

**Charles University, Faculty of Science, Department of Ecology**

Study programme: Ecology



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**Macronutrients on Glacier Surfaces: Elemental and Isotopic  
Composition of Glacier-dwelling Consumers**

Makronutrienty na povrchu ledovců: prvkové a izotopové složení ledovcových konzumentů

**PhD thesis**

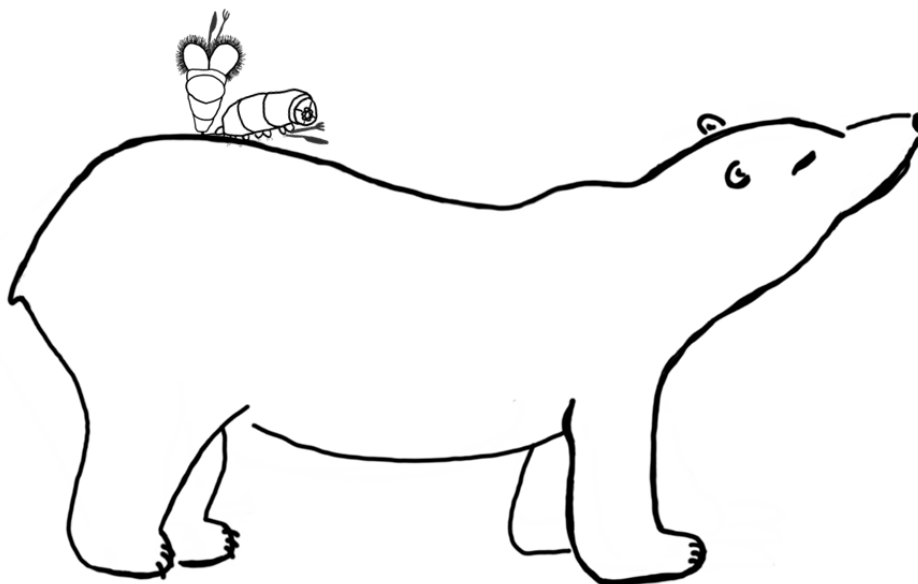
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## Věnování

Mámě a tátovi, mým všem klukům a celé (i té nepokrevní potápěčské, jachtařské a lezecké) rodině!



(Dedication to my mum and dad and the whole, even the non-blood diving, sailing, and climbing, family!)

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## Abstract

Microinvertebrate consumers are important players in carbon and nitrogen cycling on glacier surfaces (the supraglacial). They constitute the highest level of the supraglacial trophic network, and their grazing significantly affects resident microbial communities and the allochthonous organic matter (OM) pool. However, the food preferences and trophic strategies of supraglacial consumers have not been investigated to date which limits the understanding of the feedback mechanisms between carbon and nitrogen uptake and utilization on glacier surfaces. This thesis aims to reveal the mechanisms behind the supraglacial OM composition to consumers' community structure and abundance through the investigation of their trophic position and potential food preferences. A reliable methodological approach to measure carbon and nitrogen contents and their stable isotopes in supraglacial consumers was established and subsequently combined with taxonomical identification and biomass estimation. The results revealed that the different groups of supraglacial consumers (tardigrades, rotifers, and springtails) differ in their food preferences and/or in their macronutrient requirements. Differences in OM sources were reflected in the  $\delta^{13}\text{C}$  signature of the OM as well as that of the consumers themselves and indicated differences in the contribution of allochthonous and autochthonous OM between glaciers and supraglacial habitats (e.g., cryoconite vs supraglacial debris). For example, on the Forni Glacier in the Italian Alps, tardigrades were found to feed on and control the biomass of green algae. The high dynamics of the glacier surface, including meltwater pulses and precipitation events, was found to significantly affect supraglacial biota. This thesis provides the first insight into carbon and nitrogen content and stable isotopic composition in glacier-dwelling, cold-adapted microinvertebrates and so contributes to the understanding of the consumers' role in supraglacial trophic food webs.

## Abstrakt

Drobní bezobratlí živočichové jsou důležitými hráči v koloběhu uhlíku a dusíku na povrchu ledovců (supraglaciálu). Tito drobní konzumenti tvoří nejvyšší úroveň supraglaciální trofické sítě a jejich konzumace a potravní preference ovlivňuje lokální mikrobiální společenstva a množství a složení alochtonní organické hmoty. Potravní preference a trofické strategie supraglaciálních konzumentů však doposud nebyly podrobně zkoumány, což omezuje pochopení zpětnovazebních mechanismů mezi příjmem a využíváním uhlíku a dusíku na povrchu ledovců. Cílem této práce je poodhalit mechanismy, které stojí za složením organické hmoty v supraglaciálních habitatech a které ovlivňují strukturu a četnost společenstva konzumentů. Tato práce se věnuje především výzkumu trofického postavení supraglaciálních konzumentů a jejich potenciálních potravních preferencí. Jako první byla vyvinuta spolehlivá metodika k měření obsahu a stabilních izotopů uhlíku a dusíku v těchto drobných živočiších, která byla následně kombinována s dalšími metodami, jako je taxonomická identifikace nebo stanovení biomasy. Výsledky ukázaly, že různé skupiny supraglaciálních konzumentů (želvušky, vířníci a chvostoskoci) se liší v potravních preferencích a/nebo v požadavcích na makronutrienty. Rozdíly ve zdrojích organické hmoty se projeví v  $\delta^{13}\text{C}$  organické hmoty i v samotných konzumentech a naznačily rozdíly v podílu alochtonní a autochtonní organické hmoty mezi ledovci a supraglaciálními habitaty (např. mezi kryokonitovými jamkami a supraglaciální sutí). Na ledovci Forni v italských Alpách bylo zjištěno, že se želvušky živí zelenými řasami a kontrolují jejich biomasu. Také bylo zjištěno, že vysoká dynamika povrchu ledovce, včetně výkyvů v tání a srážkách, významně ovlivňuje supraglaciální biotu. Tato práce poskytuje první pohled na obsah uhlíku a dusíku a stabilní izotopové složení u bezobratlých konzumentů žijících na povrchu ledovců, a přispívá tak k pochopení role těchto živočichů v supraglaciálních trofických potravních sítích.

## **Declaration of originality**

I declare that this thesis, publications, and unpublished manuscripts have not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed in the Author's contribution statement and through the authorship order of the included publications. All literature sources used while writing this thesis have been appropriately cited.

Lyon/Prague, July 2024



Tereza Novotná Jaroměřská

## **Data statement**

All data are available in publications or upon request to the author.

## **Funding statement**

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## Poděkování

Můj postgraduální program se nenesl jen v duchu vědy, ale také v duchu rodinném. Kromě úžasného manžela v prvním roce to byly pak naše dvě děti (a třetí na cestě) v letech dalších. Lidiček, kterým vděčím za možnost pokračovat ve studiu a výzkumu, je mnoho. V první řadě je to rodina, bez jejíž existence a podpory by dokončení doktorského programu vůbec nebylo možné! Mé bláznivé nápady byly pokaždé podpořeny, o kluky bylo postaráno, když bylo potřeba, a pevně doufám, že se David po obhajobě konečně pořádně vyspí vzhledem k tomu, že jsem poslední 3 roky pracovala téměř všechny jeho volné dny po službě. Obrovský dík patří všem mým kolegům, kteří se nezalekli (nebo to alespoň nedali najevo), že hodlám pracovat i s malými dětmi, a vždy mě podpořili a pomáhali mi, když bylo potřeba. Také jim moc děkuji za to, že často pracovali mimo svou ideální pracovní dobu, aby mi vyšli maximálně časově vstříc! Velký dík patří všem oficiálním i neoficiálním školitelům a konzultantům za veškerou podporu a pomoc ve všech fázích výzkumu. Děkuji všem sestřičkám a lékařům za to, že Alberta včas dopekli a dali do kupy, aby zvládl naše ledovcová dobrodružství. Děkuji, Jony a Alberte, buďte tak fajn kluci, jako jste, i nadále! Také jsem moc ráda, že je pro vás věda zábava a berete ji jako součást našeho života!

## List of chapters and author's contribution

This thesis consists of 2 published papers and 1 submitted manuscript. I outline my contributions to each of the publications and unpublished manuscript below, following the guidelines of CRediT (Contributor Roles Taxonomy).

### Chapter 1

**Novotná Jaroměřská T.\***, Trubač J., Zawierucha K., Vondrovicová L., Devetter M., Žárský J.D. (2021): Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing divergent roles in a supraglacial trophic network. *Biogeosciences* 18, 1543–1557, doi: 10.5194/bg-18-1543-202

\*Contribution: **TNJ – conceptualization, formal analysis, investigation, writing the original draft, review and editing, funding acquisition, project administration.** JT – formal analysis, resources, methodology, review and editing. KZ, MD – investigation, review and editing. LV – formal analysis. JDŽ – conceptualization, investigation, supervision, funding acquisition, review and editing.

### Chapter 2

**Novotná Jaroměřská T.\***, Ambrosini R., Mazurkiewicz M., Franzetti A., Klimaszuk P., Rozwalak P., Poniecka E., Vondrovicova L., Zawierucha K. (2023): Spatial distribution and stable isotopic composition of invertebrates uncover differences between habitats on the glacier surface in the Alps. *Limnology* 24, 83–93, doi: 10.1007/s10201-023-00713-w

\*Contribution: **TNJ – formal analysis, investigation, writing the original draft, review and editing, funding acquisition.** RA, AF, EP, PR – investigation, writing review and editing. MM – methodology, writing review and editing. PK – formal analysis, writing review and editing. LV – formal analysis. KZ – conceptualization, formal analysis, investigation, funding acquisition, writing the original draft, review and editing.

