The role of heterotrophs in glacier surface ecosystem productivity

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Abstract

Cryoconite holes are miniature freshwater ecosystems on glacier surfaces worldwide that harbour a diverse microbial community. Microbial isolates from cryoconite holes show exceptional environmental tolerance: they are able to grow in a wide range of pH, high salinities and scavenge a wide range of organic carbon sources. All the isolates tested were facultative anaerobes The community resilience exceeds the 'normal' conditions encountered, and enables their functionality in microenvironments, and in transition environments caused by freeze-thaw. This study demonstrates for the first time that environmental conditions, in particular concentrations of oxygen, are spatially heterogenous at the microscale. Anoxic zones develop rapidly following disturbance and can be found inside cryoconite granules and in thick layers of cryoconite sediment. These microhabitats, with limited oxygen, create microniches for anaerobic metabolism. Facultatively anaerobic microorganisms isolated from cryoconite holes are capable of anaerobic biodegradation via fermentation, and anaerobic metabolism in the holes is dominated by fermentation. An excess of fermentation products, especially acetate, can be detected in defrosted cryoconite sediments and in long-term incubations, indicating incomplete metabolic pathways. Analysis of the microbial community structure revealed that terminal oxidisers are present in low abundance in some of the samples, but the terminal metabolic steps such as sulphate reduction or methanogenesis are not coupled with fermentation. This means that fermentation products could be released to downstream environments, but the extent and impact of this process requires further quantification. Anaerobic metabolism and anaerobic microorganisms are therefore important components of cryoconite ecosystem, which degrade organic matter and impact the glacier carbon cycle.

Author note and status of publications

Chapter 1 and 2 contains text and figures included in the book chapter, which is currently being prepared for publication, with the content of the book chapter distributed throughout these chapters. Chapters 4 to 5 present two papers published in international, peer-reviewed journals. While the text, figures and tables presented here are largely the same as those published in the journals, the content of the papers has been re-formatted and edited so as to be suitable for the thesis layout and avoid repetition. Additional material was also added to chapter 4, extending the material presented in the paper to include the data from Antarctic and Svalbard samples.

The status of the following papers, including co-author contributions, are as follows (correct as of thesis submission):

(Chapter 1 and 2) Poniecka, E. A. and Bagshaw, E. A. (2020, in preparation) Cryoconite biome. Chapter in *Microbial Life in the Cryosphere and Its Feedback on Global Change*.

Author contributions and declarations: EP wrote the chapter. EB was involved in discussions during the writing of the chapter, and provided constructive peer-review.

(Chapter 4) Poniecka, E. A., Bagshaw, E. A., Tranter, M., Sass, H., Williamson, C. J., and Anesio, A. M. (2018). Rapid development of anoxic niches in supraglacial ecosystems. *Arctic, Antarctic, and Alpine Research*, 50:1

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(Chapter 5) Poniecka, E. A., Bagshaw, E. A., Sass, H., Segar, A., Webster, G., Williamson, C., Anesio A. M., Tranter M. (2020). Physiological Capabilities of Cryoconite Hole Microorganisms. *Frontiers in Microbiology*, 11, 1783.

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Abbreviations

ASMA	Antarctic Specially Managed Area
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
EPS	Extracellular Polymeric Substances
ETR	Electron Transport Rate
FRR	Fast Repetition Rate (FRR) Fluorescence
GrIS	Greenland Ice Sheet
MCM	McMurdo (Dry Valleys)
MPN	Most Probable Number
NEP	Net Ecosystem Productivity
NGS	Next Generation Sequencing
NMDS	Non-Metric Multidimensional Scaling
OM	Organic Matter
OTUs	Operational Taxonomic Units
PAR	Photosynthetically Active Radiation
PCB	Polychlorinated Biphenyls
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PVDF	Polyvinylidene Fluoride
st.dev.	Standard Deviation
VFAs	Volatile Fatty Acids

