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RAPID COMMUNICATION

Note on important and novel findings

# Spatial distribution and stable isotopic composition of invertebrates uncover differences between habitats on the glacier surface in the Alps

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## Abstract

Glacier surfaces are the most biologically productive parts of glaciers with a variety of organisms and habitats. However, distinctiveness of habitats and communities of dominant invertebrate consumers on the ice surface is poorly documented. We focused on dominant consumers in three supraglacial (on the glacier surface) habitats on the alpine glacier Forni – cryoconite holes (water-filled reservoirs with a thin layer of sediment at the bottom), supraglacial debris (layer of stones and gravel covering glacier surface), and surface ice of the weathering crust. We analyzed carbon and nitrogen contents and stable isotope ratios ($\delta^{13}$C, $\delta^{15}$N), organic matter (OM) content, biomass of consumers, and the community composition of consumers to investigate differences between supraglacial habitats. In cryoconite holes, tardigrades (Tardigrada) were dominant consumers. In supraglacial debris, only springtails (Collembola) occurred mainly between stones and ice. No active animals were found in the surface ice of the weathering crust. Carbon and nitrogen contents, $\delta^{13}$C, and $\delta^{15}$N of invertebrates and OM differed between habitats. Cryoconite was enriched in OM with high $\delta^{13}$C and low $\delta^{15}$N compared to supraglacial debris likely indicating differences in major components of OM serving as food of invertebrates. Also, the OM, and carbon and nitrogen contents differed between habitats with the highest concentration in cryoconite. The dry biomass of tardigrades was similar compared to springtails. We present the first observation of differences between supraglacial habitats in the Alps based on the community composition of invertebrates, OM and stable isotopes. This initial study highlights the importance of differences in habitats and its consumers in the functioning of supraglacial ecosystem.

**Keywords** Cryoconite holes · Collembola · Supraglacial debris · Tardigrada · Stable isotopes · Weathering crust

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## Introduction

Glaciers are one of the coldest of Earth's biomes, characterized by low temperature, strong seasonality, and truncated food webs with unique organisms adapted to harsh environmental conditions (Anesio and Laybourn-Parry 2012; Hodson et al. 2008; Zawierucha et al. 2021). Due to their relatively high biological activity, as well as physical and chemical weathering of minerals, glacier surfaces are important for nutrient cycling in polar and mountain areas since they can serve as both sources and sinks of essential nutrients and compounds, particularly those containing carbon and nitrogen (Bagshaw et al. 2013; Stibal et al. 2012; Telling et al. 2011). For example, supraglacial (on the glacier surface) primary producers can have comparable production rates to those from polar lakes (Callieri and Bertoni 1999; Hood et al. 2015), and input of supraglacial organic matter transported from glaciers by meltwater can have a significant effect on downstream terrestrial and aquatic ecosystems (Bagshaw et al. 2013; Hood et al. 2015; Stibal et al. 2012). Yet, the importance of glacier ecosystems is often overlooked by broader ecological community (Stibal et al. 2020), as highlighted by Cauvy-Fraunié and Dangles (2020) largely because these ice masses are often perceived as lifeless, and their functioning remains enigmatic (e.g., Buda et al. 2021).

The surface of glaciers is a heterogeneous and dynamic landscape with variable environmental conditions which forms distinct habitats (Anesio and Laybourn-Parry 2012). The key element of supraglacial biological activity is cryoconite (Rozwalak et al. 2022). Cryoconite is a dark sediment composed of a consortium of mineral particles, bacteria, algae, and other organic matter (Langford et al. 2010; Takeuchi et al. 2010; Rozwalak et al. 2022). Cryoconite occurs on the glacier surface and through the reduction of albedo (reflection of solar radiation) which leads to greater ice melt, drives the formation of small depressions called cryoconite holes (Takeuchi et al. 2002). Cryoconite holes are formed in the ablation zone of the glacier (area with the ice loss exceeding its gain) where the surface glacier ice is exposed during the summer season, and it is snow and debris free (e.g., MacDonell and Fitzsimons 2008).

Cryoconite holes are water-filled reservoirs which serve as important stores of organic matter and are hot-spots of supraglacial biodiversity with a myriad of inhabitants from microbes to micro-invertebrates, such as tardigrades and rotifers, which dominate cryoconite holes worldwide (Pittino et al. 2018; Sommers et al. 2019; Zawierucha et al. 2021). In some regions of the world, cryoconite holes are inhabited even by macro-fauna like Plecoptera in South America or Diptera in Himalaya (Kohshima 1985; Zawierucha et al. 2022).

Apart from cryoconite holes, some parts of glacier surface are covered by a layer of gravel and stones called supraglacial debris (e.g., Azzoni et al. 2016). Due to glacier melting, supraglacial debris is a dynamic semi-aquatic environment which, depending on region and glacier, is inhabited by organisms, such as nematodes, springtails, and other minute and macro-arthropods (Buda et al. 2020; Valle et al. 2022; Zawierucha et al. 2021). The surface of the ablation zone mostly consists of weathering crust (highly porous surface ice formed by solar heating of the glacier surface) which, due to microscopic water veins, can be compared to a shallow-perched aquifer with enhanced microbial activity on the glacier surface (Christner et al. 2018; Cook et al. 2016; Patterson 1994). Surface ice of the weathering crust can be perceived as another supraglacial habitat and a transitional zone or ecotone between cryoconite holes and supraglacial debris.

Invertebrate consumers are important components in the functioning of supraglacial habitats due to their feeding on bacteria, fungi, and algae, and their ability to shape the structure of primary producer communities (Vonnahme et al. 2016; Zawierucha et al. 2022). Nevertheless, whether different taxa of glacier consumers utilize the same or different food, how they are spatially distributed on the ice surface, and what is their contribution to secondary productivity (quantifiable by estimating the biomass of heterotrophs, equivalent of net primary production by autotrophs) is still far from understood.

Considering that alpine glaciers are one of the fastest disappearing glacier systems worldwide (Gobbi et al. 2021; Lencioni et al. 2021; Zawierucha and Shain 2019), such gaps in the knowledge on supraglacial consumers and their habitats prevent the understanding of how supraglacial ecosystems may transition over time since many supraglacial habitats can change from one to another (e.g., surface ice of the weathering crust into cryoconite holes and vice versa).

We focused our study on dominant groups of consumers in three key types of supraglacial habitats: cryoconite holes, supraglacial debris, and surface ice of the weathering crust on the alpine glacier Forni (Fig. 1). We combined field observations on the distribution of consumers, estimations of their biomass, stable isotopic analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), analyses of OM, analyses of carbon and nitrogen contents, and the estimation of mineral composition in cryoconite and debris. We primarily aimed to: (i) provide an initial observation and description of spatial variation in consumer communities on an alpine glacier since most recent studies from alpine glaciers focus on microbial communities; and by analyzing organic matter contents, mineral composition, and $\delta^{13}C$ and $\delta^{15}N$, (ii) point out possible drivers of distinctiveness in their habitats.