### Chapter 3

**Novotná Jaroměřská T.\***, Ambrosini R., Richter D., Pietryka M., Niedzielski P., Souza-Kasprzyk J., Klimaszuk P., Franzetti A., Pittino F., Vondrovicová L., Senese A., Zawierucha K.: Insights into cryoconite community dynamics on the alpine glacier throughout the ablation season. (under review)

\*Contribution: **TNJ – formal analysis, investigation, writing the original draft, review and editing, funding acquisition.** RA – formal analysis, investigation, writing the original draft, review and editing. DR, MP, PN, JS-K, AS, PK – formal analysis, review and editing. AF, FP – formal analysis, investigation, review and editing. LV – formal analysis. KZ – conceptualization, formal analysis, investigation, funding acquisition, writing the original draft, review and editing.

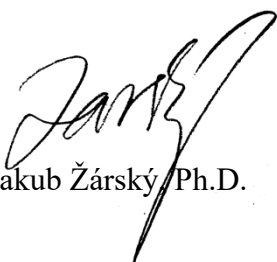


## Letter of consent

Dear members of the dissertation committee,

I hereby declare that Tereza Novotná Jaroměřská has made a significant contribution to the publication/s which I led as senior or corresponding author. I concur with the Author's contribution statement.

Sincerely,



Jakub Žárský/Ph.D.



Krzysztof Zawierucha, Ph.D.

# 1. State of the art

## 1.1. Glacier surface as an ecosystem

Glaciers and ice sheets world-wide are ecosystems whose processes are of significance to local as well as global ecology and biodiversity (e.g., Anesio and Laybourn-Perry 2012; Stibal et al. 2020). Despite the rapid deglaciation, glacier ecosystems still occupy a non-negligible part of the Earth's area and contribute significantly to global nutrient budgets and cycling (Hodson et al. 2008; Lamarche-Gagnon et al. 2019; Stibal et al. 2012a). Most biological processes in glacier ecosystems occur in supraglacial (on the glacier surface; Fig. 1) or subglacial (at the glacier bed) habitats where the abundance and activity of organisms are the highest (Hodson et al. 2008). This thesis focuses on the supraglacial habitat.

The supraglacial environment consists of several distinct habitats such as streams, ponds, snow, bare ice surface, and supraglacial debris (Hodson et al. 2008). From spring to autumn, when the glacier surface is not completely frozen and covered in snow, all these habitats come to life in the presence of liquid water. All supraglacial habitats are supplied from allochthonous as well as autochthonous sources and often interconnected by the flowing water from ice- or snow melt (Hodson et al. 2005; Stibal et al. 2012b). Despite the stable temperature around freezing, glacier surface is a very dynamic environment which is easily affected by weather (precipitation, wind, solar radiation) as well as biotic factors such as algae decreasing the albedo (degree of reflectivity) of the glacier surface (e.g., Hodson et al. 2008; Williamson et al. 2019; Zawierucha et al. 2019a). Glacier surfaces also provide a home to a plethora of organisms which have adapted to cold and dynamic conditions and thrive on within supraglacial habitats (Bradley et al. 2023; Zawierucha et al. 2021).



Figure 1. Surface of the Nordenskiöld Glacier in Svalbard, High Arctic. (photo: TNJ)

### *1.1.1. The dynamic nature of glacier surfaces*

Glacier surface is characterised by its dynamic nature and interconnected processes between various abiotic and biotic factors. During the melt season, weather conditions such as high radiation, warm winds, precipitation, and freezing can induce changes in glacier hydrology and biological activity, thus also affecting the supraglacial nutrient fluxes (e.g., Dubnick et al. 2016; Milner et al. 2017; Zawierucha et al. 2019a). The geomorphological characteristics of the glacier (slope, characteristics of adjacent areas) also contribute to the nutrient fluxes via the effect on the meltwater discharge (e.g., Dubnick et al. 2016; Hawkings et al. 2015). The activity of organisms also affects glacier dynamics and hydrology; for example, as mentioned above, glacier algal blooms significantly lower the ice surface albedo (e.g., Holland et al. 2019; Wang et al. 2020). Nutrient input and processing on glacier surfaces are highly variable on a year-to-year, seasonal, and daily basis, except for the specific cases such as ice-lidded cryoconite holes which can remain closed systems for the whole summer season (e.g., Hodson et al. 2005; Schmidt et al. 2022).

### *1.1.2. Life on the glacier surface*

Glacier surfaces host a plethora of cold-adapted organisms living in the stable low temperature around 0 °C. Communities of supraglacial organisms range from microbes to invertebrates (e.g., Winkler et al. 2022; Zawierucha et al. 2021) which form a truncated trophic network consisting of producers, consumers, and decomposers. The highest organismal diversity can be found in ablation areas of glaciers (areas where the ice loss exceeds its increase) within supraglacial depressions called cryoconite holes (Fig. 2a) that are formed by the preferential melting of ice due to the sediment coverage (Cameron et al. 2012; Nordenskiöld 1972; Zawierucha et al. 2021). Cryoconite itself is a sediment at the bottom of holes which is a conglomerate of mineral particles, microorganisms, and OM of various origins (allochthonous and autochthonous) (Rozwalak et al. 2022). Together with the liquid water column, cryoconite holes form a unique environment which is also one of the major sources of OM on glacier surfaces (e.g., Rozwalak et al. 2022). Outside cryoconite holes, organisms are also present on the bare ice surface (e.g., glacier algae, glacier ice worms (Hotaling et al. 2020), arthropods (Gobbi et al. 2006)), within mosses on the glacier ice called glacier mice (Coulson and Midgley 2012), within the supraglacial debris (e.g., springtails (Buda et al. 2020); Fig. 2b), or on the snow in glacier accumulation areas (areas where the ice increase exceeds its loss) (Shain et al. 2021). Most supraglacial habitats are characterized by high concentrations of available macronutrients with the prevalence of their organic forms (e.g., Holland et al. 2019; Stibal et al. 2008a, 2009; Telling et al. 2014).

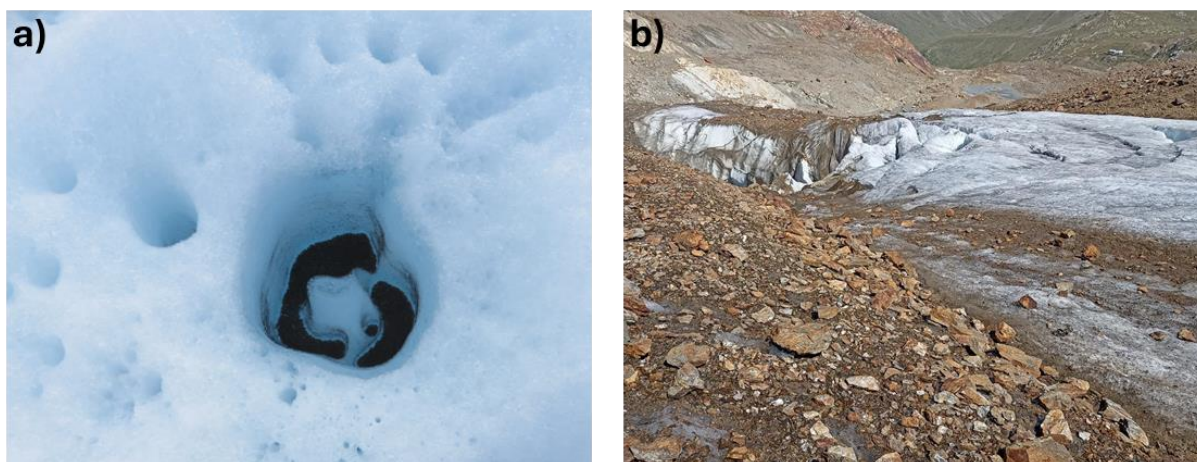


Figure 2. Supraglacial habitats of major interest in this thesis: a) cryoconite hole; b) supraglacial debris. (photo: TNJ)

### 1.1.3. *Supraglacial producers*

Photoautotrophic microbes – cyanobacteria and algae – form the basis of the supraglacial ecosystem (Stibal et al. 2012a) via fixing inorganic carbon (IC) during photosynthesis and transforming it to organic forms available to heterotrophs (e.g., Stibal et al. 2012a). Photoautotrophic microbes also assimilate ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ) and/or fix ( $\text{N}_2$ ) inorganic nitrogen (IN) like some heterotrophic microbes (Li et al. 2019; Telling et al. 2011, 2012). The abundance of cyanobacteria and algae may vary spatially and temporally on the glacier surface and is affected by nutrient availability (Stibal et al. 2006; Telling et al. 2011; Vonnahme et al. 2016). On the bare ice surface, algal communities (mostly Zygnematales) dominate the communities of primary producers (e.g., Di Mauro et al. 2020; Hoham and Remias 2020; Holland et al. 2019; Takeuchi 2013) with the net primary production often exceeding the rate of remineralization which results in the accumulation of OM (Nicholes et al. 2019; Yallop et al. 2012). Glacier algae also utilize nutrients retained within the weathering crust and may subsequently release them to other supraglacial habitats (e.g., Holland et al. 2019; Wang et al. 2020). In cryoconite holes, the abundance and community structure of primary producers vary among glaciers with dominant groups of cyanobacteria being Pseudanabaenaceae and Phormidiaceae and the dominant group of algae being Zygnematales (Rozwalak et al. 2022). The differences in the dominance of algae and cyanobacteria in cryoconite holes is related to various factors such as debris coverage, pH, irradiance, nutrient availability and concentration (e.g., Segawa et al. 2017; Stibal et al. 2012a, b; Telling et al. 2012a; Vonnahme et al. 2016). Depending on the microbial community structure and activity, cryoconite holes can act as carbon sinks or sources on glacier surfaces (e.g., Anesio et al. 2010; Hodson et al. 2010a; Lutz et al. 2014; Stibal et al. 2012a, b; Telling et al. 2012b).

