

# Is Oxygenation Related to the Decomposition of Organic Matter in Cryoconite Holes?

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## ABSTRACT

Cryoconite is a sediment occurring on glacier surfaces worldwide which reduces ice albedo and concentrates glacier surface meltwater into small reservoirs called cryoconite holes. It consists of mineral and biogenic matter, including active microorganisms. This study presents an experimental insight into the influence of sediment oxygenation on the cryoconite ability to produce and decomposition of organic matter. Samples were collected from five glaciers in the Arctic and the European mainland. Cryoconite from three glaciers was incubated in stagnant and mechanically mixed conditions to imitate inter-hole water-sediment mixing by meltwater occurring on gla-

ciers in Northern Hemisphere, and its effect on oxygen profiles and organic matter content. Moreover, we investigated short-term changes of oxygen conditions in cryoconite from four glaciers in illuminated and dark conditions. An anaerobic zone was present or approaching zero oxygen in all illuminated cryoconite samples, varying in depth depending on the origin of cryoconite: from 1500 µm from Steindalsbreen (Scandinavian Peninsula) and Forni Glacier (The Alps) to 3100 µm from Russell Glacier and Longyearbreen (Arctic) after incubation. Organic matter content varied between glaciers from 6.11% on Longyearbreen to 16.36% on Russell Glacier. The mixed sediment from the Forni Glacier had less organic matter than stagnant, the sediment from Longyearbreen followed this trend, but the difference was not statistically significant, while the sediment from Ebenferner did not differ between groups. Our results have implications for the understanding of biogeochemical processes on glacier surfaces, the adaptation of organisms to changing physical conditions due to abrupt sediment mixing, but also on the estimation of productivity of supraglacial systems.

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**Key words:** aerobic; anaerobic; decay; glacier; photoautotrophs; supraglacial.

## HIGHLIGHTS

- Anaerobic zones are common in cryoconite from the Northern Hemisphere glaciers
- Effects of oxygenation on organic matter decomposition differ between regions
- Cryoconite microorganisms are adapted to abrupt, stochastic processes

## INTRODUCTION

Understanding the productivity of ecosystems around the world is important for the predictions of ongoing global changes (Plass 1956; Chapin III and others 2008). Fragile Arctic and mountain ecosystems are the most subjected to changes related to the recent melting of glaciers and other components of the cryosphere (Kaplan and New 2006). Oxygen availability in the ecosystem might be a direct representation of its productivity by comparing the balance of photosynthesis (oxygen production) and respiration (oxygen consumption) (for example, Odum 1956; Mulholland and others 2005). During aerobic respiration, the oxygen molecule has a higher reduction potential than the other terminal electron acceptor used in anaerobic respiration. Therefore, during anaerobic conditions, the decomposition of organic matter in the environment should be significantly slower (for example, Reddy and Patrick 1975; Kristensen and others 1995). However, this process directly depends on external factors, not only the presence of oxygen itself. Therefore, the aerobic respiration will not always result in increased organic matter decomposition. For instance, Henrichs and Reeburgh (1987) showed that the labile form of organic matter might be decomposed at similar rates during aerobic and anaerobic conditions in marine sediments. Similarly, the insignificance of bottom-water oxygenation in the organic matter preservation was shown by Pedersen and others (1992), while suggesting the importance of hydrodynamic factors. Therefore, the question of whether oxygenation increases decomposition, which has broad implication in the determination of flow of elements, remains still open. Previous research on the decomposition of organic matter in aerobic and anaerobic conditions has been carried out mostly in environments that are characterised by high complexity (for example, Canfield 1994; Hedges and

Keil 1995). Here, we provide an experimental insight into understanding of the process of organic matter (OM) mineralisation in a relatively simple system occurring worldwide—cryoconite holes, which are biodiversity hotspots on glaciers. Taking into account that glaciers cover 10% of land surface and they are an important biotic and abiotic component of polar and mountainous ecosystems, the recognition of biogeochemical processes on glaciers is important to anticipate future changes in glacier adjacent habitats.

The cryoconite—dark biotic and inorganic matter on the ice surface, is a key element in nutrient cycling on glaciers (Figure 1; Sävström and others 2002; Cameron and others 2012; Stibal and others 2015). Its texture, size and composition are highly variable around the world ranging from loose sediments to spherical cryoconite granules (Rozwalak and others 2021). Cryoconite can be found dispersed across the ablation zone of glacier surfaces. However, depending on meteorological conditions and glacier topography, it may reduce albedo and melt the ice (Cook and others 2020), creating water-filled cavities called ‘cryoconite holes’ (Figure 1; McIntyre 1984; Hodson and others 2008). The continuous water flow through the cryoconite holes in the summer (with the exception of non-maritime glaciers in continental Antarctica, where cryoconite holes are constantly covered with an ice-lid; Fountain and others 2004) may result in an increased aeration of the sediment and increased supply of small particles of minerals or organic matter (Pautler and others 2013; McCutcheon and others 2021). Cyanobacteria, along with some eukaryotic algae, are responsible for most carbon and nitrogen fixation in the sediment (Stibal and Tranter 2007; Cameron and others 2012; Edwards and others 2013). Although their ecological opposite—heterotrophic bacteria constitute the majority of all heterotrophs: based on microbial counts (Margesin and others 2002) and relative reads of 16S rRNA gene copy number (Pittino and others 2018). The former dominates in the upper layer of sediment, while the latter dominates in the deeper layer (Telling and others 2012; Poniecka and others 2020). Even at the scale of cryoconite granule ecological communities assemble along the abiotic gradients: outer photic layers are dominated by photoautotrophs, while the dark interior is dominated by heterotrophs (Segawa and others 2020). Recent studies by Poniecka and others (2018) and Segawa and others (2020) showed that this spatial differentiation in microorganism distribution is directly reflected by the changes in aerobic conditions as a result of both differences in oxygen