**Fig. 1** **A** Ablation zone of the Forni Glacier; **B**, **C** Cryoconite holes on the Forni Glacier; **D** Surface ice of the weathering crust not covered by dust or algae; **E** Surface ice of the weathering crust covered by cryoconite and ice algae; **F** Supraglacial debris—a main habitat for springtails on the Forni Glacier

## Materials and methods

### Study site and sampling

Fieldwork was conducted in August 2020 on the ablation zone of the Forni Glacier in the Alps (approximately 2500 m a. s. l., 46° 24′ N 10° 35′ E, glacier surface shown in Fig. 1A). The detailed description of the Forni Glacier is provided in Azzoni et al. (2016), Citterio et al. (2007), and Senese et al. (2020). We collected metazoans and cryoconite from cryoconite holes (Fig. 1B, C), supraglacial debris (Fig. 1F), and surface ice of the weathering crust (Fig. 1D, E) at randomly chosen sites along the ablation zone of the glacier to reflect the heterogeneity and biotic diversity of the glacier surface. Sampling sites were located in the upper part of the ablation zone (area in front of the icefall, approx. 300 m from the glacier snout, where the glacier slope is mild, and the surface starts to be flat).

Cryoconite holes were a few cm deep and up to approx. 30 cm in diameter. The amount of cryoconite in the bottom of the holes did not exceed 1 cm in depth. Cryoconite granules on the Forni Glacier were approx. 0.5 mm in size (Rozwalak et al. 2022), surrounded by fine organic matter and mineral particles with and average size up to approx. 1 mm (Fig. S1C).

Supraglacial debris occured in a band composed from sediment to boulders which occurred along the entire axis of the ablation zone in front of the icefall (approx. 600 m in length). Fine sediment and smaller fraction of gravel (e.g., granules, pebbles) mostly form a thin layer which is wet or even submerged in some places. Pebbles and cobbles are dry, but the border between their surface and ice forms a wet layer inhabited by springtails (Fig. S1A). Sample of supraglacial debris consisted of fine organic matter and mineral particles with size < 1 mm up to rocks approx. 1.5 cm long (Fig. S1B). This material consists mostly of gravel fraction without cryoconite. Supraglacial debris originates most likely from both moraines and surrounding mountain slopes.

Surface ice of the weathering crust differed between sample types, clean ice was mostly solid while ice covered by cryoconite and, most likely, ice algae was characterized by relatively big crumbling crystals. The thickness of crystals was approx. 7 cm.

In total, one pooled sample of cryoconite (approx. 1 L of sediment from more than 15 cryoconite holes), one pooled sample of supraglacial debris (approx. 1 kg in total collected from many sites in the middle and upper part of supraglacial debris band), and 12 independent samples of surface ice of the weathering crust from which 6 were clean ice and 6 were ice covered by algae and cryoconite (potential food for animals) were collected for the characterization of invertebrate communities and analyses of their carbon and nitrogen contents, $\delta^{13}C$ and $\delta^{15}N$, and the organic matter content.

Additionally, cryoconite for the estimation of biomass of tardigrades was collected from 8 randomly chosen cryoconite holes (in the upper part of the ablation zone, see the description above). For this purpose, independent cryoconite samples were collected from the bottom of holes by Pasteur pipette. We collected approx. 1 $cm^2$ of sediment from the bottom of the holes (then the biomass of tardigrades in cryoconite per 1 $cm^2$ for each sample was estimated). Sampled cryoconite holes for the biomass estimation were located in the same area of the ablation zone where cryoconite for isotopic analyses was sampled. Sampled cryoconite holes were of diverse shapes (from irregular to oval), and in size range of approx. 10 cm to 30 cm. Such holes reflected the heterogeneity of hole shapes on the Forni Glacier.

All samples were collected in sterile falcon tubes or plastic bags using aseptic spoons, clean plastic pipettes, and nitrile gloves. Cryoconite was collected by a pipette, debris was collected with a spoon, and surface ice of the weathering crust was collected by scratching a few centimeters of the surface ice (area of 10 cm × 10 cm) by chisel. Springtails were collected into a falcon tube by air flow made by a big pipette from the stone surface under randomly chosen supraglacial stones. Immediately after collection, all samples were frozen and transported to Adam Mickiewicz University in Poznań and kept at –20 °C until further analyses.

## Stable isotopic analyses

### Preparation of invertebrate consumers

For analyses of tardigrades, approx. 200 $cm^3$ of cryoconite from the pooled sample was slowly melted and tardigrades were collected by glass Pasteur pipettes under a light microscope. During the collection, tardigrades were constantly cooled by a cooling pad. Individuals were cleaned from superficial mineral and organic particles in distilled water and transferred into a clean Eppendorf tube. Thereafter, the sample was stored at –20 °C until further processing started. Springtails were already separated from supraglacial debris during the field sampling and immediately frozen in a conical tubes. Thus, no preparation before lyophilization was needed. After all samples were prepared, tardigrades and springtails were melted, and individuals were cleaned at least two times in a drop of distilled water under a light microscope (Leica DM750). Overall 546 individuals of tardigrades and 193 individuals of springtails were cleaned, and transferred into pre-weighed tin capsules (Elemental Microanalysis, 8 × 5 mm, D1013) using a glass Pasteur pipette for tardigrades and stainless-steel lab spatula for springtails. The total number of individuals was divided into two subsamples for tardigrades and three subsamples for springtails. Afterward, all samples were stored at –20 °C and at –72 °C half an hour before the lyophilization. The duration of lyophilization was 3.5 h. Thereafter, samples were weighed (Mettler Toledo Excellence Plus XP6; linearity = 0.0004 mg) and tin capsules were closed, wrapped, and stored in a desiccator at 22 °C with 32% humidity for approx. 1 h before, they were analyzed. The average dry weight of subsamples was ~ 0.109 mg for tardigrades and ~ 1.727 mg for springtails. During the lyophilization, a few individuals of tardigrades (5−10) were scattered around the tin capsule, probably because of their low dry weight.