### 1.1.4. *Supraglacial decomposers*

OM from autochthonous and allochthonous sources is mineralized by heterotrophic decomposers such as bacteria and fungi (including yeasts) which are also abundant components of supraglacial communities (Bhatia et al. 2010; Hodson et al. 2007; Margesin et al. 2002; Perini et al. 2019; Pittino et al. 2021; Telling et al. 2011; Uetake et al. 2012). Heterotrophic bacteria

preferably utilize substrates of autochthonous origin (i.e., organic carbon (OC) derived from photosynthesis) and contribute significantly to the supraglacial respiration which varies with the size of the glacier, the distance from the glacier margin, and the availability of dissolved organic matter (DOM) (e.g., Stibal et al. 2012a, b). However, as shown by Anesio et al. (2010) from Arctic, Antarctic, and Alpine cryoconite holes, only up to 7 % of cryoconite organic carbon is utilized by heterotrophic bacteria, indicating that OC accumulation exceeds its loss by respiration in these habitats. Fungi are capable of enzymatic activity to degrade complex organic substrates such as proteins, esters, carbohydrates, pectin, cellulose, lignin or tannin, and contribute to the weathering of phosphate and silicate (e.g., Sanyal et al. 2020; Singh et al. 2016). This ability allows fungi to process more complex OC from allochthonous sources which is also a significant compound of the supraglacial OC pool (Bhatia et al. 2010; Stibal et al. 2008b). Although little is known about the processing of photoautotrophic exudates, they also represent an important source for decomposers (Cole et al. 1982; Smith et al. 2017).

#### *1.1.5. Viruses*

The activity of photoautotrophic microbes and decomposers (specifically bacteria) is also highly affected by viruses which can considerably influence bacterial secondary production and carbon cycling in the microbial loop (Bellas et al. 2015; Rassner et al. 2016). As modelled by Rassner et al. (2016), up to third of carbon from the bacterial secondary production can be shunt to the dissolved OM due to viral lysis on bacterial cells. As such, viruses can negatively affect the carbon flow to higher trophic levels, but they also contribute to trophic food webs by the release of nutrients back into the circulation by the cell lysis. Also, supraglacial viruses evolved mechanisms to survive glacier conditions, such as low decay helping them to overcome low bacterial abundances and thus play a crucial role in the supraglacial microbial loop (e.g., Rassner 2017).

#### *1.1.6. Supraglacial consumers*

Supraglacial consumers are a diverse group of various organisms, mostly microinvertebrates, which form abundant communities on the bare glacier ice or weathering crust (porous surface glacier ice), cryoconite holes, and within supraglacial debris (Buda et al. 2020; Zawierucha et al. 2021). On most polar glaciers, only one level of consumers occurs, while on mountain glaciers, other levels (secondary consumers and predators), such as beetles, spiders, or birds feeding on ice worms might be present (Gobbi et al. 2006; Hotaling et al. 2020).

In cryoconite holes world-wide, the most common groups of consumers are the microinvertebrates tardigrades (Fig. 3a) and rotifers (Fig. 3b), which also represent the highest level in cryoconite food webs (Zawierucha et al. 2021). Both groups of consumers are adapted to life in the dynamic supraglacial environment with constantly low temperature and periodic freezing, using various mechanisms such as e.g., ametabolic dormant state called cryptobiosis (Guidetti et al. 2011; Ricci 2001), resistance to starvation (Marotta et al. 2012), or resistant dormant eggs (Nelson et al. 2015). All the species of tardigrades identified in supraglacial samples to date are glacier specialists. At least five species of tardigrades have been detected on Arctic glaciers (Zawierucha et al. 2020), while only one species is known from glaciers in the Alps (Zawierucha et al. 2019b). The species richness of cryoconite rotifers is still not fully

described, mostly due to their morphological similarities and the necessity of molecular identification. Tardigrades and rotifers use various feeding strategies likely including bacterivory, herbivory, and omnivory, but most findings on the diet of supraglacial species are assumptions based on the feedback mechanisms with the community structure of producers or studies on their non-glacier counterparts. The exact food of supraglacial tardigrades and rotifers is still under investigation (Vonnahme et al. 2016; Zawierucha et al. 2022). Based on findings from laboratory analyses and non-glacier polar studies, tardigrades likely feed on algae or other size-suitable particles (Almela et al. 2019, Bryndová et al. 2020) while rotifers prefer smaller particles than tardigrades (Almela et al. 2019).

Supraglacial springtails (Fig. 3c) are also considered common supraglacial consumers inhabiting various supraglacial habitats worldwide (Coulson and Midgley 2012; Fjellberg 2010; Gobbi et al. 2010; Kohshima et al. 2002; Makowska et al. 2016). The species of springtails identified in supraglacial habitats include both glacier specialists and common temperate species which most probably use “warmer” supraglacial habitats such as glacier mice as a refugia to survive when they land on the glacier surface (Coulson and Midgley 2012). On alpine glaciers, springtails occur mostly in the stone-ice border within supraglacial debris (e.g., Buda et al. 2020; Valle et al. 2023). Due to their high abundance, especially in the Alps, supraglacial springtails are considered important consumers, mostly detritivores, significantly contributing to secondary production and energy flow (e.g, Buda et al. 2020; Rusek 1998; Valle et al. 2022). Nevertheless, detailed information on their ecological role in supraglacial food webs is sparse.

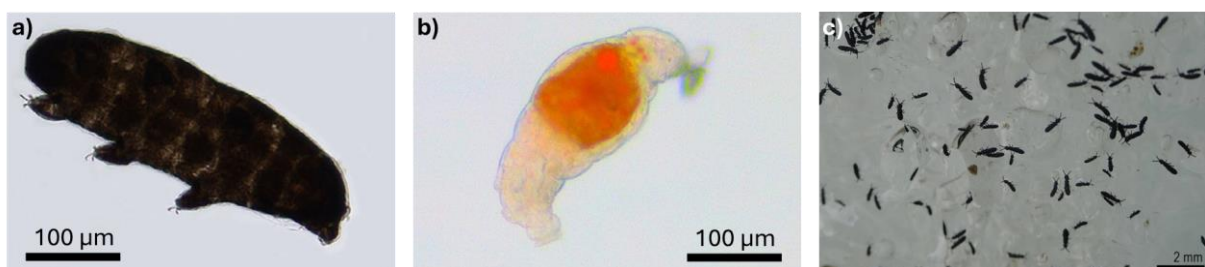


Figure 3. Supraglacial consumers: a) the tardigrade *C. klebelsbergi*; b) a rotifer from an Arctic cryoconite hole; c) supraglacial springtails. Photos adapted from Zawierucha et al. (2021) and Novotná Jaroměřská et al. (2023).

## 1.2. Supraglacial biota and their role in carbon and nitrogen cycling

### 1.2.1. The carbon and nitrogen sources important for supraglacial biota

Carbon and nitrogen are deposited on the glacier surface in the form of both inorganic and organic compounds (e.g., Koziol et al. 2019; Stibal et al. 2012a). Atmospheric inorganic forms,  $\text{CO}_2$ ,  $\text{N}_2$ , or nitrate ( $\text{NO}_3^-$ ) are taken up by microbial communities and transformed into organic forms available for consumers (Hodson et al. 2010b; Segawa et al. 2014; Stibal et al. 2012a; Telling et al. 2011). *In situ* microbial production and N fixation significantly contribute to nutrient cycling on glacier surfaces (Anesio et al. 2009; Telling et al. 2011, 2012). The rate of photosynthesis can be high on glaciers, turning the glacier surface into a carbon sink when allochthonous OM or other forms of dissolved inorganic carbon (DIC) are not available and the



abundance of phototrophic organisms is high (Anesio et al. 2009; Stibal et al. 2012a). The uptake of nitrogen by N fixation on glacier surfaces is relatively low since other N sources ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ) are usually available at sufficient concentrations (Telling et al. 2011, 2012). However, N fixation becomes important during the colonization of nutrient-poor supraglacial sediment and in the absence of dissolved inorganic nitrogen (DIN) from precipitation and meltwater (Telling et al. 2011, 2012). Allochthonous sources comprise inorganic as well as readily available organic compounds originating from the adjacent but also distant areas (Stibal et al. 2008b, Vonnahme et al. 2016). The majority of the available allochthonous OC compounds are wind-blown terrestrial OM including plant and microbial residua, but also animal excrements, remains of animals, and nutrients contained in aerosols (Barbaro et al. 2021; Vonnahme et al. 2016; Zawierucha et al. 2016, 2019b). Anthropogenic contaminants such as black carbon from fossil fuel burning may also be deposited on glacier surfaces, but their availability (the potential of organisms to their degradation) is still under investigation (Hodson 2014; Pittino et al. 2018). On various glaciers, the contribution of allochthonous and autochthonous sources may vary according to their size, morphology, climate and the characteristics of their adjacent areas (e.g., Liu et al. 2022; Stibal et al. 2008b, 2012).

#### 1.2.2. Carbon: from sources to $\delta^{13}\text{C}$ signatures in supraglacial consumers

The processes preceding the formation of OC in organisms drives their specific stable isotopic signature ( $\delta^{13}\text{C}$ ). The whole journey of such specificity starts with the uptake of atmospheric  $\text{CO}_2$ , when enzymatic reactions during photosynthesis discriminate heavier  $^{13}\text{C}$  and deplete photoautotrophs in  $^{13}\text{C}$  compared to the atmosphere (Michener and Lajtha 2008; Šantrůček et al. 2018). The following biochemical processes in organisms change the isotopic signature only by approx. 1 ‰, so in the end, “organisms are isotopically what they eat” for  $\delta^{13}\text{C}$  with some known exceptions of particular cellular compounds, such as depleted  $\delta^{13}\text{C}$  in lipids (e.g., Boecklen et al. 2011).