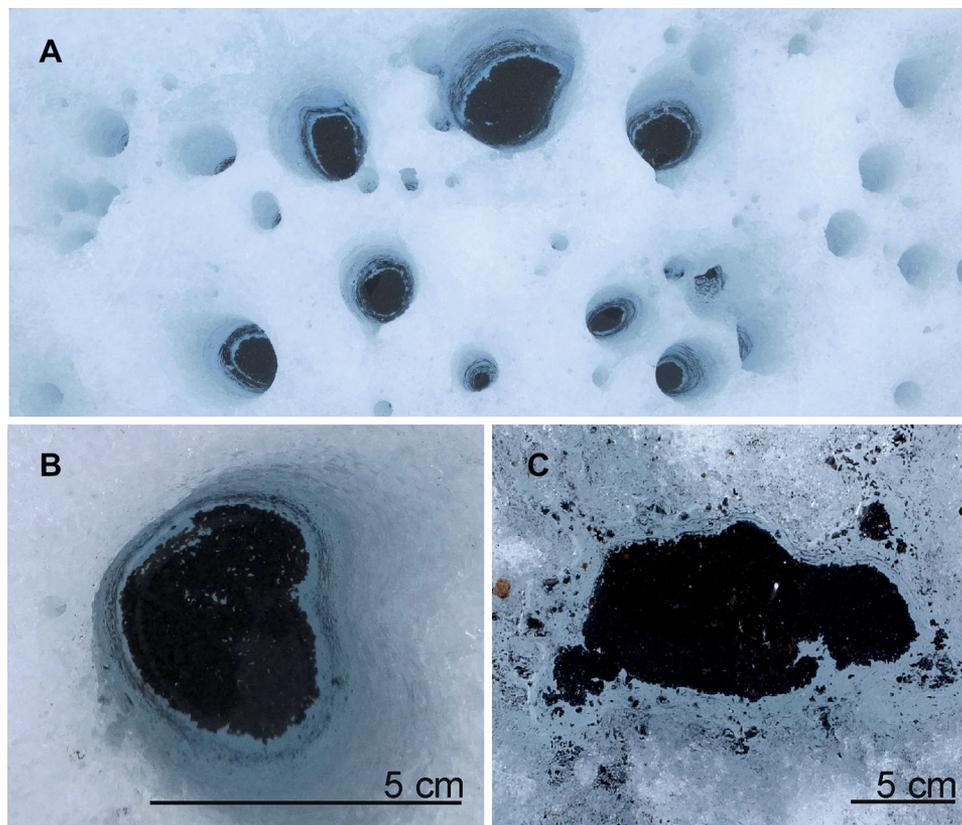


Figure 1. In situ cryoconite holes from: **A**–GrIS, **B**–Longyearbreen, **C**–Forni Glacier.

production (also related to the penetration of solar radiation) and consumption by organisms.

We used two approaches to test whether the oxygenation of the sediment as a simulation of water flow on a glacier may result in increased decomposition of organic matter: indirect—by measuring oxygen along with a depth of the cryoconite thickness, and direct—by measuring the changes of OM. Taking into account that aeration of cryoconite after mixing is relatively short (Poniecka and others 2018), daily aeration of cryoconite in long-term could result in the decrease in OM, because of the high advantage of aerobic respiration in terms of energy efficiency. We measured oxygen in vertical profiles to determine whether the difference in the OM would be due to oxygen supply or stress experienced by the photoautotrophic community. The above actions could help to understand whether oxygen availability in the environment is one of the main factors which influence the organic matter decomposition on the glacier's surface. Moreover, we present an insight into the understanding of the formation of anaerobic zones in cryoconite on four glaciers different in their biotic (photoautotrophic community compo-

sition, organic matter content) and abiotic (altitude, glacier thermal regime, cryoconite granule size) parameters. Finally, we present and discuss potential factors related to water flow through cryoconite holes that may influence the Net Ecosystem Productivity (NEP) of glaciers.