### Preparation of cryoconite and supraglacial debris

For analyses of cryoconite and supraglacial debris, all animals were removed from the material and both samples were frozen in –20 °C before further processing started. For analyses of cryoconite, approx. 6 ml of material from pooled sample was melted and homogenized using an agate pestle and mortar and dried on a Petri dish at 45 °C for 19 h. For analyses of supraglacial debris, approx. 30 ml of material from pooled sample was melted and divided into 3 size fractions using: (i) plastic Pasteur pipette with a 1 mm diameter of the syringe; (ii) plastic Pasteur pipette with a 2 mm diameter of the syringe; (iii) plastic spoon for debris with an average size of 5 mm. Thereafter, all three fractions were separately homogenized using an agate pestle and mortar and dried on Petri dishes at 45 °C for approximately 18 h. We partially covered all Petri dishes by an aluminum film during the drying.

For analyses of $\delta^{15}N$ in OM, dry cryoconite and supraglacial debris were transferred without any other preparation into pre-weighed tin capsules (Elemental Microanalysis, 8 × 5 mm, D1013) and weighed. For all samples, three subsamples (from one pooled sample) on average 30.7 mg were prepared. For analyses of $\delta^{13}C$ in the OM, on average 11.7 mg of material was transferred into pre-weighed silver capsules (Elemental Microanalysis, 8 × 5 mm, D2008) and carbonates were dissolved using 10% HCl moistened with $diH_2O$. The acid was pipetted into the capsules followed by additions of 20, 30, 50 or 10, 10, 30, 50 and 100 µL 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 to dry at 50 °C for

17 h. After drying, silver capsules were inserted into tin capsules and put into a desiccator for 22 d.

### Analytical approach

For stable isotopic analyses, the method according to Novotná Jaroměřská et al. (2021) was used and the carbon and nitrogen contents, $\delta^{13}C$, and $\delta^{15}N$ in all samples were analyzed using a Flash 2000 elemental analyser (ThermoFisher Scientific, Bremen, Germany) and isotope-ratio mass spectrometer Delta V Advantage (ThermoFisher Scientific, Bremen, Germany), respectively. Released gasses ($NO_x$, $CO_2$) separated in a GC (gas chromatography) column were transferred to an isotope-ratio mass spectrometer through a capillary by Continuous Flow IV system (ThermoFisher Scientific, Bremen, Germany). The stable isotopic values were expressed in standard delta notation (δ) with samples measured relative to Pee Dee Belemnite for carbon isotopes and atmospheric $N_2$ for nitrogen isotopes. All values were normalized to a regression 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 (International Atomic Energy Agency, Vienna) for nitrogen. The regression curve of the total gas for analyses of cryoconite was based on the international standard Soil Standard Clay OAS (Elemental Microanalysis, UK). Analytical precision as a long reproducibility for standards was within ± 0.03 ‰ for $\delta^{13}C$ and ± 0.02 ‰ for $\delta^{15}N$.

For cryoconite and supraglacial debris, the $\delta^{13}C$ of decarbonized material and $\delta^{15}N$ of material without any treatment were used as a reference to the isotopic composition of potential food source for tardigrades and springtails. Carbon content in decarbonized samples and nitrogen content in samples without treatment were used as a reference to OC (organic carbon) and ON (organic nitrogen).

### X-ray diffraction

For analyses of mineral composition of cryoconite and supraglacial debris, homogenized sediment from remaining samples used for isotopic analyses was analyzed by an X-ray diffraction. Analyses were performed on the Panalytical X'Pert Pro (PW3040/60) with an X'Celerator detector. The measurements were conducted under the following conditions: radiation – CuKα, 40 kV, 30 mA; angular range – 3 to 70° 2*θ*; step 0.02°/200 s. The results were evaluated using an X'Pert HighScore Plus 1.0d software program with a JCPDS PDF-2 (ICDD 2002) database.

### Organic matter measurements

The amount of OM in cryoconite and debris was measured as a percentage weight loss through the combustion at 550 °C for 3 h following drying at 50 °C for 24 h (Wang et al. 2011). Supraglacial debris was divided into two fractions, the small below 2 mm as a reference to the majority of consumable OM in the debris and the bigger one which contained debris without any size selection.

### Observation of animals, their identification, and biomass estimation

Animals collected from three sampled habitats were investigated under stereo microscopes in the laboratory. Tardigrades and springtails were identified by comparisons with the original literature (Buda et al. 2020; Dastych et al. 2003; Fjellberg 2010; Zawierucha et al. 2019a).

For the biomass estimation of tardigrades, body length and width of fresh (wet) individuals were measured from photographs (Fig. S2A, B) taken by Quick PHOTO Camera 3.0 software (Promicra, Prague, Czech Republic) under an Olympus BX53. Animals were measured using ImageJ 1.53 software (Schneider et al. 2012). Photographs were turned into grayscale and binary defined (Fig. S2C). Thereafter, the "analyze particle command" (this command counts and measures objects in binary or thresholded images) was used to fit an ellipse onto each organism (Fig. S2D). Primary and secondary axis of each ellipse marked body length and body width of tardigrades. All these steps were run as separate macros (a simple program that automates a series of ImageJ commands) to provide a better control on the whole analysis. Biomass (wet weight, *W*) of each specimen was calculated based on the formula of Hallas and Yates (1972): if body length (*L*) and width (*D*) were 4:1; $W = L3 \times 0.051 \times 10^{-6}$, or 5:1; $W = L3 \times 0.033 \times 10^{-6}$. The wet weight was converted to dry weight based on De Bovée and Labat (1993).

Data on the biomass of springtails from the supraglacial debris of the Forni Glacier were taken from Buda et al. (2020). Dry biomass of springtails was calculated based on a linear equation with coefficients proposed by Tanaka (1970). Detailed description of sampling and calculations for the estimation of biomass of springtails in the supraglacial debris can be found in Buda et al. (2020). Weight of tardigrades is expressed as dry biomass per approx. 1 $cm^2$, and weight of springtails is expressed as dry biomass per 1 $cm^2$ of stone surface in a band of supraglacial debris.

### Statistical analyses and graphical illustration of results

The graphic visualizing of stable isotopic values was created using the R version 3.5.3 (R Development Core Team 2018) package ggplot2 (Wickham 2009).