As mentioned above, glacier surfaces balance between being sinks or sources of carbon (Stibal et al. 2012a). The photoautotrophic microbial communities, mostly cyanobacteria and algae, uptake carbon in the form of  $\text{CO}_2$  or DIC and convert it into organic carbon compounds (Anesio et al. 2009; Stibal and Tranter 2007). On the other side, heterotrophic microbes and consumers utilize organic carbon and  $\text{CO}_2$  is a product of their respiration (e.g., Hodson et al. 2007). On smaller glaciers, the allochthonous input of mostly terrestrial OM is an important source of carbon to supraglacial organisms (Stibal et al. 2008b). *In situ* microbial production is considered higher in  $^{13}\text{C}$  compared to allochthonous OM (Musilova et al. 2015) due to the preferred utilization of DIC than the atmospheric  $\text{CO}_2$  (Stibal and Tranter 2007). The food of supraglacial consumers can contain OM of both autochthonous and allochthonous origins with diet preferences within each group as mentioned in the Section 1.1.6. (e.g., Bryndová et al. 2020, Buda et al. 2020). Therefore, to provide a relevant interpretation of  $\delta^{13}\text{C}$  signatures of supraglacial OM and its connection to supraglacial consumers, their species composition, abundance, and proportions of co-existing species must be considered. Even more since non-species-specific pooled samples are used to obtain a sufficient dry weight of consumers for carbon stable isotopic analyses (e.g., Shaw et al. 2018).

### 1.2.3. Nitrogen: from sources to $\delta^{15}\text{N}$ in consumers

The stable nitrogen isotopic composition ( $\delta^{15}\text{N}$ ) is a useful tool for the evaluation of sources of nitrogen and the trophic position of consumers (e.g., Michener and Lajtha 2008; Minagawa and Wada 1984). Similar to carbon, the main source of nitrogen on the glacier surface is the atmosphere, as described in the Section 1.2.1. During the uptake, organisms incorporate IN into organic compounds or release it back to the environment as products of their metabolisms in the form of ammonium, nitrate, or nitrogen oxides (e.g., Segawa et al. 2014). N fixation is known to have minimal fractionation factor and the fixed nitrogen remains close to atmospheric  $\text{N}_2$  with respect to its  $\delta^{15}\text{N}$  signature (e.g., Michener and Lajtha 2008). All other reactions decrease the content of the heavier isotope  $^{15}\text{N}$  (high fractionation against  $^{15}\text{N}$  in most metabolic processes), which should lead to the overall depleted  $\delta^{15}\text{N}$ . Nevertheless, due to preferential release or excretion of isotopically depleted nitrogen compounds (e.g., nitrogen oxides,  $\text{NO}_3^-$ , urea) and the consequent enrichment of organic compound in organisms (e.g. proteins) or soils (Adams and Sterner 2000; Cantalapiebra-Hijar et al. 2015; Šantrůček et al. 2018), the final isotopic signature is often enriched in  $^{15}\text{N}$ . The  $^{15}\text{N}$ -depleted nitrogen can also remain within the systems, be utilized again by microorganisms, with the overall  $\delta^{15}\text{N}$  signature unchanged. Typically, the  $\delta^{15}\text{N}$  of consumers is higher compared to their diet (DeNiro and Epstein 1981; Kling et al. 1992; Zah et al. 2001) with the fractionation factor equal to or higher than 3 ‰ (DeNiro and Epstein 1981).

The availability and content of nitrogen follow the dynamic nature of processes on glacier surfaces which are highly variable thorough the season (Hodson et al. 2010b). Such variability can be reflected in  $\delta^{15}\text{N}$  of photoautotrophic communities, as for example  $\delta^{15}\text{N}$  of algae depends on the availability of nitrogen and  $\delta^{15}\text{N}$  of their nitrogen source (Adams and Sterner 2000; Gu and Alexander 1993). The major IN input in form of nitrites and nitrates usually occurs at the beginning of the melting season or during episodic precipitation or meltwater events (Hodson et al. 2010b; Telling et al. 2011). When the episodic events of nitrogen input (e.g., rain) occur,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are rapidly assimilated by supraglacial microbial communities (Hodson et al. 2010b) which may influence their  $\delta^{15}\text{N}$  and consequently the  $\delta^{15}\text{N}$  of consumers.

In polar environments, the  $\delta^{15}\text{N}$  is usually lower compared to temperate habitats where soil  $\delta^{15}\text{N}$  values can range from 5 ‰ in temperate regions to 13 ‰ in the tropics (Amundson et al. 2003). The depletion of  $\delta^{15}\text{N}$  in polar soils is likely caused by low temperature and the consequent slower volatilization of  $^{15}\text{N}$  depleted products of denitrification and nitrification which remain in higher concentration in soils decreasing the overall  $\delta^{15}\text{N}$  of organisms (Šantrůček et al. 2018; Sigman and Casciotti 2001). Specifically,  $\delta^{15}\text{N}$  of non-glacier polar soils or microbial mats often do not exceed 1 ‰ (e.g., Oelbermann et al. 2008; Shaw et al. 2018) when analysed on bulk samples. The low  $\delta^{15}\text{N}$  in non-glacier polar soils likely reflects the effect of low temperature and potential nitrogen limitation triggering higher content of  $^{15}\text{N}$ -depleted compounds as a source of nitrogen for metabolic processes (Adams and Sterner 2000).



### 1.3. Carbon and nitrogen contents and stable isotope composition as a useful tool in ecological studies

#### 1.3.1. Ecological stoichiometry

Carbon and nitrogen are dominant macronutrients (nutrient macro elements) in organisms and essential as well as limiting for many metabolic processes (Sternner and Elser 2002). The ecological studies which focus on the role of macronutrients within ecosystems often use the carbon:nitrogen:phosphorus (C:N:P) molar ratio of 106:16:1 (Redfield 1934), known as the Redfield ratio. This ratio was originally established for the ocean and its organisms but found its use as an optimal proportion of macronutrients within an environment and its biota in overall (e.g., Sternner and Elser 2002). Deviations from the Redfield C:N:P ratio often imply limitations or excess in some macronutrient. However, biochemical variability within organisms and their specific nutrient requirements may deviate the ratio from the 'Redfield' one (e.g., Cleveland and Liptzin 2007, Sardans et al. 2012; Sternner and Elser 2002).

Carbon is the most abundant macronutrient in dry weight of organisms as the main constituent of all cells building blocks such as carbohydrates, proteins, lipids, or nucleic acids. Some of these compounds (e.g., carbohydrates, lipids) are variable in nitrogen content which shape the proportion of each element in cells as well as the overall C:N of the whole organism. The changes in the nutrient pool within the environment can affect the C:N proportion and requirements in organisms or vice versa and lead up to changes in the community composition (Sternner and Elser 2002). For example, when organisms with high requirements for nitrogen increase their abundance, they lower the amount of available nitrogen from the environment, which can lead to nitrogen limitation. In case of absence of a new pulse of available nitrogen (by input or release), the community structure of organisms will change and so the nutrient requirements. As such, macronutrient homeostasis is not common in the long term within biological systems, although many animals are able to maintain stable C:N:P composition (Sternner and Elser 2002).

#### 1.3.2. Stable isotopes

Both carbon and nitrogen have atoms differing in the number of neutrons called isotopes. Isotopes can be stable or radioactive. For carbon and nitrogen in ecological studies, we use their stable isotopes which considerably differ in their proportions in the nature. For C, the lighter  $^{12}\text{C}$  represents 98.89 % and the heavier  $^{13}\text{C}$  1.11 % in the occurrence. For nitrogen, the lighter isotope  $^{14}\text{N}$  represents 99.64 % and the heavier  $^{15}\text{N}$  0.36 %. To quantify the representation of stable isotopes in nature or within organisms, the dimensionless isotopic proportion (R) which expresses proportion of minor isotope / proportion of major isotope is used. Nevertheless, for the description of results, most studies use different, and more reader friendly explanation of the relative isotopic proportion related to international standards ( $\delta$ ) which is:

$$\delta = \left[ \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \right] \times 1000, (\delta) = \text{‰}$$

Artificially derived PeeDee Belemnite (originally fossils from the PeeDee formation in United States) is used as a standard for C and atmospheric N<sub>2</sub> establishes the 0 ‰ for N. If  $\delta > 0$ , the sample is enriched in the heavier isotope,  $\delta < 0$  means that sample is depleted in the heavier isotope. The process which changes the proportion of isotopes in biochemical reactions is called isotopic fractionation. The most important drivers of isotopic proportions in organisms are enzymes which prefer or discriminate light or heavy isotopes. Thereafter, isotopic composition is related to the importance of various enzymes (e.g., incorporating or excreting), their isotopic selectivity and role in particular processes and organisms (Michener and Lajtha 2008; Šantrůček et al. 2018).