## MATERIAL AND METHODS

### Cryoconite Collection

The cryoconite samples for testing the effect of oxygenation on organic matter decomposition were collected on the three glaciers: one in Svalbard (Longyearbreen) and two in The Alps (Forni Glacier and Ebenferner). Moreover, to determine oxygen conditions in cryoconite, sediment was collected from four glaciers located in different altitudes, latitudes and elevation, differing in morphology, thermal regime and geological settings: Russell Glacier of Greenland Ice Sheet (hereafter GrIS), Longyearbreen (Svalbard), Steindalsbreen (Scandinavian Peninsula; Northern Norway), Forni (the Alps; Ortles Cevedale group). Detailed information on the sampled glaciers is presented in Table 1. The material was collected by a clean plastic

**Table 1.** Sediment-Related Features of cryoconite used for determination of oxygen conditions

Glacier name	Organic matter	Granule size	Photoautotroph biomass
Russell Glacier	16.36% [16.20, 16.52]	0.52 mm [0.48, 0.56]	3.84 mg L <sup>-1</sup> [2.78, 4.90]
Longyearbreen	6.11% [5.78, 6.45]	1.70 mm [1.49, 1.97]	0.07 mg L <sup>-1</sup> [0.05, 0.09]
Steindalsbreen	15.84% [15.47, 16.22]	0.59 mm [0.50, 0.68]	0.15 mg L <sup>-1</sup> [0.08, 0.22]
Forni Glacier	10.69% [10.45, 10.93]	0.54 mm [0.50, 0.58]	0.26 mg L <sup>-1</sup> [0.16, 0.37]

Mean value of the content of organic matter and phycobiont biomass after 7-days incubation. The granules size as the diameter of the granules measured for glacier based on samples from the same sampling campaign. Values in brackets are 95%-confidence interval.

pipette or stainless spoon into the plastic bags or 50-cm<sup>3</sup> falcon tubes, then frozen and kept at -20°C until further analysis. To reflect the biological and geological diversity of the sediment on the glacier scale, the final pooled sample consisted of material collected from several cryoconite holes.

## Experiment design

Initially, we incubated cryoconite to determine the variance in oxygen production and consumption between sampled glaciers (hereafter, the light conditions experiment). To do so, slowly melted and homogenised cryoconite was transferred to the beakers (7 beakers for each glacier, in each beaker 1 cm<sup>3</sup> of cryoconite). These incubations were kept in dark conditions to acclimatise, in a cold room at 1–3 °C. After 48 h, the oxygen concentration was measured in the vertical profiles (see “[Oxygen measurements](#)” Section for details). Next, the beakers were incubated for seven days in a 24 h/0 h light–dark cycle (The Russell Glacier, Longyearbreen and Steindalsbreen) or 12 h/12 h light–dark cycle (Forni Glacier) in order to reflect natural light availability. Then, the oxygen measurements were repeated in each beaker. Since the vegetative period on glaciers is rather short, we assumed that microbial communities in fully melted and open cryoconite holes are highly active from the beginning of the season, when the first holes form. Therefore, we considered a time span of one week sufficient to detect any significant changes in their activity.

A second experiment tested the effect of a mechanical disturbance of sediment to organic matter decomposition (Longyearbreen, Forni Glacier and Ebenferner) and oxygen conditions (Longyearbreen, Forni Glacier) (hereafter mechanical disturbance experiment). In the control group, 10 beakers per glacier were kept in

stable condition (not mixed and not oxygenated), while in the experimental group 10 beakers per glacier were mixed (oxygenated) thoroughly and rapidly aerated by ambient air using Pasteur pipette for 15 s or mixed with glass rod once a day (all treatments have been treated by the same method at the same time). The beakers were incubated for eight weeks in a 12/12 h dark–light cycle for Forni Glacier and in a 24 h/0 h cycle for Longyearbreen. Moreover, independently, the sediment from Ebenferner was kept in similar conditions by eight weeks in a 24 h/0 h cycle. After this experiment, the organic matter content of each beaker was measured and compared with the organic matter content at the start of the experiment.

All above experiments were carried out in beakers with a capacity of 5 cm<sup>3</sup>, with a final sediment thickness of 4–6 mm. The outside of the beaker surrounding the cryoconite layer was covered with aluminium foil to mimic the light-reflecting ice surrounding cryoconite holes. Sediment was mixed with demineralised water with no microbial medium added. The water level was kept constant at approximately 3 cm by trickling in additional demineralised water down the beaker wall as required throughout the experiment. The photon flux density during incubation in the light conditions was equal to 50 μmol quanta s<sup>-1</sup> m<sup>-2</sup> of photosynthetically active radiation, while in the rest it was about 30 μmol quanta s<sup>-1</sup> m<sup>-2</sup>.

## Oxygen Measurements

Oxygen concentrations in the cryoconite were measured by a Clark-type oxygen micro-cathode (Revsbech 2021) with a tip diameter of 200 μm (Unisense, Denmark). In the light conditions experiment, the measurements began in the water ca. 500 μm above the sediment and continued every 200 μm until the anoxic layer was reached. At

least four measurements were taken in the anoxic layer to assure that we did not encounter a local oxygen minimum. In the mechanical disturbance experiment (for Longyearbreen and Forni Glacier), the oxygen was measured from 3000  $\mu\text{m}$  above the water and continued every 500  $\mu\text{m}$ , until a depth of 4 mm into the sediment.