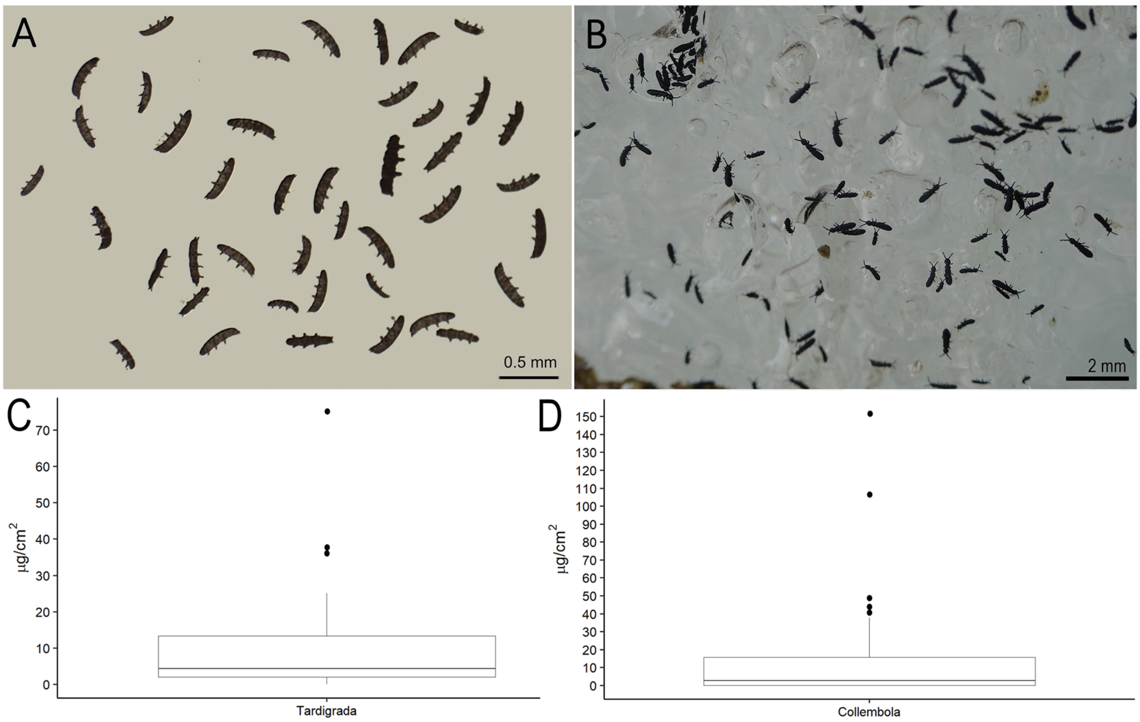


**Fig. 2** **A** Tardigrades (Tardigrada); **B** Springtails from the family Isotomidae (Collembola); **C** Dry biomass of tardigrades from cryoconite holes; **D** Dry biomass of springtails from the supraglacial debris (data on springtails are handled from Buda et al. (2020))

## Results

Cryoconite holes on the Forni Glacier were inhabited by a single morpho-species of tardigrade (*Cryobiotus klebelsbergi*) (Fig. 2A). We found only few individual rotifers among hundreds of tardigrades. In supraglacial debris, particularly between stones and ice surface, only springtails belonging to the family Isotomidae (Fig. 2B) occurred and no other invertebrates were found. Occasionally, springtails were found on the surface water of cryoconite holes, but their densities were low compared to those in the supraglacial debris. Samples of surface ice of the weathering crust were devoid of active invertebrates, and only a few carcasses of tardigrades and springtails were found. Thus, surface ice of the weathering crust was not further investigated. The biomass of tardigrades in cryoconite holes ranged from 0 to 75 µg/cm$^2$, with an average of 12.28 µg/cm$^2$ (Fig. 2C). The biomass of springtails was 0–151.82 µg/cm$^2$ of stones, with an average of 12.67 µg/cm$^2$ (Fig. 2D) (Buda et al. 2020).

Results of stable isotopic analyses showed differences between both groups of invertebrate consumers as well as between organic matter in different fractions of supraglacial debris and cryoconite. The $\delta^{13}C$ and $\delta^{15}N$ of tardigrades and springtails was considerably lower compared to OM in cryoconite and fractions of supraglacial debris (Fig. 3). The differences in mean isotopic values revealed by stable isotopic analysis were 1.72 ‰ for the $\delta^{13}C$ and 0.92 ‰ for the $\delta^{15}N$ between tardigrades and springtails with higher (more enriched in heavier isotope) values for springtails (Fig. 3). Cryoconite revealed higher $\delta^{13}C$ compared to all size fractions of supraglacial debris. Also, each size fraction of debris revealed different isotopic values. Smaller fractions had lower $\delta^{15}N$ and higher $\delta^{13}C$ values compared to bigger fractions. The biggest size fraction (approx. 5 mm) had highest $\delta^{15}N$ and lowest $\delta^{13}C$ from all size fractions of debris.

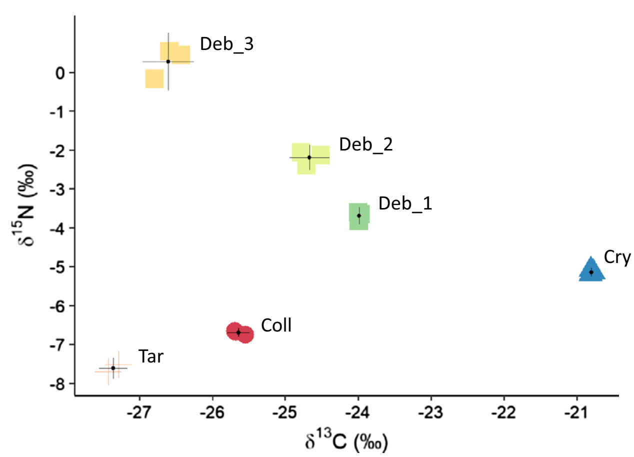


**Fig. 3** The map of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopic values of tardigrades (Tar), springtails (Coll), cryoconite (Cry), and size fractions of supraglacial debris (Deb) with mean and error bars for their subsamples. The size fractions of debris are Deb_1 < 1 mm, Deb_2 < 2 mm, Deb_3 ≤ 5 mm

The organic matter content differed between cryoconite and supraglacial debris. The OM content in cryoconite varied from 11 to 12.5%. The OM content in debris was 2.05% in the fraction below 2 mm and 0.62% in all size fractions together. Cryoconite had higher amount of OC and ON, and lower OC:ON (mol/mol) ratio compared to all fractions of supraglacial debris. In supraglacial debris, OC and ON contents decreased with increasing size of fraction (Fig. 4). The detailed results of stable isotopic ratios, carbon and nitrogen contents, and OC:ON ratios in consumers (tardigrades and springtails), organic matter in each size fraction of supraglacial debris (Deb 1 < 1 mm, Deb 2 < 2 mm, Deb 3 ≤ 5 mm), cryoconite, and their subsamples are shown in Table 1.

Results of X-ray diffraction showed that all samples contained quartz, plagioclase, muscovite or illite, chlorite and orthoclase. The composition of mineral phases was the same in samples, but every sample differed in the proportion of each phase. The proportion of plagioclase (albite) increased with decreasing size of the debris and the proportion of quartz decreased with increasing size of the debris. Cryoconite was rich in organic matter which increased the background of the X-ray recording (Fig. S3).