Chapter 1

Introduction

1.1. Microbial life on ice

The cryosphere covers 20% of the Earth's surface and consists of the regions where water is found mainly in the frozen state (Boetius et al., 2015). Global climate change is causing significant changes to the cryosphere, including mass loss from ice sheets and glaciers, reduced snow cover, reduced sea ice extent and thickness, and increased permafrost temperatures (Pörtner et al., 2019). The icy habitats of the cryosphere were long thought as lifeless because water in the solid state of ice and snow was not available for biological processes (Boetius et al., 2015; Hodson et al., 2015). Despite growing evidence and studies on microorganisms dating back to 1930s (reviewed in Boetius et al., 2015), only recently have glaciers and snow packs begun to be recognized as a distinct biome of Earth (Anesio and Laybourn-Parry, 2012; Hodson et al., 2015). Since ice sheets and glaciers comprise the biggest freshwater ecosystem on the planet (Edwards et al., 2013b) and are undergoing rapid changes (Stocker et al., 2013), it is crucial to understand the biogeochemical processes occurring within the cryosphere in order to predict future changes and their impacts on surrounding environments, as well as potential losses of cryospheric biodiversity.

Glacial runoff is a significant source of labile carbon and nutrients to downstream subglacial and marine ecosystems (Hood et al., 2009; Bhatia et al., 2013; Hawkings et al., 2016; Musilova et al., 2017; Wadham et al., 2019). A decrease in the volume of glaciers worldwide affects the quantity and quality of the dissolved organic nutrients, particularly carbon, delivered to the coastal ecosystems (Hood et al., 2009). There is large variability in the quality and quantity of nutrients released. In higher melt years, the concentration of nutrients released increases, including labile macronutrients such as phosphorus, nitrogen and silica (Hawkings et al., 2015, 2018; Wadham et al., 2016). It is expected the transport of nutrients off the glaciers will steadily increase in the foreseeable future, but their origin and behaviour remains poorly understood.

A major source of the bioavailable nutrients is assigned to microbial activity on and beneath glaciers and depends on active primary production and carbon recycling during the melt season (Lawson et al., 2014; Musilova et al., 2017). These icy habitats are characterized by truncated

food webs, dominated by microorganisms (Anesio and Laybourn-Parry, 2012) and therefore serve as a natural laboratory to study simplified microbial and biogeochemical processes in habitable niches. An example of such niches are cryoconite holes (Fig. 1.1), regarded as hotspots of microbial processes on glacier surfaces (Stibal et al., 2006; Anesio et al., 2009; Cook et al., 2016). Cryoconite is a matrix of mineral particles and biological material deposited on glaciers by wind and meltwater, most likely of local origin (Porazinska et al., 2004). Having lower albedo than surrounding ice, it absorbs heat and melts downwards, creating a suitable habitat for microbial life in the supraglacial environment (McIntyre, 1984; Tranter et al., 2004; Cook et al., 2016). First observed in Greenland, cryoconite holes occur on glaciers surfaces all over the world, including polar (Arctic and Antarctic) and temperate ice (reviewed in Kaczmarek et al., 2015). They are typically found in the lower part of the glaciers, where ablation dominates, rather than the upper part which accumulates snow (Porazinska et al., 2004). In some locations the cryoconite material forms granules, but the mechanisms and factors responsible for such aggregation are not fully understood (Langford et al., 2010; Zawierucha et al., 2020, in press). The structure of cryoconite holes ensures that the organisms that inhabit them have access to liquid water throughout the ablation season (Fountain et al., 2004; Hodson et al., 2008) and ensures a relative high density of different life forms when compared to other supraglacial habitats (Edwards et al., 2011).