Prior to every measurement, the probe was left for 10 s which allowed the signal to stabilise. Afterwards, two to three measurements were made every 3 s. In the authors' previous measurements, the procedure was repeated three times in each beaker per treatment, once in the middle of the beaker and twice close to the beaker wall to attempt to capture spatial variability in a community activity. However, the data clearly showed low spatial variation, thus in the mechanical disturbance experiment, only one profile was measured in the middle of the beaker. In total, 112 and 40 profiles were taken for the light and mechanical disturbances experiments. The measurements were logged using a Unisense Microsensor Multimetric and SensorTrace Profiling software (Unisense, Denmark).

### Estimation of Organic Matter Content, Cyanobacteria and Algae Biomass, and Granules Size

After the light conditions experiment, the sediment in each beaker was separately homogenised and split into two portions, one for organic matter estimation, and the other for algae and cyanobacteria biomass estimation, whereas before the mechanical disturbance experiment, five replicates were taken from pooled sediment of each glacier to assess the organic matter content before incubation, which was compared with the final organic matter content in each tested beaker after eight weeks of incubations. The organic matter content was measured as a percentage weight loss through combustion at 550 °C for 3 h, following drying at 50 °C for 24 h (Wang and others 2011).

Biomass of algae for each incubated sediment was conducted in 5 technical replications. During qualitative analyses of cyanobacteria and algae were conducted under a light microscope, the taxonomy of species was based on Hindák (1996), Komárek and Anagnostidis (2005), Coesel and Meesters (2007). Quantitative analyses, individual cells or filaments, were done with a Nikon Eclipse TE2000-S digital microscope equipped with a Nikon DS-Fi1 camera. Calculating the biomass of phototrophs was conducted on the basis of cell volume measurements as presented in Pliński and

others (1984). Only dominant taxa ( $> 0.0002 \text{ mg L}^{-1}$ ) in particular cryoconite were taken into account for biomass analyses.

One pooled sample of cryoconite was analysed to measure the cryoconite granules' size. The sediment was suspended in the water and about 3 ml of it was transferred to a Petri dish with a pasture pipette. Then, all the visible granules were measured using a stereomicroscope with a camera (OLYMPUS SZ camera and Quick PHOTO CAMERA 3.0 software).

### Statistical Methods

Altogether, two clear outliers resulting from measurement errors were removed from further analyses. Those measurements of oxygen concentration were higher than the other two measurements in technical replication by 500% and 2400%. For each experiment, the oxygen concentrations were averaged within each depth, then for all three profiles in a beaker (if present). As the oxygen concentrations did not reach zero but only approached it as a consequence of measuring error, a threshold for anaerobic conditions was set at  $0.05 \mu\text{mol L}^{-1}$ . To assess the total amount of oxygen in the measured zone of the beaker, we decided to use the definite integral of oxygen concentration as an approximated function of depth. To do it, we converted micrometre as depth to microlitre assuming that each measurement represented a 'slice' of the beaker with 10- $\mu\text{m}$  depth. This approximation let us calculate the relative difference in oxygen amount, because all measurement in each experiment was done with the same method. Therefore, this approximation might be used only for comparison within experiments in this study, while it cannot be used in the direct comparison of oxygen amount with the other ecosystems.

In the mechanical disturbance experiment, the sediment coming from the Forni Glacier after about 4 weeks started to turn orange-brown, and the hydrogen sulphide smell was detectable. It was impossible to verify whether this originated from organisms in the sediment or was a result of contamination, thus the data about final oxygen measurements were removed from further analysis, while the organic matter data should be treated carefully during interpretation, the latter one might be partially related to the changes which were in the first 4 weeks of the experiment.

Linear mixed models were used to test whether the total oxygen amount and depth of the aerobic zone are different in dark and light conditions