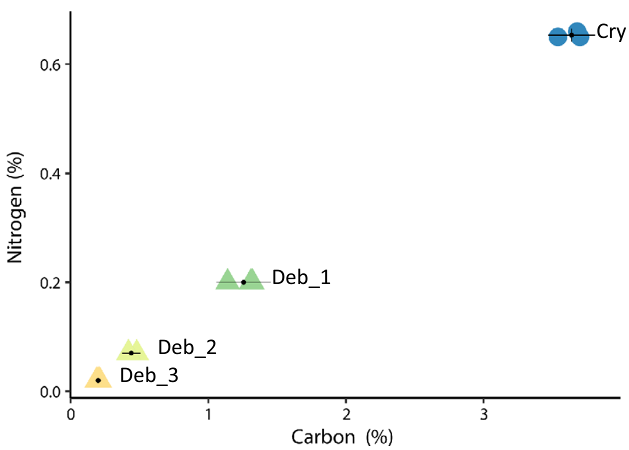


**Fig. 4** The proportion (%) of carbon (C) and nitrogen (N) in organic matter of cryoconite and size fractions of supraglacial debris (Deb) with mean and error bars (standard deviation) for their subsamples. The size fractions of debris are Deb_1 < 1 mm, Deb_2 < 2 mm, Deb_3 ≤ 5 mm

## Discussion

Results of this study revealed differences in communities of dominant supraglacial consumers among three supraglacial habitats on the alpine Forni Glacier. Cryoconite holes were inhabited by tardigrades while the band of supraglacial debris was inhabited by springtails. The dominance of tardigrades in cryoconite holes corroborates results of Zawierucha et al. (2019a, 2021) who described this phenomenon over four ablation seasons since 2012. Such stability in spatial distribution of consumers indicates their high adaptability to dynamic conditions, and stability of their niches, given that the surface of a small valley glacier is a dynamic environment, mostly controlled by physical forces (radiation, rain, wind) which often cause unexpected mixing, redistribution and transport of cryoconite, OM produced in situ, and windblown OM over the whole supraglacial zone (author's personal observation; Azzoni et al. 2016; Hodson et al. 2008; Stibal et al. 2006; Takeuchi et al. 2010, 2018; Vonnahme et al. 2016; Zawierucha et al. 2019b).

The absence of tardigrades in supraglacial debris can be triggered by low survival rate in high temperatures (Zawierucha et al. 2019a). Although tardigrades were observed in sparse, fine debris mixed with cryoconite in area of the formation of cryoconite holes on the Forni Glacier, their abundance was significantly lower compared to cryoconite holes (Zawierucha et al. 2019a). Moreover, they were still absent in the band of supraglacial debris where springtails occur. Whereas most supraglacial habitats fluctuate around 0 °C, supraglacial debris can reach much higher temperature with strong diurnal fluctuations (Conway and Rasmussen

**Table 1** Stable isotopic values ($\delta^{13}C$, $\delta^{15}N$), carbon and nitrogen contents, and mean OC:ON molar ratio of tardigrades, springtails, cryoconite (Cry), and three different size fractions of supraglacial debris (Deb_1 < 1 mm, Deb_2 < 2 mm, Deb_3 ≤ 5 mm). OC:ON molar ratio is averaged across all subsamples

| Sample type | Tardigrades | Springtails | Deb_1 | Deb_2 | Deb_3 | Cry |
|---|---|---|---|---|---|---|
| $\delta^{13}C$ (‰) | –27.29 | –25.68 | –23.99 | –24.71 | –26.79 | –20.81 |
| | –27.43 | –25.55 | –23.97 | –24.78 | –26.59 | –20.78 |
| | | –25.69 | –24.00 | –24.51 | –26.43 | –20.81 |
| $\delta^{15}N$ (‰) | –7.52 | –6.66 | –3.82 | –2.39 | 0.44 | –5.20 |
| | –7.71 | –6.76 | –3.66 | –2.07 | 0.53 | –5.14 |
| | | –6.68 | –3.60 | –2.14 | –0.17 | –5.09 |
| C (%) | 54.64 | 57.37 | 1.14 | 0.48 | 0.20 | 3.54 |
| | 56.44 | 56.90 | 1.31 | 0.42 | 0.19 | 3.70 |
| | | 57.01 | 1.32 | 0.42 | 0.21 | 3.68 |
| N (%) | 9.19 | 10.64 | 0.20 | 0.07 | 0.02 | 0.65 |
| | 9.24 | 10.83 | 0.20 | 0.07 | 0.02 | 0.65 |
| | | 10.41 | 0.20 | 0.07 | 0.02 | 0.66 |
| OC:ON | 7.03 | 6.27 | 7.44 | 7.30 | 10.39 | 6.50 |

2000). Springtails are not known to survive in fully aquatic conditions. Thus, their absence at the bottom of cryoconite holes is not surprising. Springtails found in cryoconite holes were likely transported by wind or meltwater from supraglacial debris. The absence of active animals from algae and cryoconite covered samples of surface ice of the weathering crust was unexpected since active invertebrates were previously found in glacier surface ice (Shain et al. 2016, 2021). Thus, other environmental factors, such as high doses of UV radiation (e.g., Morgan-Kiss et al. 2006), short-term stability (e.g., Zawierucha et al. 2019a, b), or absence of preferred food, could prevent alpine species of supraglacial consumers from expanding their distribution to the surface ice of the weathering crust on the Forni Glacier.

The mineral composition of cryoconite and supraglacial debris was similar suggesting similar source of material in both habitats. However, supraglacial debris was approximately ten times lower in organic matter content compared to cryoconite. The lower amount of OC and ON was noticeable, likewise from OC:ON ratio. Overall, OC:ON ratios of cryoconite and debris were lower than those previously reported from arctic cryoconite holes (Stibal et al. 2008, 2010) which may be related to the different composition of OM, lower input of nutrient-rich OM, or lower atmospheric nitrogen deposition (precipitation) in the Arctic compared to the Alps.

Cryoconite was higher in $\delta^{13}C$ and lower in $\delta^{15}N$ compared to all fractions of OM in supraglacial debris likely indicating different composition of carbon and nitrogen sources and their utilization by microbes and consumers. The high $\delta^{13}C$ of cryoconite could be related to the high proportion and photosynthetic activity of primary producers (Musilova et al. 2015). Lower $\delta^{13}C$ of OM in supraglacial debris could indicate a higher input of allochthonous OC originated from terrestrial plants (Musilova et al. 2015; O'Leary 1981). The higher OC:ON in debris supports this assumption since terrestrial biomass often invests more into structural compounds with higher requirements for carbon (e.g., Cleveland and Liptzin 2007). The differences in $\delta^{15}N$ between debris and cryoconite are likely a result of their different origin and composition (e.g., Michener and Lajtha 2008). However, the decreasing $\delta^{13}C$ and increasing $\delta^{15}N$ with the size of the debris fraction may also be the result of a higher abundance of cyanobacterial biofilms on the rocks analyzed as the biggest fraction (e.g., Kohler et al. 2018) or a decline in recent organic matter and its replacement by an old organic matter in pores of minerals, such as chlorite and illite (Hayatdavoudi and Ghalambor 1996; McKirdy and Powell 1974).