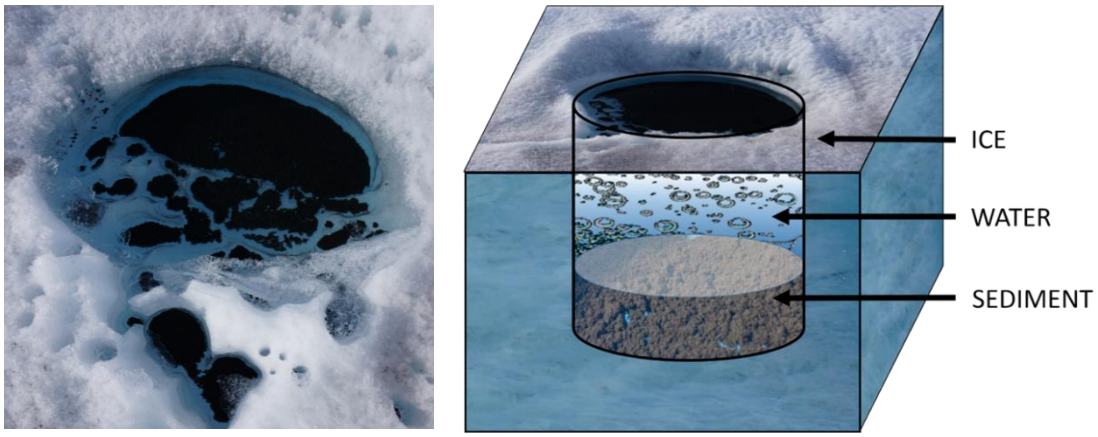

Figure 1.1. A cryoconite hole on Greenland Ice Sheet (left) and a conceptual cross-section of unlidded cryoconite hole (right).

Much of the research on polar cryoconite holes has been focused on geochemistry, net ecosystem productivity and carbon cycling (Stibal et al., 2008; Cook et al., 2012; Bagshaw et al., 2013). There has been some research into microbial community structure (Porazinska et al., 2004; Cameron et al., 2012; Edwards et al., 2013a, 2013b; Musilova et al., 2015; Lutz et al., 2017), but the actual functionality remains largely unidentified and physiological limits are untested. The detailed roles of individual groups of organisms within the holes are poorly understood, particularly with respect to the heterotrophs. Existing data assess various characteristics of the

cultivable heterotrophic microbes from Antarctica (Sanyal et al., 2018), Svalbard (Singh and Singh, 2012; Singh et al., 2014), Greenland (Singh et al., 2020), Himalaya (Sanyal et al., 2018; Singh et al., 2020) and Alps (Margesin et al., 2002; Lee et al., 2011), but no study focuses on the most abundant representatives. The most abundant bacteria likely have a bigger influence impact on the cryoconite hole biogeochemistry than the species growing the fastest in culture.

Some of the fundamental assumptions on cryoconite hole geochemistry, such as their oxygen status and spatiotemporal variability, have remained unchallenged since intensive research began. Cryoconite holes are generally believed to be oxygen-rich (Christner et al., 2003; Stibal and Tranter, 2007). Point measurements of oxygen saturation in the water column of Antarctic ice-lidded cryoconite holes ranged from 70 to 160% (Tranter et al., 2004). Continuous measurements of cryoconite hole water over a four-week period on the Canada glacier in the McMurdo Dry Valleys (Antarctica) showed the oxygen saturation remained in the range of 50 to 80% (Bagshaw et al., 2011), with short periods of over- and under-saturation. Despite the assumption that cryoconite holes are broadly oxic, anaerobic microorganisms were detected in the cryoconite holes from Ecology Glacier in Antarctica, although no corresponding oxygen measurements were taken (Zdanowski et al., 2017). The occurrence of anoxic zones in the cryoconite granules in the Arctic was speculated (Uetake et al., 2016), but no direct measurements of the oxygen saturation in the Arctic or temperate cryoconite were published prior to the results of this study.

Another underrepresented aspect of cryoconite research are comparisons between different regions. Whilst it is generally accepted that there are physical differences between cryoconite holes in different geographical locations (Hodson et al., 2008), detailed geochemical and microbial analyses are lacking. Because of the logistical challenges, costs and difficult access, studies on cryoconite are usually limited to snapshots of single glaciers and most comparative studies comprise laboratory incubations and experiments. One of the first studies showed that Antarctic and Canadian communities were dominated by cyanobacteria and algae respectively; they also differed in the abiotic characteristics of the holes (Mueller et al., 2001). Organic matter composition also differed between Antarctic, Greenland and Canadian glaciers (Pautler et al., 2013). Analysis of the differences in microbial communities both by molecular methods (Cameron et al., 2012; Kleinteich et al., 2017; Lutz et al., 2017; Darcy et al., 2018) and cultivation methods (Sanyal et al., 2018) has been attempted, but a global picture of cryoconite microbial communities remains elusive.