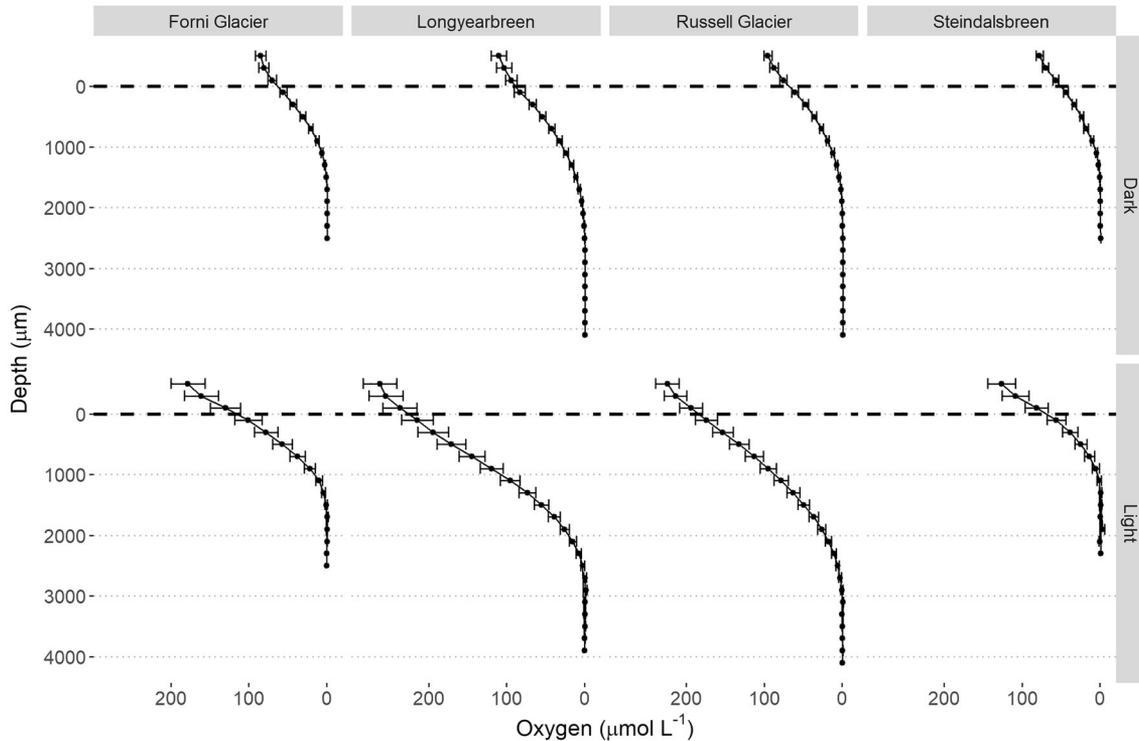
(possible applications of LMMs are presented in Jiang 2007). The first model (L1) included the depth at which the anaerobic zone started as the response variable, treatment (light vs dark) as a fixed effect and sample ID nested in glacier ID as random intercept effects. The second model (L2) included the total amount of oxygen (calculated as above) as the response variable, treatment (light vs dark) as a fixed effect and sample ID nested in glacier ID as random intercept effects. The total amount of oxygen was square-root transformed before the analysis to improve the normality of the model residuals. To assess whether oxygen amount and anaerobic zone depth differed in mechanical disturbances a two-sided t test was performed. Then, to test the differences between organic matter content in mixed and stagnated groups, we performed a Wilcoxon rank-sum test separately for each glacier. The choice of the test depended on the distribution of the variable and the homogeneity of the variance in the tested groups.

Linear mixed models were implemented in R 3.6.3 software (R Core Team 2020), using the glmmTMB package (Brooks and others 2017) and built-in functions. The identity-link function was used for Gaussian family models. Likelihood-ratio

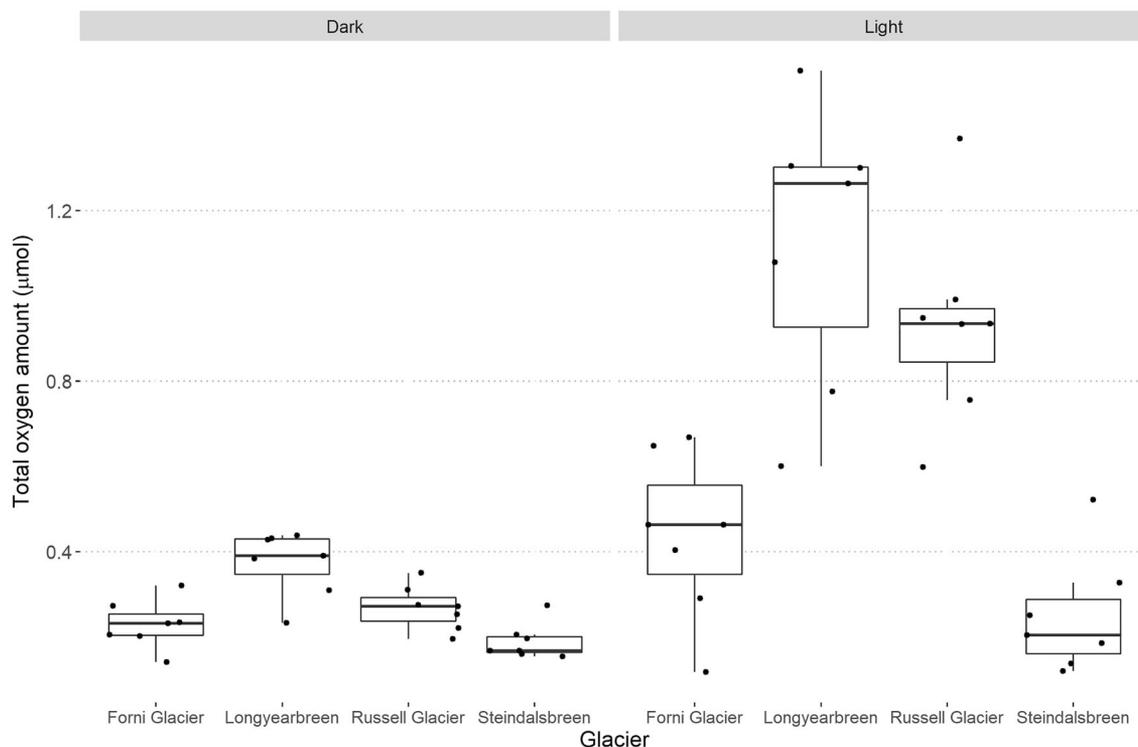
test was performed for models to identify whether the built model has significantly different goodness of fit than the null model. Finally, all models were checked for any violation of assumptions based on diagnostic plots.