Springtails and tardigrades on the Forni Glacier are herbivorous/detritivorous groups (Guidetti et al. 2012; Hao et al. 2020) which likely utilize algae and detritus (organic matter, microbes), as described in their non-glacier counterparts (Bryndová et al. 2020; Endlweber et al. 2009; Hao et al. 2020; Rusek 1998). Results of carbon and nitrogen isotopic analyses showed that springtails had higher $\delta^{13}C$ and $\delta^{15}N$ values compared to tardigrades. The $\delta^{13}C$ of consumers is often close (approx. $\pm 1$ ‰) to $\delta^{13}C$ of consumed food, while its $\delta^{15}N$ usually increases by approximately 3 ‰ with trophic level (e.g., Peterson and Fry 1987). The differences between tardigrades and springtails were less than one per mil 1 ‰, which indicates that they are not different trophic levels (Peterson and Fry 1987).

The $\delta^{13}C$ and $\delta^{15}N$ of tardigrades were considerably lower than those of their potential food, which is contrary to the general pattern of isotopic fractionation between consumer and food (Peterson and Fry 1987). A similar distribution of $\delta^{13}C$ and $\delta^{15}N$ between tardigrades and OM in cryoconite was reported by Novotná Jaroměřská et al. (2021). Based on these facts, we assume that tardigrades likely prefer to select some compounds of cryoconite OM (e.g., specific algae, cyanobacteria, or compounds of detritus) which may be a sufficient source of carbon and nitrogen, but their $\delta^{13}C$ and $\delta^{15}N$ could be affected by nutrient limitation or specific fractionation due to metabolic processes connected with low temperature.

The $\delta^{13}C$ of springtails could indicate the consumption of microbial biofilms or the selective consumption of bigger particles of OM which were not present or dominant in the smallest fraction of debris. Also, compared to the results of studies on species of both tardigrades and springtails and their food from soil, polar microbial mats, or Arctic cryoconite holes (Almela et al. 2019; Novotná Jaroměřská et al. 2021; Potapov et al. 2016; Potapov and Tiunov 2016; Shaw et al. 2018; Velázquez et al. 2017), consumers on the Forni Glacier had lower $\delta^{13}C$ and $\delta^{15}N$ than their potential food. However, a reliable method for analyses of debris and cryoconite compounds separately has not been established yet (mostly because cryoconite is a consortium of autotrophs, heterotrophs, and mineral particles), and we can only open the discussion on differences in isotopic values of consumers caused by different food, or by other mechanisms such as deviation from the usual fractionation between food and consumer due to enrichment or limitations of nutrients in the food (e.g., Haubert et al. 2005).

The biomass of springtails and tardigrades was similar. Biomass and size of consumers often indicates rates of consumers' secondary production (e.g., Edgar 1990), and it is a pivotal indicator of response of invertebrates to changes and availability of resources (Reed et al. 1994). Competition for food could also be one of the crucial factors driving the spatial distribution of both groups of invertebrates and formation of different niches on the glacier surface. Considering that debris and cryoconite covered area on the Forni Glacier is approximately 1/3 of the ablation zone (Ambrosini et al. 2019), differences in the distribution of invertebrates and their biological activity may have potential impact to the functioning of the Forni Glacier supraglacial environment.

## Conclusions

This study reports the distribution and trophic niches of dominant supraglacial consumers in three microhabitats (supraglacial debris, cryoconite holes, and surface ice of the weathering crust) on the alpine valley glacier Forni. Differences in $\delta^{13}C$, $\delta^{15}N$, OC:ON ratio and carbon and nitrogen contents between cryoconite and fractions of supraglacial debris indicate differences in organic matter source (autochthonous/allochthonous) and its usability as food source for consumers. The $\delta^{13}C$ and $\delta^{15}N$ of springtails which mainly inhabited supraglacial debris were higher compared to tardigrades which inhabited cryoconite holes likely indicating differences in food sources or fractionation. The biomass of tardigrades in cryoconite and springtails in debris indicates similar secondary production. Our results indicate that studies on differences between supraglacial habitats using invertebrates and stable isotopic analyses can be useful in investigations on supraglacial ecosystem functioning and changes in nutrient pathways during the glacier retreat since one habitat can become another.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

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



## References

Almela P, Velázquez D, Rico E, Justel A, Quesada A (2019) Carbon pathways through the food web of a microbial mat from Byers Peninsula, Antarctica. Front Microbiol 10:628

Ambrosini R, Azzoni S, Pittino F, Diolaiuti G, Franzetti A, Parolini M (2019) First evidence of microplastic contamination in the supraglacial debris of an alpine glacier. Environ Pollut 253:297–301

Anesio AM, Laybourn-Parry J (2012) Glaciers and ice sheets as a biome. Trends Ecol Evol 27:219–225

Azzoni RS, Senese A, Zerboni A, Maugeri M, Smiraglia C, Diolaiuti GA (2016) Estimating ice albedo from fine debris cover quantified by a semi-automatic method: the case study of Forni Glacier, Italian Alps. Cryosphere 10:665–679

Bagshaw EA, Tranter M, Fountain AG, Welch K, Basagic HJ, Lyons WB (2013) 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

Brodie CR, Leng MJ, Casford JS, Kendrick ChP, Lloyd JM, Yongqiang Z, Bird MI (2011) Evidence for bias in C and N concentrations and $\delta^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. Chem Geol 282:67–83

Bryndová M, Stec D, Schill RO, Michalczyk Ł, Devetter M (2020) Dietary preferences and diet effects on life-history traits of tardigrades. Zool J Linn Soc 188:865–877

Buda J, Azzoni RS, Ambrosini R, Franzetti A, Zawierucha K (2020) Effects of locality and stone surface structure on the distribution of Collembola inhabiting a novel habitat—the stone-ice border on an alpine glacier. Acta Oecol 108:103629

Buda J, Poniecka EA, Rozwalak P, Ambrosini R, Bagshaw EA, Franzetti A, Klimaszyk P, Nawrot A, Pietryka M, Richter D, Zawierucha K (2021) Is oxygenation related to the decomposition of organic matter in cryoconite holes? Ecosystems 2:1–12

Callieri C, Bertoni R (1999) Organic carbon and microbial food web assemblages in an oligotrophic alpine lake. J Limnol 58:136–143

