analyses of~~ mineral composition of cryoconite ~~among glaciers located in different parts of Billefjorden Fault Zone. Especially in samples from Svenbreen,~~ we detected a ~~very low~~ high amount of amphibole and dolomite ~~which were common~~ on Ebbabreen and Nordenskiöldbreen. ~~Considering which are both located in a geologically younger zone of the Billefjorden Fault Zone compared to Svenbreen located in an older part of the Billefjorden Fault Zone. Considering a~~ higher potential solubility of minerals due to acidic pH of cryoconite holes (4.48–5.9) and differences in mineral composition of cryoconite aggregates among glaciers, the differences in the community structure of microbial communities and consequent isotopic signatures ~~could also~~ may 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~~ 2019c). 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 by presence of  $\text{NO}_3^-$  in the meltwater (Hodson et al., 2005).

## 5 Conclusions

475 This study presents the first description of  $\delta^{13}\text{C}$  carbon and  $\delta^{15}\text{N}$  nitrogen isotopic ~~compositions~~ signatures of cryoconite consumers (tardigrades and rotifers) and their potential food. Despite the variability in distribution of isotopic values, we showed that  $\delta^{15}\text{N}$  differs ~~among~~ between tardigrades and rotifers in all samples which points to their different roles in cryoconite trophic network. The  $\delta^{13}\text{C}$  values ~~reveal~~ revealed variability in their distribution among the animal taxa as well as between 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 suggesting~~ 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 consumers. We also revealed a significant correlation between organic carbon from decarbonized cryoconite and rotifers, which ~~indicates~~ may indirectly indicate that rotifers ~~may be~~ are related more to cryoconite carbon from bacteria than tardigrades, which are likely considered to be more herbivorous. Nevertheless, further research is required to elucidate and explain the cryoconite trophic levels network, the entire diet of the consumers and their contribution to supraglacial nutrient pathways. ~~Another outcome of this study is the introduction of modified technique of sample preparation avoiding procedures such as sugar centrifugation or oven drying.~~

490 **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  $\leftrightarrow(-)$  means that the mineral was not ~~present~~detected.

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

495 *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 Láska and all requests must be sent to him.

500 *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.

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