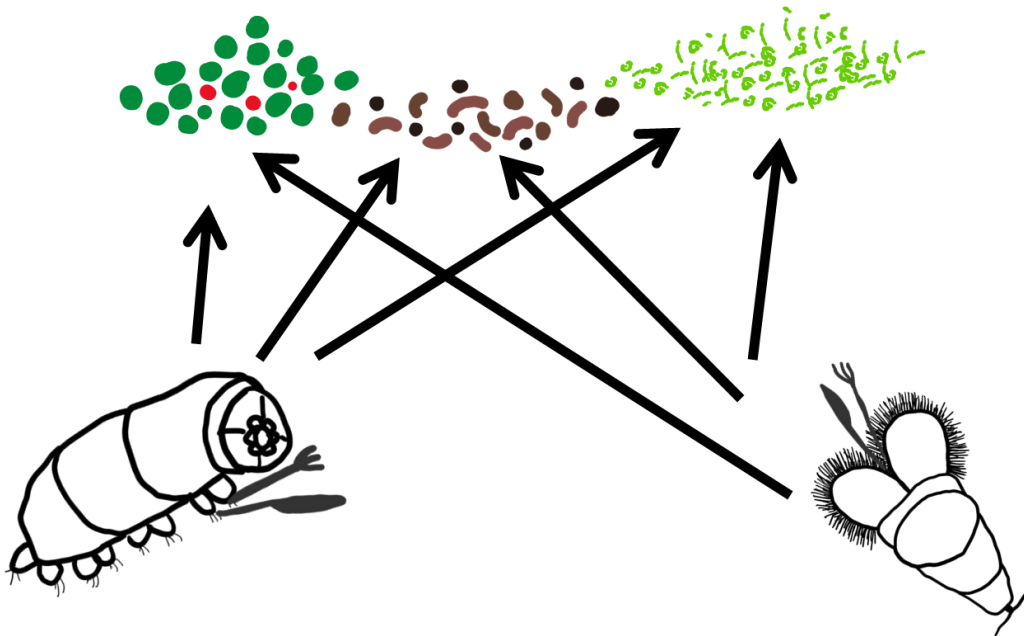
Stable isotopes are commonly used to reconstruct the food web and characterize trophic relationships between organisms (Peterson and Fry 1987). Carbon and nitrogen are due to their abundance in organisms the most common food web tracers in ecological studies (Michener and Lajtha 2008). However, despite the variety of studies focusing on numerous ecosystems (McCutchan et al. 2003; O'Reilly et al. 2003; Wada 2009; Yoshii et al. 1999), glaciers and their consumers have been outside the scope of most isotopic and trophic food web investigations and information on their carbon and nitrogen isotopic composition and trophic relationships is missing.

## 2. Aims

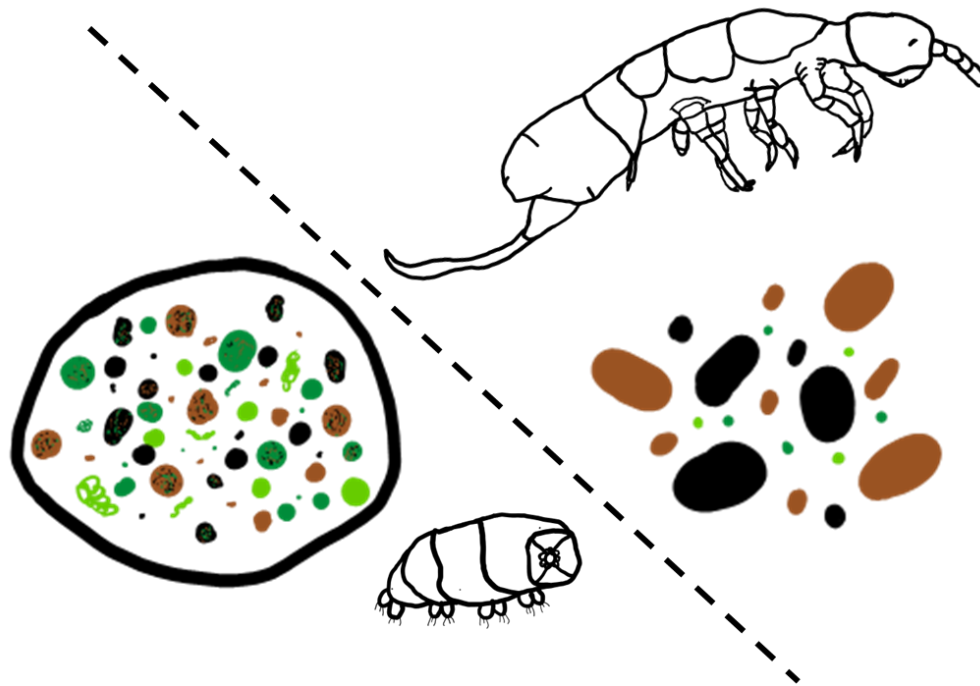
This thesis focuses on carbon and nitrogen and their stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the three most common supraglacial consumer groups – tardigrades, rotifers, and springtails. All these consumers contribute to supraglacial nutrient utilization and cycling as grazers at the highest level of the supraglacial trophic network. However, the role of supraglacial consumers in the feedback mechanisms between carbon and nitrogen uptake and its utilization by microbes or even data on their elemental and isotopic composition are lacking. Since information on macronutrients and their stable isotopes in consumers can provide an insight into food sources and the role of consumers in the microbial community composition, trophic status, and the availability of carbon and nitrogen in the environment, this thesis aims **to reveal the trophic position of supraglacial consumers and their potential food preferences**, which is essential for understanding the role of consumers in supraglacial nutrient cycling.

The research presented in this thesis had the following two major objectives: **(i) to establish reliable methodological approaches to measuring carbon (C) and nitrogen (N) contents and their stable isotopes in supraglacial microinvertebrates**, and **(ii) reveal the contribution and role of grazers in OM composition on glacier surfaces**.

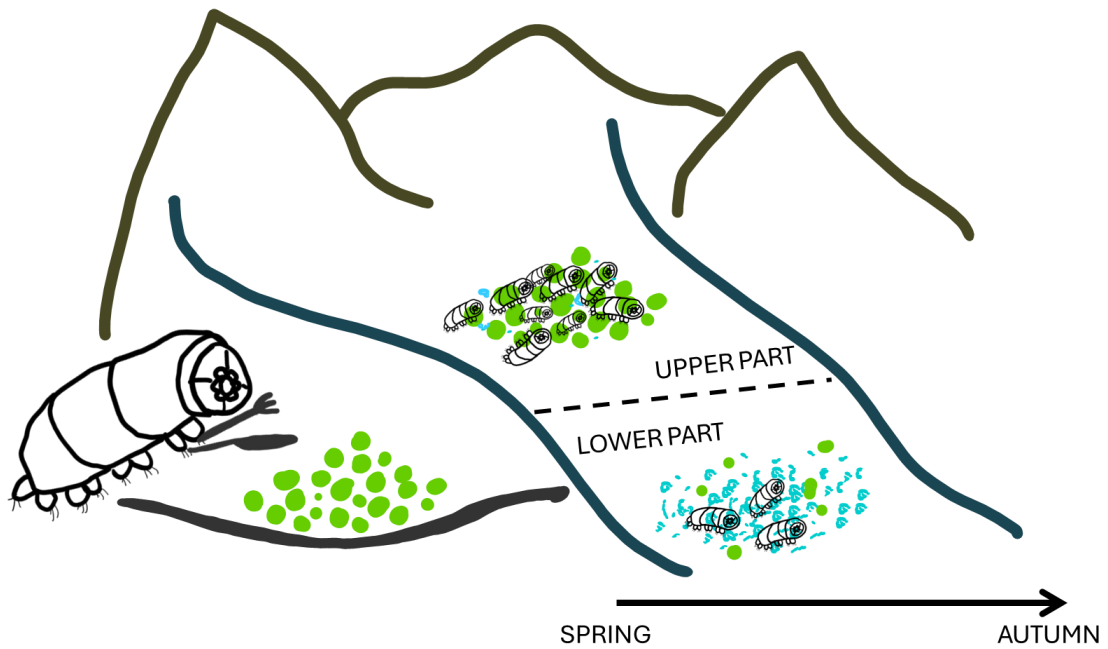
**3. Chapter 1: Stable isotopic composition of top consumers in Arctic cryoconite holes: revealing divergent roles in a supraglacial trophic network**



**4. Chapter 2: Spatial distribution and stable isotopic composition of invertebrates uncover differences between habitats on the glacier surface in the Alps**



## 5. Chapter 3: Insights into cryoconite community dynamics on the alpine glacier throughout the ablation season



## 6. Results and discussion

**Chapter 1** reveals the differences between supraglacial tardigrades and rotifers in their  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures, indicating their different feeding strategies and position in the cryoconite food web. Both groups of consumers are known to consume bacteria, algae, or other size-suitable OM (Almela et al. 2023; Bryndová et al. 2020, Velázquez et al. 2017; Vonnahme et al. 2016). In the presented study, rotifers were enriched in  $^{15}\text{N}$  compared to tardigrades, suggesting differences in  $\delta^{15}\text{N}$  in their respective food. However, the differences in  $\delta^{15}\text{N}$  of both consumers were too low (less than 3 ‰) to indicate different trophic levels (e.g., Minagawa and Wada 1984). Even though the uptake of nitrogen by the main compound of their diet (algae, bacteria, allochthonous terrestrial OM) may differ (e.g., Gu and Alexander 1993; Hadas et al. 2009), each group of consumers may also differ in their N requirements and metabolic processing which can affect their  $\delta^{15}\text{N}$ .

Since cryoconite OM could not be analysed for the isotopic composition of each compound separately, it was not possible to accurately assess the exact food source of the consumers. However, the studied glaciers were chosen based on the differences in their size and geomorphological characteristics and so expected different OM sources. The results showed that on smaller glaciers, where a higher proportion allochthonous OM was expected (e.g. Stibal et al. 2008b), the  $\delta^{13}\text{C}$  of cryoconite, supraglacial OM, and consumers was lower (depleted in  $^{13}\text{C}$ ) compared to larger glaciers. Both consumer groups, tardigrades and rotifers, followed fluctuations in  $\delta^{13}\text{C}$  of cryoconite OM, which suggests that cryoconite OM serves as their food source.