## RESULTS

Profiles of oxygen concentrations are presented in Figures 2 and 3. In the light conditions experiment the oxygen concentration progressively decreased and reached an anaerobic status in all samples (Figure 2). The median depth of the anaerobic zone was 1800  $\mu\text{m}$  (IQR = 650) in dark conditions and 1900  $\mu\text{m}$  in light conditions (IQR = 1400). Results of model L1 have shown that the aerobic zone was deeper in the light condition than the dark ( $\chi^2 = 15.954$ ,  $df = 1$ ,  $p < 0.001$ ). For the dark treatment, total oxygen amount was 0.265  $\mu\text{mol}$  with 95% confidence interval (CI) [0.232, 0.297], while for the light it was 0.685  $\mu\text{mol}$  95% CI [0.528, 0.842]. Differences in the total oxygen amount between glaciers and treatments are presented in Figure 3. Model L2 showed that oxygen amount was higher in light treatment than in dark ( $\chi^2 = 32.98$ ,  $df = 1$ ,  $p < 0.001$ ).



**Figure 2.** Oxygen concentration profiles in the sediment for light and dark treatment separately for each glacier. Dots represent the mean value of oxygen concentration per each depth within the glacier-treatment sample, while whiskers represent standard error of the mean. The dashed line indicates the sediment surface.



**Figure 3.** Total oxygen amount in a profile between treatments and glaciers based on raw data. Boxes denote 25th, 50th, and 75th percentiles while whiskers represent the lowest and highest datum within the 1.5 interquartile range of the lower and upper quartile. The dots represent jittered observation on the x- and y-axis.

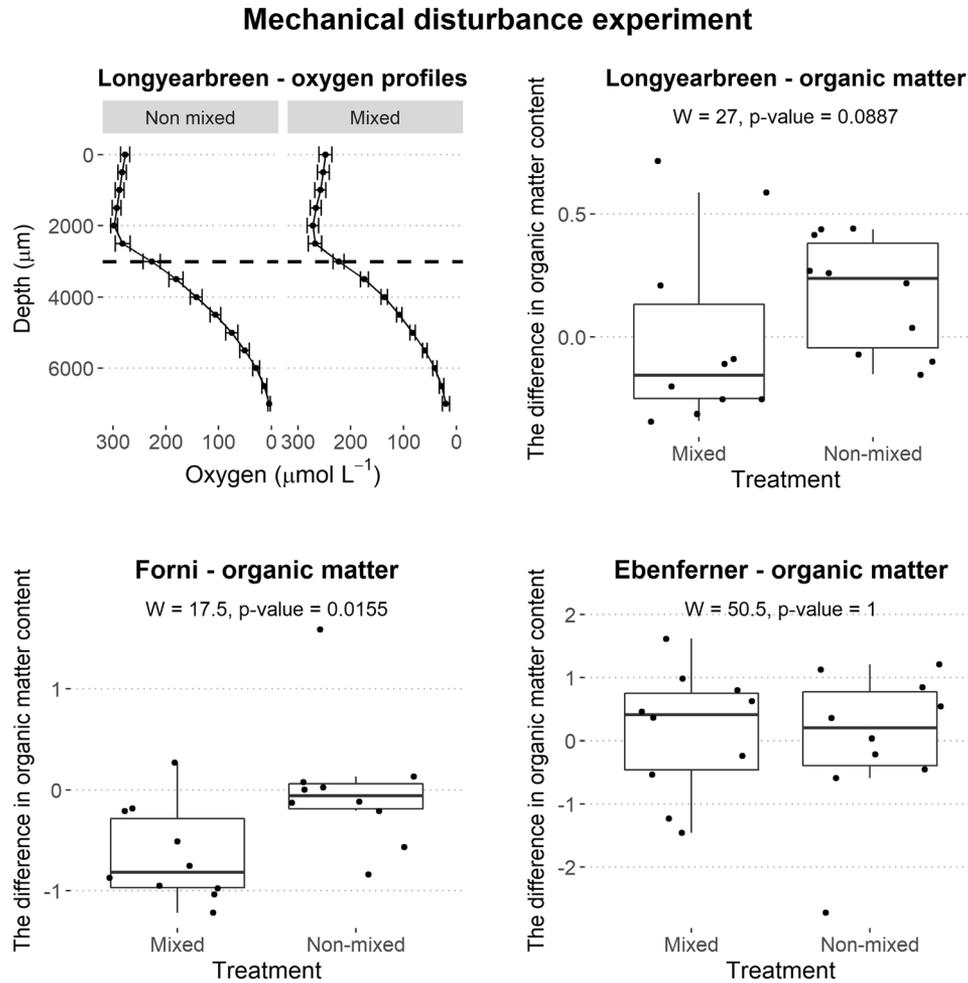
In the mechanical disturbance experiment (Longyearbreen), the anaerobic zone was mostly absent; however, we stopped measuring oxygen at about 1 mm above the bottom of a beaker to avoid destroying the fragile sensor, so it is possible that we did not reach the anaerobic zone, which could be limited to the deepest 1 mm of sediment (Figure 4). The mean oxygen amount per beaker for the stagnant treatment was equal to 31.1  $\mu\text{mol}$  95% CI [28.9, 33.3], whereas for the mixed one it was 27.9  $\mu\text{mol}$  95% CI [25.9, 29.9]. The amount of oxygen in the stagnant treatment did not differ from that of the mixed ( $t = 0.82$ ,  $df = 18$ ,  $p = 0.43$ ).