Most of the existing studies on geochemistry and microbial diversity have focused on the cryoconite sediment as a whole, disregarding its small-scale complexity and dynamics. Takeuchi

et al. (2001) first observed that phototrophs are present only on the surface of cryoconite granules from Himalaya. Similarly, microstructure analysis of the Greenland and Svalbard granules have also showed differences in the location and quantity of photosynthetic and heterotrophic microorganisms, as well as organic matter (Langford et al., 2010). Uetake et al. (2016) discovered that smaller and larger granules originating from the same holes in Greenland had different C and N contents and varying bacterial community structures. Unexplained significant differences in distribution of microscopic invertebrates (e.g. rotifers, tardigrades) in subsamples of the same cryoconite holes were observed on glaciers from Svalbard, Antarctica and the Alps (Zawierucha et al., 2019). There are no data on heterogeneity of cryoconite sediment from Antarctica.

Despite these efforts, some gaps in cryoconite research remain: the oxygen dynamics in the cryoconite sediment, the occurrence and impact of anoxia, the role of the heterotrophic community in ecosystem processes, the physical, biogeochemical and microbiological diversity of cryoconite systems in different locations, and the effect of micro-scale changes on microbial community form and function.

Chapter 2

Literature review

2.1. Life in cold environments

The cryosphere biome has specific physical and environmental characteristics (Anesio and Laybourn-Parry, 2012); temperatures are low, reaching about −85 °C in winter at the south pole (Carpenter et al., 2000), and precipitation consists mainly of snow. Microorganisms that inhabit the cryosphere must endure these conditions, which are described in the following sections. Total cell numbers of microorganisms (Table 2.1) are comparable to non-cryospheric freshwater habitats (Boetius et al., 2015).

Table 2.1. Bacteria and archaea cell counts from cold habitats on Earth (adapted from Boetius et al., 2015)

Habitat	Average area (10^6 km^2)	Average volume (10^3 km^3)	Cell number per ml	Total cell numbers
seasonal snow	47	2	$10^2 – 10^5$	$10^{20} – 10^{23}$
sea ice	25	50	$10^4 – 10^7$	$10^{23} – 10^{26}$
supraglacial	17	0.02	$10^4 – 108$	$10^{23} – 10^{27}$
englacial	17	33 000	$10^1 – 10^3$	$10^{23} – 10^{25}$
subglacial	17	0.02	$10^3 – 10^5$	$10^{22} – 10^{24}$
subglacial lakes	>0.05	16	$10^2 – 10^5$	$10^{21} – 10^{24}$
permafrost	23	300	$10^5 – 10^8$	$10^{25} – 10^{28}$
total	**112**	**33 400**	**$10^1 – 10^8$**	**$10^{25} – 10^{28}$**

2.1.1. Snow

The snow habitat is characterized by extremes of low temperature, high UV radiation, and a lack of liquid water in the porous structure. It was traditionally regarded as a reservoir of surviving, but not metabolically active cells (Maccario et al., 2015), yet recent seasonal fluctuations were presented as evidence that snow is a habitable environment for bacteria (Carpenter et al., 2000; Boetius et al., 2015). Most studies focused on red snow, coloured by pigment-carrying algae (Margesin and Miteva, 2011; Anesio and Laybourn-Parry, 2012). Red-pigmented green algae appear seasonally on the surface of thawing snow worldwide, with *Chlamydomonas* cf. *nivalis*

regarded as most common cosmopolitan species (Segawa et al., 2018). Heterotrophic bacteria can be found throughout the depth of snowpack and likely remain active in winter (Maccario et al., 2015; Holland et al., 2020) The abundance of bacteria varied in different studies, from as low as 10^2 cells per 1 ml of melted snow, up to 10^5 cells (Margesin and Miteva, 2011). The number of cells often correlated with calcium ion concentrations, which can be treated as a proxy for dust content (Margesin and Miteva, 2011). Significant diversity of snow microorganisms was revealed by molecular and cultivation methods, but surprisingly, no new species were described from the snow (Margesin and Miteva, 2011). Low levels of bacterial activity (e.g. DNA and protein synthesis) have been detected in the snowpack of South Pole (Carpenter et al. 2000), as well as reactive nitrogen species, most likely produced by microbial activity (Amoroso et al., 2009). All these findings point to snow being a biologically dynamic environment.