In **Chapter 2**, various supraglacial habitats (supraglacial debris, cryoconite holes, surface ice of the weathering crust) on the Forni Glacier in the Italian Alps were investigated. The results of this study showed that despite the dynamic conditions occurring on the surface of this relatively small alpine glacier, supraglacial habitats revealed distinct environmental characteristics with differences in their OM content as well as the abundance of their major consumers. Cryoconite holes and supraglacial debris differed in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , OC:ON (organic carbon:organic nitrogen) ratio and contents as well as their consumers. In cryoconite, higher proportion of  $^{13}\text{C}$  enriched autochthonous OM likely resulted in higher  $\delta^{13}\text{C}$  compared to supraglacial debris. Such elevated  $\delta^{13}\text{C}$  corresponds with the observation of  $\delta^{13}\text{C}$  in cryoconite with prevailing autochthonous OM in Greenland (Musilova et al. 2015). Within the debris, higher input of terrestrial OM likely depleted the  $\delta^{13}\text{C}$  signature as observed elsewhere (Musilova et al. 2015; O'Leary 1981). These results were supported by higher OC:ON in supraglacial debris indicating higher content of OM likely originating from C-rich terrestrial plants (Cleveland and Liptzin 2007). The  $\delta^{15}\text{N}$  differed between both habitats, with lower values in cryoconite compared to supraglacial debris. Both habitats differ in their average temperature with higher  $\delta^{15}\text{N}$  values in supraglacial debris (Conway and Rasmussen 2000). Temperature is known to affect the  $\delta^{15}\text{N}$  of the nitrogen source to microbes due to slower metabolic rates and volatilization of  $^{15}\text{N}$  depleted products of metabolism such as  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (e.g., Šantrůček et al. 2018; Sigman and Casciotti 2001). Therefore, consumers in cryoconite holes may be depleted  $\delta^{15}\text{N}$  in OM compared to those in supraglacial debris. Similarly to Chapter 1, it was not possible to analyse each OM compound separately and

investigate the food of both consumers in detail. However, the results indicate that springtails might consume microbial biofilms or selectively feed on bigger particles in OM which were not present or dominant in the smallest fraction of debris.

**Chapter 3** focuses on the seasonal changes in cryoconite biota on the Forni Glacier. Results of this study revealed seasonal variations in the biota and the cryoconite OM in both the lower and upper part of the ablation zone. The tardigrade community on the glacier was only composed of one species (*Cryobiotus klebelsbergi*). Measurements of the total biomass of consumers and photoautotrophs indicated that in the upper part of the ablation zone, grazing of tardigrades likely controlled the biomass of green algae, which is supported by the assumed algivory of *C. klebelsbergi* (Bryndová et al. 2020; Zawierucha pers. observ.). The lower part of the ablation zone on the Forni Glacier was characterized by higher debris coverage and the occurrence of meltwater pulses which likely affected the abundance and biomass of tardigrades. The overall biomass of tardigrades was lower here compared to the upper part, with relatively small fluctuations in their abundance over the season. The community of photoautotrophs shifted from algae- to cyanobacteria-dominated over the season in the lower part suggesting that high supraglacial debris coverage served as a substrate allowing cyanobacterial growth (Telling et al. 2012a; Uetake et al. 2016; Wejnerowski et al. 2023) and the firm attachment of cyanobacterial communities to debris avoided their removal by meltwater pulses compared to algae (e.g., Takeuchi et al. 2001; Telling et al. 2012a) as observed on snow algae on Alaskan glacier (Takeuchi et al. 2001). The abundance and the community structure of microbes in cryoconite OM mirrored its  $\delta^{13}\text{C}$  in all samples with the highest  $\delta^{13}\text{C}$  at the beginning of the ablation season in the upper part when the biomass of all photoautotrophs and green algae was the highest. This pattern likely indicates high photosynthetic activity of green algae, which could result in carbon limitation, and consequently high  $\delta^{13}\text{C}$  due to minor fractionation against  $^{13}\text{C}$  (Michener and Lajtha 2008; Musilova et al. 2015; Stibal and Tranter 2007). This result also supports the hypothesis of the significant effect of the dominant cryoconite OM compound on its overall  $\delta^{13}\text{C}$  signature. In the lower part of the ablation zone, the change of the photoautotrophic community from algae to cyanobacteria mirrored the increase in the  $\delta^{13}\text{C}$  of cryoconite OM likely reflecting increasing *in situ* microbial production preferably utilizing isotopically heavy DIC, described previously from other glaciers (Musilova et al. 2015; Stibal and Tranter 2007). No correlation between  $\delta^{13}\text{C}$  of consumers and cryoconite OM was observed in the lower part indicating that cyanobacteria are likely not the preferred food for tardigrades.



## 7. Conclusions

The results of this thesis show that analysis of the carbon and nitrogen stable isotopic composition of supraglacial biota is a powerful tool for the investigation of OM input and the community structure and abundance of consumers and photoautotrophs on glacier surfaces. The food preferences of different groups of supraglacial consumers (tardigrades, rotifers, springtails) likely differ, albeit within the same level of the supraglacial trophic network. Differences in the sources of the OM are reflected in the  $\delta^{13}\text{C}$  signature of the OM as well as that of the consumers themselves. Observations of the relationships between tardigrades and photoautotrophs on the Forni Glacier significantly deepen the current knowledge on biological (i.e. grazing) and physico-chemical (i.e. precipitation) controls of the community structure and biomass of cryoconite biota. This thesis provides the first insight into carbon and nitrogen content and stable carbon and nitrogen isotopic composition in glacier-dwelling, cold-adapted microinvertebrates and corroborates the importance of grazers in supraglacial nutrient cycling. Moreover, the establishment of reliable methodological approaches for isotopic and elemental analyses of microinvertebrates opens new possibilities for further investigations on supraglacial consumers and their role in carbon and nitrogen cycling on glaciers.

## 8. Future directions

Glacial ecosystems vanish rapidly together with their unique fauna (e.g., Stibal et al. 2020). Therefore, their deeper research and understanding of ecological processes is more than timely. Here, I present several future research directions, some already under the review or investigation and some currently included in project proposals, arisen from the research presented in this thesis.

1. The food preferences of supraglacial consumers have not been determined in a sufficiently precise way with the methodological approach established and presented in this thesis. This represents a major gap which should be considered in further research, for example through isotopic labelling.
2. Very few ecological studies of supraglacial consumers have used seasonal sampling. Seasonal data in future ecological studies on glacier surfaces will considerably improve the knowledge of supraglacial biota and also increase the reliability of obtained results since some patterns (e.g., occurrence of rare species) can be overlooked in snapshot studies.
3. There is little knowledge on biomass of supraglacial producers and consumers which is crucial for the estimation of secondary productivity. Also, the knowledge on interactions between supraglacial consumers with different feeding modes (e.g., tardigrade species with the same or different food preferences) have not been investigated to the detail yet which prevents a deeper understanding of supraglacial food webs and trophic interactions.
4. Supraglacial organisms are very tricky to be cultivated which makes *in vitro* experiments difficult. Yet they should be attempted, as such experiments would considerably advance our knowledge on various feedback mechanisms between biota and supraglacial OM composition and nutrient cycling processes.
5. Phosphorus content of supraglacial microinvertebrates is unknown. Estimation of the whole C:N:P of supraglacial consumers and the organic matter would deeply contribute to the understanding of nutrient cycling on glacier surfaces.
6. Various studies have investigated the export of supraglacial OM to downstream ecosystems. However, to our knowledge, there is no study which would prove and quantify the contribution of supraglacial OM to consumers in downstream habitats. This knowledge is especially important for further modelling of the impact of climate change to polar and mountain ecosystems and their nutrient pathways.

## References

- Adams, T. S. and Sterner, R. W. (2000). The effect of dietary nitrogen content on trophic level  $^{15}\text{N}$  enrichment. *Limnol. Oceanogr.*, 45, 601–607.
- Almela, P., Velázquez, D., Rico, E., Justel, A., and Quesada, A. (2019). Carbon pathways through the food web of a microbial mat from Byers Peninsula, Antarctica. *Front. Microbiol.*, 10, 628.
- Almela, P., Rico, E., Velázquez, D., Verleyen, E., and Quesada, A. (2023). Soil moisture drives differences in the diversity and trophic complexity of high Arctic tundra soils. *FEMS Microbiol. Ecol.*, 99, fiad050.
- Amundson, R. et al. (2003). Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochem. Cy.*, 17.
- Anesio, A. M., Hodson, A. J., Fritz, A., Psenner, R., and Sattler, B. (2009). High microbial activity on glaciers: importance to the global carbon cycle. *Glob. Chang. Biol.*, 15, 955–960.
- Anesio, A. M., Sattler, B., Foreman, C., Telling, J., Hodson, A., Tranter, M., and Psenner, R. (2010). Carbon fluxes through bacterial communities on glacier surfaces. *Ann. Glaciol.*, 51, 32–40.
- Anesio, A. M., and Laybourn-Parry, J. (2012). Glaciers and ice sheets as a biome. *Trends Ecol. Evo.*, 27, 219–225.
- Barbaro, E. et al. (2021). Measurement report: Spatial variations in ionic chemistry and water-stable isotopes in the snowpack on glaciers across Svalbard during the 2015–2016 snow accumulation season. *Atmos. Chem. Phys.*, 21, 3163–3180.
- Bellas, Ch. M., Anesio A. M., and Barker, G. (2015). Analysis of virus genomes from glacial environments reveals novel virus groups with unusual host interactions. *Front. Microbiol.*, 6, 656.
- Bhatia, M. P., Das, S. B., Longnecker, K., Charette, M. A., and Kujawinski, E. B. (2010). Molecular characterization of dissolved organic matter associated with the Greenland ice sheet. *Geochim. Cosmochim. Ac.*, 74, 3768–3784.
- Boecklen, W. J., Yarnes, C. T., Cook, B. A., and James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annu. Rev. Ecol. Evol. Syst.*, 42, 411–440.
- Bradley, J. A., et al. (2023). Active and dormant microorganisms on glacier surfaces. *Geobiology*, 21, 244–261.
- Bryndová, M., Stec, D., Schill, R. O., Michalczyk, Ł., and Devetter, M. (2020). Dietary preferences and diet effects on life-history traits of tardigrades. *Zool. J. Linn. Soc.*, 188, 865–877.