Cauvy-Fraunié S, Dangles O (2020) Reply to: Glacial ecosystems are essential to understanding biodiversity responses to glacier retreat. Nat Ecol Evol 4:688–689

Christner BC, Lavender HF, Davis CL, Oliver EE, Neuhaus SU, Myers KF, Hagedorn B, Tulaczyk SM, Doran PT, Stone WC (2018) Microbial processes in the weathering crust aquifer of a temperate glacier. Cryosphere 12:3653–3669

Citterio M, Diolaiuti G, Smiraglia C, Verza GP, Meraldi E (2007) Initial results from the automatic weather station (AWS) on the ablation tongue of Forni Glacier (Upper Valtellina, Italy). Geogr Fis e Din Quat 30:141–151

Cleveland CC, Liptzin D (2007) C:N: P stoichiometry in soil: is there a ‘Redfield ratio’ for the microbial biomass? Biogeochemistry 85:235–252

Conway H, Rasmussen LA (2000) Summer temperature profiles within supraglacial debris on Khumbu Glacier. Nepal IAHS Publication 264:89–98

Cook JM, Hodson AJ, Irvine-Fynn TDL (2016) Supraglacial weathering crust dynamics inferred from cryoconite hole hydrology. Hydrol Process 30:433–446

Dastych H, Kraus J, Thaler K (2003) Redescription and notes on the biology of the glacier tardigrade *Hypsibius klebelsbergi* Mihelcic, 1959 (Tardigrada), based on material from Ötztal Alps, Austria. Mitteilungen Aus Dem Hamburgischen Zoologischen Museum Und Institut 100:77–100

De Bovée F, Labat JP (1993) A simulation model of a deep meiobenthic compartment: a preliminary approach. Marine Ecol 14(2):159–173

Edgar GJ (1990) The use of the size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production. J Exp Mar Biol Ecol 137:195–214

Endlweber K, Ruess L, Scheu S (2009) Collembola switch diet in presence of plant roots thereby functioning as herbivores. Soil Biol Biochem 41:1151–1154

Fjellberg A (2010) Cryophilic Isotomidae (Collembola) of the Northwestern Rocky Mountains, U.S.A. Zootaxa 2513:27–49

Gobbi M, Ambrosini R, Casarotto C, Diolaiuti G, Ficetola GF, Lencioni V, Seppi R, Smiraglia C, Tampucci D, Valle B, Caccaniga M (2021) Vanishing permanent glaciers: climate change is threatening a European Union habitat (Code 8340) and its poorly known biodiversity. Biodivers Conserv 30:2267–2276

Guidetti R, Altiero T, Marchioro T, Amade LS, Avdonina AM, Bertolani R, Rebecchi L (2012) Form and function of the feeding apparatus in Eutardigrada (Tardigrada). Zoomorphology 131:127–148

Hallas TE, Yeates GW (1972) Tardigrada of the soil and litter of a Danish beech forest. Pedobiologia 12:287–304

Hao C, Chen TW, Wu Y, Chang L, Wu D (2020) Snow microhabitats provide food resources for winter-active Collembola. Soil Biol Biochem 143:107731

Haubert D, Langel R, Scheu S, Ruess L (2005) Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. Pedobiologia 49:229–237

Hayatdavoudi A, Ghalambor A (1996) A study of formation damage of selective clay and other minerals caused by bacterial plugging. SPE Drill Complet 11:160–167

Hodson A, Anesio A, Tranter M, Fountain AG, Osborn M, Priscu JC, Laybourn-Parry J, Sattler B (2008) Glacial ecosystems. Ecol Monogr 78:41–67

Hood E, Battin TJ, Fellman J, O’neel S, Spencer RG, (2015) Storage and release of organic carbon from glaciers and ice sheets. Nat Geosci 8:91–96

International Centre for Diffraction Data (2002) JCPDS PDF-2 Database, ICDD, Newtown Square, Pennsylvania, USA

Kohler TJ, Stanish LF, Liptzin D, Barrett JE, McKnight DM (2018) Catch and release: hyporheic retention and mineralization of N-fixing *Nostoc* sustains downstream microbial mat biomass in two polar desert streams. L&O Letters 3:357–364

Kohshima S (1985) Patagonian glaciers as insect habitats. In: Nokajima C (ed) Glaciological studies in Patagonia Northern Icefield. Data Center for Glacier Research Japanese Society of Snow and Ice, Kyoto, pp 94–99

Langford H, Hodson A, Banwart S, Bøggild C (2010) The microstructure and biogeochemistry of Arctic cryoconite granules. Ann Glaciol 51:87–94

Lencioni V, Franceschini A, Paoli F, Debiasi D (2021) Structural and functional changes in the macroinvertebrate community in Alpine stream networks fed by shrinking glaciers. Fundam Appl Limnol 194:237–258

MacDonell S, Fitzsimons S (2008) The formation and hydrological significance of cryoconite holes. Prog Phys Geogr 32:595–610

McKirdy DM, Powell TG (1974) Metamorphic alteration of carbon isotopic composition in ancient sedimentary organic matter: new evidence from Australia and South Africa. Geology 2:591–595

Michener R, Lajtha K (2008) Stable isotopes in ecology and environmental science. John Wiley & Sons, New Jersey

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Molec Biol Rev 70:222–252

Musilova M, Tranter M, Bennett SA, Wadham J, Anesio AM (2015) Stable microbial community composition on the Greenland Ice Sheet. Front Microbiol 6:193

Novotná Jaroměřská T, Trubač J, Zawierucha K, Vondrovicová L, Devetter M, Žárský JD (2021) Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing divergent roles in a supraglacial trophic network. Biogeosciences 18:1543–1557

O’Leary MH (1981) Carbon isotope fractionation in plants. Phytochemistry 20:553–567

Patterson WSB (1994) The physics of glaciers, 3rd edn. Elservier, Oxford

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Pittino F, Maglio M, Gandolfi I, Azzoni RS, Diolaiuti G, Ambrosini R, Franzetti A (2018) Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. Ann Glaciol 59:1–9

Potapov AM, Tiunov AV (2016) Stable isotope composition of mycophagous collembolans versus mycotrophic plants: Do soil invertebrates feed on mycorrhizal fungi? Soil Biol Biochem 93:115–118

Potapov AA, Semenina EE, Korotkevich AY, Kuznetsova NA, Tiunov AV (2016) Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biol Biochem 101:20–31

R Development Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Reed JL, Cambell IC, Bailey PCE (1994) The relationship between invertebrate assemblages and available food at forest and pasture sites in three southeastern Australian streams. Freshw Biol 32:641–650