2.1.2. Sea Ice

Another challenging environment for microbes, due to fluctuating conditions throughout the year, is sea ice. Sea ice is characterized by a wide range of UV radiation, temperatures and salinities. Solar radiation levels change with the seasons and depth, varying from very low in the deeper layers and in winter to very high on the surface (Mader et al., 2006; Arrigo, 2014). The existence of water in brine channels enables habitability of ice and activity of bacteria. Extensive photosynthesis during the sunlit season increases pH values in the brine. The temperature of the surface ice, which is more susceptible to atmospheric conditions, can drop to -30 °C in winter and raise above zero in the summer, and salinities of liquid brine channels within the ice vary from 24% to as low as 0.05%. Bacteria can be found in the entire column of sea ice (Boetius et al., 2015), but the highest abundance (up to 10^8 bacterial cells per mg of algal mass) is concentrated around algal aggregates or mats forming on the underside of the sea ice. Primary production by algae and cyanobacteria on the surface similarly supports high numbers of heterotrophic bacteria and eukaryotes - protists (ciliates, flagellates and foraminifera) and invertebrates (nematodes, rotifers, copepods and polychaetes) (Gradinger, 2001; Arrigo, 2014). Ice melting during late summer releases organic compounds accumulated during sunlit, net-autotrophic season, supplying underlying communities. Productivity of sea ice can vary from a few, up to several hundred mg of carbon per m^2 per day (Boetius et al., 2015). Bacterial activity in sea ice cores under *in situ* conditions was detected down to -20 °C on the dispersed mineral particles, demonstrating the importance of the mineral surface and liquid inclusions as habitat for microbes at subzero temperatures (Junge et al., 2004).

The photosynthetic community in sea ice is dominated by eukaryotes, mainly diatoms, with rarely occurring cyanobacteria and purple sulphur bacteria. Amongst heterotrophic bacteria, Flavobacteria and Gammaproteobacteria are dominant classes, followed by Alphaproteobacteria (Boetius et al., 2015). This is different to cold lakes, where Betaproteobacteria seem to be dominant and from the underlying seawater where the most abundant phylotype is Alphaproteobacteria . The unstable habitat of sea ice generates significant physiological plasticity of microorganisms, allowing quick adaptation in changing environment (Margesin and Miteva, 2011); this is likely true for other cold niches.

2.1.3. Soils

Soils present a distinct habitat in the cold biosphere, with comparatively large input of ions from mineral particles and high microbial abundance (Table 2.1). They show great variability, depending on the distance from the sea, precipitation patterns, organic matter (OM) content and water availability. When the upper horizons melt during summer, extensive biological activity occurs (Bradley et al., 2014) dominated by bacteria (e.g. Proteobacteria, Actinobacteria and Acidobacteria). It is useful to consider soil habitats as seeding grounds for glacier surface ecosystems, but their physical characteristics and microbial communities will not be explored here.

2.1.4. Lakes

Polar lakes and streams provide a broad range of aquatic habitats in the cryosphere. They exhibit a large variety of chemical and physical conditions, varying from freshwater to hypersaline, from acidic to alkaline, from rich in oxygen to anoxic (Sattler and Storrie-Lombardi, 2009; Margesin and Miteva, 2011). This means they are an ideal system to address general questions regarding the biogeography of microorganisms, factors influencing the biological production and responses of different aquatic ecosystems to climate change (Vincent and Laybourn-Parry, 2009). They also differ in the duration of ice cover, maintaining minimal, annual or perennial ice covers. The inhabiting organisms include both pelagic and benthic organisms, which include microbial mat communities (Sattler and Storrie-Lombardi, 2009; Margesin and Miteva, 2011). Benthic photosynthetic communities support the highest rates of productivity in these ecosystems because of the stable physical environment and nutrients supplied from sedimentation (Vincent and Laybourn-Parry, 2009). Active microbial communities are also found in the ice covering polar lakes, for example in the accretion ice of Lake Bonney in McMurdo (MCM) Dry Valleys (Porazinska et al., 2004).