- Buda, J., Azzoni, R. S., Ambrosini, R., Franzetti, A., and 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.
- Cameron, K. A., Hodson, A. J., and Osborn, M. (2012). Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities. *Polar Biol.*, 35, 1375–1393.
- Cantalapiedra-Hijar, G., Ortigues-Marty, I., Sepchat, B., Agabriel, J., Huneau, J. F., and Fouillet, H. (2015). Diet–animal fractionation of nitrogen stable isotopes reflects the efficiency of nitrogen assimilation in ruminants. *Br. J. Nutr.*, 113, 1158–1169.
- Cleveland, C. C. and Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry*, 85, 235–252.
- Cole, J. J. (1982). Interactions between bacteria and algae in aquatic ecosystems. *Annu. Rev. Ecol. Syst.*, 13, 291–314.
- Conway, H. and Rasmussen, L. A. (2000). Summer temperature profiles within supraglacial debris on Khumbu Glacier. *Nepal IAHS Publication*, 264, 89–98.
- Coulson, S. J., and Midgley, N. G. (2012). The role of glacier mice in the invertebrate colonisation of glacial surfaces: the moss balls of the Falljökull, Iceland. *Polar Biol.*, 35, 1651–1658.
- DeNiro, M. J. and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals, *Geochim. Cosmochim. Ac.*, 45, 341–351.
- Di Mauro, B. et al. (2020). Glacier algae foster ice-albedo feedback in the European Alps. *Sci. Rep.*, 10, 4739.
- Dubnick, A. et al. (2017). Trickle or treat: The dynamics of nutrient export from polar glaciers. *Hydrol. Process.*, 31, 1776–1789.
- Fjellberg, A. (2010). Cryophilic Isotomidae (Collembola) of the Northwestern Rocky Mountains, USA. *Zootaxa*, 2513, 27–49.
- Gobbi, M., De Bernardi, F., Pelfini, M., Rossaro, B., and Brandmayr, P. (2006). Epigeal arthropod succession along a 154-year Glacier foreland chronosequence in the Forni Valley (Central Italian Alps). *Arct. Antarct. Alp. Res.*, 38, 357–362.
- Gobbi, M., Isaia, M., and De Bernardi, F. (2011). Arthropod colonisation of a debris-covered glacier. *The Holocene*, 21, 343–349.
- Gu, B. and Alexander, V. (1993). Estimation of N<sub>2</sub> fixation based on differences in the natural abundance of <sup>15</sup>N among freshwater N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing algae. *Oecologia* 96, 43–48.
- Guidetti, R., Altiero, T., and Rebecchi, L. (2011). On dormancy strategies in tardigrades. *J. Insect Physiol.*, 57, 567–576.

- Hadas, O., Altabet, M. A., and Agnihotri, R. (2009). Seasonally varying nitrogen isotope biogeochemistry of particulate organic matter in Lake Kinneret, Israel. *Limnol. Oceanogr.*, 54, 75–85.
- Hawkings, J. R. et al. (2015). The effect of warming climate on nutrient and solute export from the Greenland Ice Sheet. *Geochem. Perspect. Lett.*, 1, 94–104.
- Hodson, A. J., Mumford, P. N., Kohler, J., and Wynn, P. M. (2005). The High Arctic glacial ecosystem: new insights from nutrient budgets. *Biogeochemistry*, 72, 233–256.
- Hodson, A. et al. (2007). A glacier respire: quantifying the distribution and respiration CO<sub>2</sub> flux of cryoconite across an entire Arctic supraglacial ecosystem. *J. Geophys. Res.: Biogeosciences*, 112, G4.
- Hodson, A. et al. (2008). Glacial ecosystems, *Ecol. Monogr.*, 78, 41–67.
- Hodson, A., Bøggild, C., Hanna, E., Huybrechts, P., Langford, H., Cameron, K., and Houldsworth, A. (2010a). The cryoconite ecosystem on the Greenland ice sheet. *Ann. Glaciol.*, 51, 123–129.
- Hodson, A., Roberts, T. J., Engvall, A. C., Holmén, K., and Mumford, P. (2010b). Glacier ecosystem response to episodic nitrogen enrichment in Svalbard, European High Arctic. *Biogeochemistry*, 98, 171–184.
- Hodson, A. (2014). Understanding the dynamics of black carbon and associated contaminants in glacial systems. *WIREs Water*, 1, 141–149.
- Hoham, R. W. and Remias, D. (2020). Snow and glacial algae: a review. *J. Phycol.*, 56, 264–282.
- Holland, A. T. et al. (2019). Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet). *Biogeosciences*, 16, 3283–3296.
- Hotaling, S., Wimberger, P. H., Kelley, J. L., and Watts, H. E. (2020). Macroinvertebrates on glaciers. *Ecology*, 101, 1–3.
- Kling, G. W., Fry, B., and O'Brien, W. J. (1992). Stable isotopes and planktonic trophic structure in arctic lakes, *Ecology*, 73, 561–566.
- Kohshima, S., Yoshimura, Y., and Takeuchi, N. (2002). Glacier ecosystem and biological ice-core analysis. In: Casassa, G., Sepúlveda, F. V., Sinclair, R. M. (eds). *The Patagonian Icefields. Series of the Centro de Estudios Científicos*. Springer, pp. 1–8.
- Koziol, K. A., Moggridge, H. L., Cook, J. M., and Hodson, A. (2019). Organic carbon fluxes of a glacier surface: a case study of Foxfonna, a small Arctic glacier. *Earth Surf. Process. Landf.*, 44, 405–416.
- Lamarche-Gagnon, G. et al. (2019). Greenland melt drives continuous export of methane from the ice-sheet bed. *Nature*, 565, 73–77.

- Li, Y. et al. (2019). Reconstruction of the functional ecosystem in the high light, low temperature union glacier region, Antarctica. *Front. Microbiol.*, 10, 2408.
- Liu, Y. et al. (2022). A comprehensive dataset of microbial abundance, dissolved organic carbon, and nitrogen in Tibetan Plateau glaciers. *Earth Syst. Sci. Data Discuss.*, 14, 1–15.
- Lutz, S., Anesio, A. M., Jorge Villar, S. E., and Benning, L. G. (2014). Variations of algal communities cause darkening of a Greenland glacier. *FEMS Microbiol. Ecol.*, 89, 402–414.
- Makowska, N., Zawierucha, K., Mokracka, J., and Koczura, R. (2016). First report of microorganisms of Caucasus glaciers (Georgia). *Biologia*, 71, 620–625.
- Margesin, R., Zacke, G., and Schinner, F. (2002). Characterization of heterotrophic microorganisms in alpine glacier cryoconite. *Arct. Antarct. Alp. Res.*, 34, 88–93.
- Marotta, R., Uggetti, A., Ricci, C., Leasi, F., and Melone, G. (2012). Surviving starvation: changes accompanying starvation tolerance in a bdelloid rotifer. *J. Morphol.*, 273, 1–7.
- McCutchan, J. H., Lewis, W. M., Kendall, C., and McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102, 378–390.
- Michener, R. and Lajtha, K. (Eds.). (2008). *Stable isotopes in ecology and environmental science*. John Wiley & Sons.
- Milner, A. M. et al. (2017). Glacier shrinkage driving global changes in downstream systems. *Proc. Natl. Acad. Sci.*, 114, 9770–9778.
- Minagawa, M. and Wada, E. (1984). Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim. Cosmochim. Ac.*, 48, 1135–1140.
- Musilova, M., Tranter, M., Bennett, S. A., Wadham, J., and Anesio, A. M. (2015). Stable microbial community composition on the Greenland Ice Sheet. *Front. Microbiol.*, 6, 193.
- Nelson, D. R., Guidetti, R., and Rebecchi, L. (2015). Phylum Tardigrada. In: Thorp, J. H., Rogers, D. Ch. (eds.). *Thorp and Covich's Freshwater Invertebrates*. Academic Press, pp. 347–380.
- Nicholes, M. J. et al. (2019). Bacterial dynamics in supraglacial habitats of the Greenland Ice Sheet. *Front. Microbiol.*, 10, 1366.
- Nordenskiöld, A. E. (1872). I.—Account of an Expedition to Greenland in the year 1870. *Geological Magazine* 9, 289–306.
- Oelbermann, M., English, M., and Schiff, S. L. (2008). Evaluating carbon dynamics and microbial activity in arctic soils under warmer temperatures. *Can. J. Soil Sci.*, 88, 31–44.
- O'Leary, M. H. (1981). Carbon isotope fractionation in plants. *Phytochemistry*, 20, 553–567.