Rozwalak P, Podkowa P, Buda J, Niedzielski P, Kawecki S, Ambrosini R, Azzoni RS, Baccolo G, Ceballos JL, Cook J, Di Mauro B, Ficetola GF, Franzetti A, Ignatiuk D, Klimaszyk P, Łokas E, Ono M, Parnikoza I, Pietryka M, Pittino F, Poniecka E, Porazinska DL, Richter D, Schmidt SK, Sommers P, Souza-Kasprzyk J, Stibal M, Szczuciński W, Uetake J, Wejnerowski Ł, Yde JC, Takeuchi N, Zawierucha K (2022) Cryoconite – From minerals and organic matter to bioengineered sediments on glacier’s surfaces. Sci Total Environ 807:150874

Rusek J (1998) Biodiversity of Collembola and their functional role in the ecosystem. Biodivers Conserv 7:1207–1219

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Senese A, Manara V, Maugeri M, Diolaiuti GA (2020) Comparing Measured Incoming Shortwave and Longwave Radiation on a Glacier Surface with Estimated Records from Satellite and Off-Glacier Observations: A Case Study for the Forni Glacier. Italy Remote Sens 12:3719

Shain DH, Halldórsdóttir K, Pálsson F, Aðalgeirsdóttir G, Gunnarsson A, Jónsson Þ, Lang SA, Pálsson HS, Steinþórssson S, Arnason E

(2016) Colonization of maritime glacier ice by bdelloid Rotifera. Mol Phylogenet Evol 98:280–287
Shain DH, Novis PM, Cridge AG, Zawierucha K, Geneva AJ, Dearden PK (2021) Five animal phyla in glacier ice reveal unprecedented biodiversity in New Zealand's Southern Alps. Sci Rep 11:3898
Shaw EA, Adams BJ, Barrett JE, Lyons WB, Virginia RA, Wall DH (2018) 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
Sommers P, Porazinska DL, Darcy JL, Zamora F, Fountain AG, Schmidt SK (2019) Experimental cryoconite holes as mesocosms for studying community ecology. Polar Biol 42:1973–1984
Stibal M, Šabacká M, Kaštovská K (2006) Microbial communities on glacier surfaces in svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb Ecol 52:644–654
Stibal M, Tranter M, Telling J, Benning LG (2008) Speciation, phase association and potential bioavailability of phosphorus on a Svalbard glacier. Biogeochemistry 90:1–3
Stibal M, Lawson EC, Lis GP, Mak KM, Wadham JL, Anesio AM (2010) Organic matter content and quality in supraglacial debris across the ablation zone of the Greenland ice sheet. Ann Glaciol 51:1–8
Stibal M, Šabacká M, Žárský J (2012) Biological processes on glacier and ice sheet surfaces. Nat Geosci 5:771–774
Stibal M, Bradley JA, Edwards A, Hotaling S, Zawierucha K, Rosvold J, Lutz S, Cameron KA, Mikucki JA, Kohler TJ, Šabacká M, Anesio AM (2020) Glacial ecosystems are essential to understanding biodiversity responses to glacier retreat. Nat Ecol Evol 4:686–687
Takeuchi N (2002) Optical characteristics of cryoconite (surface dust) on glaciers: the relationship between light absorbency and the property of organic matter contained in the cryoconite. Ann Glaciol 34:409–414
Takeuchi N, Nishiyama H, Li Z (2010) Structure and formation process of cryoconite granules on Ürümqi glacier No. 1, Tien Shan. China Ann Glaciol 51:9–14
Takeuchi N, Sakaki R, Uetake J, Nagatsuka N, Shimada R, Niwano M, Aoki T (2018) Temporal variations of cryoconite holes and cryoconite coverage on the ablation ice surface of Qaanaaq Glacier in northwest Greenland. Ann Glaciol 59:21–30
Tanaka Z (1970) Ecological studies on communities of soil Collembola in Mt. Sobo, southwest Japan. Jpn J Ecol 20:102–110
Telling J, Anesio AM, Tranter M, Irvine-Fynn T, Hodson A, Butler C, Wadham J (2011) Nitrogen fixation on Arctic glaciers. Svalbard J Geophys Res 116:G03039
Valle B, di Musciano M, Gobbi M, Bonelli M, Colonnelli E, Gardini G, Migliorini M, Pantini P, Zanetti A, Berrilli E, Frattaroli AR, Fugazza D, Invernizzi A, Caccianiga M (2022) Biodiversity and ecology of plants and arthropods on the last preserved glacier of the Apennines mountain chain (Italy). Holocene: 32:853–865
Velázquez D, Jungblut AD, Rochera C, Rico E, Camacho A, Quesada A (2017) Trophic interactions in microbial mats on Byers Peninsula, maritime Antarctica. Polar Biol 40:1115–1126
Vindušková O, Jandová K, Frouz J (2019) Improved method for removing siderite by in situ acidification before elemental and isotope analysis of soil organic carbon. J Plant Nutr Soil Sci 182:82–91
Vonnahme TR, Devetter M, Žárský JD, Šabacká M, Elster J (2016) Controls on microalgal community structures in cryoconite holes upon high Arctic glaciers, Svalbard. Biogeosciences 13:659–674
Wang Q, Li Y, Wang Y (2011) Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. Environ Monit Assess 174:241–257
Wickham H (2009) Elegant graphics for data analysis. Media 35:10–1007
Zawierucha K, Shain DH (2019) Disappearing Kilimanjaro snow—Are we the last generation to explore equatorial glacier biodiversity? Ecol Evol 9:8911–8918
Zawierucha K, Buda J, Azzoni RS, Niśkiewicz M, Franzetti A, Ambrosini R (2019a) Water bears dominated cryoconite hole ecosystems: densities, habitat preferences and physiological adaptations of Tardigrada on an alpine glacier. Aquat Ecol 53:543–556
Zawierucha K, Buda J, Nawrot A (2019b) Extreme weather event results in the removal of invertebrates from cryoconite holes on an Arctic valley glacier (Longyearbreen, Svalbard). Ecol Res 34:370–379
Zawierucha K, Porazinska D, Ficetola GF, Ambrosini R, Baccolo G, Buda J, Ceballos J, Devetter M, Dial R, Franzetti A, Fuglewicz U, Gielly L, Łokas E, Janko K, Novotná Jaroměřská T, Kościński A, Kozłowska A, Ono M, Parnikoza I, Pittino F, Poniecka E, Sommers P, Schmidt SK, Shain D, Sikorska S, Uetake J, Takeuchi N (2021) A hole in the nematosphere: tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing. J Zool 313:18–36
Zawierucha K, Trzebny A, Buda J, Bagshaw E, Franzetti A, Dabert M, Ambrosini R (2022) Trophic and symbiotic links between obligate-glacier water bears (Tardigrada) and cryoconite microorganisms. PLoS ONE 17:e0262039