The difference in OM content between the start and the end of the experiment varied between glaciers (Figure 4). On the Arctic glacier—the Longyearbreen—organic matter content tended to be lower in the mixed sediments, but the difference was not significant ( $W = 27$ ,  $p = 0.0887$ ). In samples from Forni, the organic matter content was lower in the mixed than in non-mixed treatment (Forni:  $W = 17.5$ ,  $p = 0.0155$ ), whereas in Ebenferner samples there was no difference between groups ( $W = 50.5$ ,  $p = 1$ ).

Sediment from Russell Glacier consisted predominantly of granular cryoconite largely overgrown by

filamentous cyanobacteria. The Longyearbreen cryoconite consisted of bright, large oval granules with smooth edges or granules degraded into fine particles looking like mud. Cryoconite from Steindalsbreen and Forni consisted mostly of irregularly shaped granules with some loose mineral particles. Summary of cryoconite granular size, mean organic matter content and photoautotrophic composition is provided in Table 1 and Supplementary Figures 1 and 2 (Except Ebenferner).

In each sample set, the biomass was dominated by a specific composition of photoautotroph species (Supplementary Figure 2). On GrIS, the dominant species counted was *Porphyrosiphon natarisii* Kützing ex Gomont 1892—filamentous cyanobacteria with very thick, firm, lamellated, brown sheaths. The biggest granules were observed in Longyearbreen, where the green algae *Klebsormidium* sp. dominated, occurring primarily as individual cells, not forming filaments. Filamentous species that occurred in the cryoconite from Forni were dominated by cyanobacteria *Wilmottia murrayi* (W. et G.S.West) together with *Klebsormidium* sp. forming filamentous structures. On Steindalsbreen, the dominant phototrophs were *Klebsormidium* sp. and *Leptolyngbya* sp. 3, but due to small cell size had a



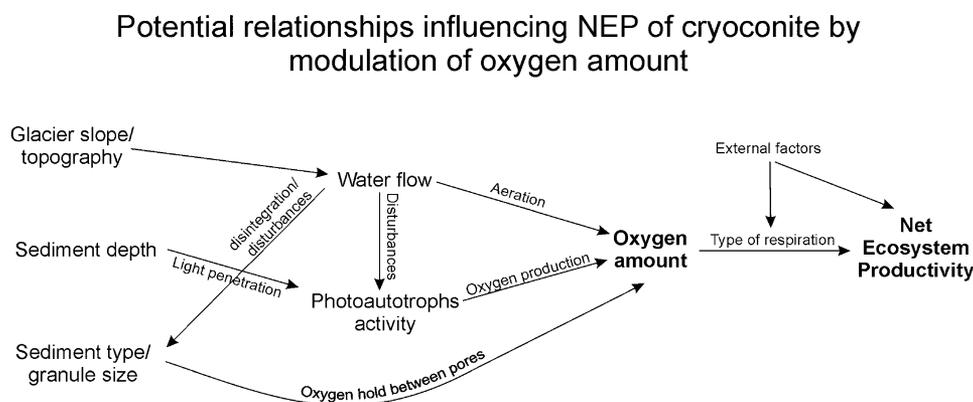
**Figure 4.** Oxygen profiles and the comparison of organic matter decomposition between mixed and non-mixed treatment in mechanical disturbance experiment. *Profiles:* Dots represent the mean value of oxygen concentration per each depth within the glacier-treatment sample, while whiskers represent standard error of the mean. The dashed line indicates the sediment surface. *Boxplots:* Boxes denote 25th, 50th, and 75th percentiles, while whiskers represent the lowest and highest datum within the 1.5 interquartile range of the lower and upper quartile. The dots represent jittered observation on the x- and y-axis.

small share in the total biomass of photoautotrophic organisms in the sediment. Count data of cells are presented in Supplementary Table 2 (Except Ebenferner).