- O'Reilly, C. M., Verburg, P., Hecky, R. E., Plisnier, P. D., and Cohen, A. S. (2003). Food web dynamics in stable isotope ecology: time integration of different trophic levels. In: *Handbook of Scaling Methods in Aquat. Ecol.* CRC Press, pp. 145–154.
- Perini, L., Gostinčar, C., Anesio, A. M., Williamson, C., Tranter, M., and Gunde-Cimerman, N. (2019). Darkening of the Greenland Ice Sheet: Fungal abundance and diversity are associated with algal bloom. *Front. Microbiol.*, 10, 439176.
- Peterson, B. J. and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.*, 18, 293–320.
- Pittino, F., Ambrosini, R., Azzoni, R. S., Diolaiuti, G. A., Villa, S., Gandolfi, I., and Franzetti, A. (2018). Post-depositional biodegradation processes of pollutants on glacier surfaces. *Condens. Matter*, 3, 24.
- Rassner, S. M., Anesio, A. M., Girdwood, S. E., Hell, K., Gokul, J. K., Whitworth, D. E., and Edwards, A. (2016). Can the bacterial community of a high Arctic glacier surface escape viral control? *Front. Microbiol.*, 7, 206151.
- Rassner, S. M. E. (2017). Viruses in Glacial Environments. In: Margesin, R. (eds). *Psychrophiles: From Biodiversity to Biotechnology*. Springer, 111–131.
- Redfield, A. C. (1934). On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *James Johnstone Memorial*, 1, University Press of Liverpool, 176–192.
- Ricci, C. (2001). Dormancy patterns in rotifers. *Hydrobiologia*, 446, 1–11.
- Rozwalak, P. et al. (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.
- Šantrůček, J. et al. (2018). Stabilní izotopy biogenních prvků: použití v biologii a ekologii. *Academia*.
- Sanyal, A., Antony, R., Ganesan, P., and Thamban, M. (2020). Metabolic activity and bioweathering properties of yeasts isolated from different supraglacial environments of Antarctica and Himalaya. *Antonie van Leeuwenhoek*, 113, 2243–2258.”
- Sardans, J., Rivas-Ubach, A., and Peñuelas, J. (2012). The C:N:P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. *Perspect. Pl. Ecol. Evol. Syst.*, 14, 33–47.
- Schmidt, S. K. et al. (2022). Microbial biogeochemistry and phosphorus limitation in cryoconite holes on glaciers across the Taylor Valley, McMurdo Dry Valleys, Antarctica. *Biogeochemistry* 158, 313–326.

- Segawa, T. et al. (2014). The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environ. Microbiol.*, 16, 3250–3262.
- Segawa, T. et al. (2017). Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers. *J. Biogeogr.*, 44, 2849–2861.
- Shain, D. H., Novis, P. M., Cridge, A. G., Zawierucha, K., Geneva, A. J., and Dearden, P. K. (2021). Five animal phyla in glacier ice reveal unprecedented biodiversity in New Zealand's southern Alps. *Sci. Rep.*, 11, 3898.
- Shaw, E. A., Adams, B. J., Barrett, J. E., Lyons, W. B., Virginia, R. A., and Wall, D. H. (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.
- Sigman, D. M. and Casciotti, K. L. (2001). Nitrogen isotopes in the ocean. *Encyclopedia of Ocean Sciences*, 3, 1884–1894.
- Singh, P., Roy, U., and Tsuji, M. (2016). Characterisation of yeast and filamentous fungi from Brøggerbreen glaciers, Svalbard. *Polar Rec.*, 52, 442–449.
- Smith, H. J., Foster, R. A., McKnight, D. M., Lisle, J. T., Littmann, S., Kuypers, M. M., and Foreman, C. M. (2017). Microbial formation of labile organic carbon in Antarctic glacial environments. *Nat. Geosci.*, 10, 356–359.
- Sterner, R. and Elser, J. J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press.
- Stibal, M., Šabacká, M., and 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. and Tranter, M. (2007). Laboratory investigation of inorganic carbon uptake by cryoconite debris from Werenskioldbreen, Svalbard. *J. Geophys. Res.: Biogeosciences*, 112, G4.
- Stibal, M., Tranter, M., Telling, J., and Benning, L. G. (2008a). Speciation, phase association and potential bioavailability of phosphorus on a Svalbard glacier. *Biogeochemistry*, 90, 1–13.
- Stibal, M., Tranter, M., Benning, L. G., and Řehák, J. (2008b). Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environ. Microbiol.*, 10, 2172–2178.
- Stibal, M., Anesio, A. M., Blues, C. J. D., and Tranter, M. (2009). Phosphatase activity and organic phosphorus turnover on a high Arctic glacier. *Biogeosciences*, 6, 913–922.
- Stibal, M., Šabacká, M., and Žárský, J. (2012a). Biological processes on glacier and ice sheet surfaces. *Nat. Geosci.*, 5, 771.



- Stibal, M., Telling, J., Cook, J., Mak, K. M., Hodson, A., and Anesio, A. M. (2012b). Environmental controls on microbial abundance and activity on the Greenland ice sheet: a multivariate analysis approach. *Microb. Ecol.*, 63, 74–84.
- Stibal, M. et al. (2020). Glacial ecosystems are essential to understanding biodiversity responses to glacier retreat. *Nat. Ecol. Evol.*, 4, 686–687.
- Takeuchi, N. (2001). The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). *Hydrol. Process.*, 15, 3447–3459.
- Takeuchi, N. (2013). Seasonal and altitudinal variations in snow algal communities on an Alaskan glacier (Gulkana glacier in the Alaska range). *Environ. Res. Lett.* 8, 035002.
- Telling, J., Anesio, A. M., Tranter, M., Irvine-Fynn, T., Hodson, A., Butler, C., and Wadham, J. (2011). Nitrogen fixation on Arctic glaciers, Svalbard. *J. Geophys. Res.: Biogeosciences*, 116, G3.
- Telling, J. et al. (2012a). Microbial nitrogen cycling on the Greenland Ice Sheet. *Biogeosciences*, 9, 2431–2442.
- Telling, J. et al. (2012b). Controls on the autochthonous production and respiration of organic matter in cryoconite holes on high Arctic glaciers. *J. Geophys. Res.: Biogeosciences*, 117, G1.
- Telling, J. et al. (2014). Spring thaw ionic pulses boost nutrient availability and microbial growth in entombed Antarctic Dry Valley cryoconite holes. *Front. Microbiol.*, 5, 694.
- Uetake, J., Yoshimura, Y., Nagatsuka, N., and Kanda, H. (2012). Isolation of oligotrophic yeasts from supraglacial environments of different altitude on the Gulkana Glacier (Alaska). *FEMS Microbiol. Ecol.*, 82, 279–286.
- Uetake, J., Tanaka, S., Segawa, T., Takeuchi, N., Nagatsuka, N., Motoyama, H., and Aoki, T. (2016). Microbial community variation in cryoconite granules on Qaanaaq Glacier, NW Greenland. *FEMS Microbiol. Ecol.*, 92, fiv127.
- Valle, B. et al. (2022). Biodiversity and ecology of plants and arthropods on the last preserved glacier of the Apennines mountain chain (Italy). *The Holocene*, 32, 853–865.
- Valle, B., Gobbi, M., Brambilla, M., Borgatti, M. S., and Caccianiga, M. (2023). Finding the optimal strategy for quantitative sampling of springtails community (Hexapoda: Collembola) in glacial lithosols. *Pedobiologia*, 101, 150914.
- Velázquez, D., Jungblut, A. D., Rochera, C., Rico, E., Camacho, A., and Quesada, A. (2017). Trophic interactions in microbial mats on Byers Peninsula, maritime Antarctica. *Polar Biol.*, 40, 1115–1126.
- Vonnahme, T. R., Devetter, M., Žárský, J. D., Šabacká, M., and Elster, J. (2016). Controls on microalgal community structures in cryoconite holes upon high-Arctic glaciers, Svalbard. *Biogeosciences*, 13, 659–674.

- Wada, E. (2009). Stable  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope ratios in aquatic ecosystems. *P. Jpn. Acad. B.*, 85, 98–107.
- Wang, S., Tedesco, M., Alexander, P., Xu, M., and Fettweis, X. (2020). Quantifying spatiotemporal variability of glacier algal blooms and the impact on surface albedo in southwestern Greenland. *The Cryosphere*, 14, 2687–2713.
- Wejnerowski, Ł. et al. (2023). Empirical testing of cryoconite granulation: Role of cyanobacteria in the formation of key biogenic structure darkening glaciers in polar regions. *J. Phycol.*, 59, 939–949.
- Williamson, C. J., Cameron, K. A., Cook, J. M., Zarsky, J. D., Stibal, M., and Edwards, A. (2019). Glacier algae: A dark past and a darker future. *Front. Microbiol.*, 10, 436973.
- Winkel, M., Trivedi, C. B., Mourot, R., Bradley, J. A., Vieth-Hillebrand, A., and Benning, L. G. (2022). Seasonality of glacial snow and ice microbial communities. *Front. Microbiol.*, 13, 876848.
- Yallop, M. L. et al. (2012). Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. *ISME J.*, 6, 2302–2313.
- Yoshii, K., Melnik, N. G., Timoshkin, O. A., Bondarenko, N. A., Anoshko, P. N., Yoshioka, T., and Wada, E. (1999). Stable isotope analyses of the pelagic food web in Lake Baikal. *Limnol. Oceanogr.*, 44, 502–511.
- Zah, R., Burgherr, P., Bernasconi, S. M., and Uehlinger, U. (2001). Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. *Freshwater Biol.*, 46, 871–882.
- Zawierucha, K., Ostrowska, M., Vonnahme, T. R., Devetter, M., Nawrot, A., Lehmann, S., and Koliczka, M. (2016). Diversity and distribution of Tardigrada in Arctic cryoconite holes. *J. Limnol.*, 75, 545–559.
- Zawierucha, K., Buda, J., and Nawrot, A. (2019a). 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., Buda, J., Azzoni, R. S., Niškiewicz, M., Franzetti, A., and Ambrosini, R. (2019b). 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., Novotna Jaromerska, T., Janko, K., and Gąsiorek, P. (2020). Integrative approach reveals new species of water bears (*Pilatobius*, *Grevenius*, and *Acutuncus*) from Arctic cryoconite holes, with the discovery of hidden lineages of *Hypsibius*. *Zool. Anz.*, 289, 141–165.
- Zawierucha, K. et al. (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., and Ambrosini, R. (2022). Trophic and symbiotic links between obligate-glacier water bears (Tardigrada) and cryoconite microorganisms. *Plos One*, 17, e0262039.