## DISCUSSION

Cryoconite samples used in this study came from five glaciers that differ in their topography and geographical position (the Arctic, subarctic and alpine regions). They also differ in organic matter content, photoautotrophic community composition, as well as dimensions of the cryoconite granules, and thus the size of pores between the sediment particles (Segawa and others 2017; Rozwalak and others 2021). The cryoconite variety is

reflected in the oxygen conditions in the vertical profiles, both in the depth of the aerobic zone and in the total oxygen amount (Figures 2 and 3). Most samples became anoxic, but the depth at which this occurred varied between samples as well as between experimental treatments. The total oxygen amount was universally greater in the light samples, which testifies that our method of sample collection, preservation and incubation allow survival of phototrophic community members. The results were consistent with those presented by Poniecka and others (2018) for GrIS as well Segawa and others (2020) for Ürumqi Glacier no. 1 in Central Asia. The maximum depth of the aerobic zone was no more than 1500  $\mu\text{m}$  for Forni Glacier and Steindalsbreen cryoconite, while for the GrIS



**Figure 5.** Scheme presenting potential relationships influencing NEP of cryoconite by modulation of oxygen amount in the sediment.

and Longyearbreen it reached 3100  $\mu\text{m}$  depth (Figure 2). Neither organic matter content, photoautotrophic biomass nor its combination can clearly explain the difference between oxygen profiles inter glaciers. It proves that oxygen conditions in cryoconite are under the control of multiple factors. Nevertheless, the anaerobic zone occurs in the cryoconite from the Northern Hemisphere, and observed changes in OM content between glaciers and regions in both mixed and stagnant conditions have broad implications in understanding ecological and evolutionary processes shaping microbial communities on glaciers.

### Implication for NEP of Cryoconite

In the samples from Forni Glacier, the decomposition of organic matter was higher in mixed treatment, for Longyearbreen the trend was consistent but not significant, while for Ebenferner differences between groups were not present (Figure 4). Our results suggest that intense water flow through cryoconite, which results in a mechanical disturbance of the benthic environment, could influence the decomposition of organic matter by aeration of the sediment, but this process has to be modulated by sediment-related factors. Canfield (1994), based on the observation of marine ecosystems, stressed that the advantage of aerobic OM decomposition or its lack does not have to be mutually exclusive, but it directly depends on the circumstance of organic matter deposition and its chemical composition. Kristensen and others (1995) showed that oxygen has a significant effect on the decomposition of old and complex organic matter, but not on fresh and labile organic form. These results were also supported by Hulthe and others (1998) on aged plant material buried in coastal sediment. Complex or-

ganic matter, unlike small particles that can be transported into bacterial cells, requires extracellular hydrolysis, which is an additional step depending on the aerobic conditions outside the cell and thus limits the degradation process under anaerobic conditions (Kristensen and others 1995; Qu and others 2021). Therefore, the diverse response of cryoconite to oxygenation by mechanical disturbance could be due to different microbial communities as well chemical and structural composition of deposited organic matter on the particular glaciers. On glaciers, where circumstances favour aerobic decomposition, final productivity of a glacier could be under the control of factors related to glacier features, which directly influence water flow and so oxygenation of the sediment (Figure 5). Further research is needed to reveal which external factors have the major effect on the above process on glaciers.

### Adaptation of Cryoconite Organisms to the Fast-Changing Environment

Lack of difference in oxygen conditions between mixed and non-mixed sediment, suggests that even if light availability is a key limiting factor of the photoautotrophic growth (Bagshaw and others 2016; Perkins and others 2017), the cryoconite photoautotrophs are adapted to live in such a fast-changing environment. It is also indirectly evident from the minor differences in oxygen conditions between mixed and stagnant sediment measured for Longyearbreen. It may indirectly testify that the evolution of microorganisms in glacial ecosystems was driven by stochastic processes. If the ancestors of glacier organisms were under the influence of stochastic processes, they might be selected directionally for diverse metabolism, while weakening

the strength of the selection factor. Such adaptation has been also indirectly shown in Poniecka and others (2018), where the aerobic zone became anaerobic within 120 min after perturbation. Moreover, based on cryoconite from the Karakoram and the Alps, Franzetti and others (2016) used molecular tools to show that microbial communities in cryoconite holes are capable of using different carbon sources. Similar results were also found in microcosm experiments from Antarctica and the Himalaya (Sanyal and others 2018), and in incubations of Greenland, Svalbard and Antarctic samples (Poniecka and others 2020). These findings suggest the complexity of metabolic pathways in cryoconite which, contrary to our expectations, testify to the adaptation of these organisms to live in such conditions.

## CONCLUSION

Cryoconite holes, although considered simple, turned out to be a complex system in which the decomposition of organic matter regulated by oxygen varies greatly between glaciers in the Northern Hemisphere. One of the main factors which likely shapes cryoconite productivity is water flow through sediment, which in turn can be modified by weather and topography of glacier. Moreover, sediment-related features like cryoconite granule size and morphology, microorganism community and chemical composition potentially modulate the decomposition of organic matter itself. Therefore, the cryoconite holes seems to be a good model in further research related to biogeochemical processes, abiotic–biotic relations connected with spatial changes, such as evolution in a fast-changing environment or decomposition of organic matter by microbial communities.

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