In Antarctica, epiglacial, subglacial and supraglacial lakes can be found. Epiglacial lakes are situated on the bare ground, usually in the proximity of the ocean or on nunataks. They are often found in the ice-free dry valleys, like MCM or Schirmacher Oasis. Subglacial lakes are located underneath the ice sheet (Cavicchioli, 2015). They are covered with glacial ice and may be isolated from the atmosphere for prolonged periods. Supraglacial lakes form on glaciers surfaces and microbiologically resemble cryoconite holes (Sattler and Storrie-Lombardi, 2009; Keskitalo et al., 2013). Antarctic lakes are known as hot spots or oases for life in the barren landscape of polar deserts. They support stable microbial communities, with a relatively simple food-web structure when compared to temperate lakes (Vincent and Laybourn-Parry, 2009). Microbial abundance reaches 10^5-10^6 cells per ml (Margesin and Miteva, 2011), similar to summer abundances in Arctic and Alpine lakes. Antarctic epiglacial lakes are often perennially ice-covered and sometimes develop extreme salinity and pH values (e.g. Sonic Lake, pH 10.6, Framnes Mountains near Mawson station, (Sattler and Storrie-Lombardi, 2009)). The perennial ice cover supports not only development of unusual geochemical conditions, but also unique microbial populations (Margesin and Miteva, 2011). For example Ace Lake (Vestfold Hills, East Antarctica) is dominated by green sulphur bacteria, but these are absent from Lake Fryxell (MCM) in favour of purple sulphur bacteria (Cavicchioli, 2015).

Subglacial lakes provide an even more unique habitat for microbial life, devoid of atmospheric inputs and access to light (Priscu et al., 2009). Over 380 subglacial lakes have been identified in Antarctica (Cavicchioli, 2015). The best known example is Lake Vostok, one of the largest lakes on Earth with a surface area of 14 000 km^2. It is buried under 4km of glacial ice and it may have been isolated for 14 million years (Sattler and Storrie-Lombardi, 2009). Little is known about the microbial assemblage of the lake's water, but cores analysed from accretion ice overlying the lake revealed 18 unique bacterial rRNA phylotypes (Margesin and Miteva, 2011). The analyses revealed as little as 400 cells per ml and gave insight into lake geochemistry, being extremely oligotrophic and highly oxygenated. Although lake water was sampled twice – in 2012 and 2015 – due to contamination issues only one phylotype was identified as an unknown type of bacterium, showing around 86% sequence similarity to other currently known taxa. Results of 2015 (Bulat, 2016) drilling are still not publicly available.

There are eight times more lakes in Arctic than in the Antarctic (Margesin and Miteva, 2011), meaning they are an important microbial habitat within the wider ecosystem. Bacterial abundance in winter is smaller than in Alpine lakes, averaging 1 x 10^5 cells per ml, and peaks in summer reaching 7 x 10^5 cells per ml (Garneau et al., 2008). Microbial community is dominated by Cyanobacteria thanks to resistance to desiccation, UV radiation and freeze-thaw cycles.

Several cyanobacterial phylotypes from Arctic lakes closely resemble Alpine and Antarctic ones (Margesin and Miteva, 2011). Allochthonous sources of organic carbon such as plant material are relatively more important in Arctic lakes in comparison to their Antarctic counterparts, which rely on autochthonous cycling of organic matter (Vincent and Laybourn-Parry, 2009)

Alpine lakes, by contrast, do not develop such extreme physical and geochemical conditions as high latitude Arctic and Antarctic lakes. They are temporarily ice-covered, for up to 3-6 months per year (Margesin and Miteva, 2011). The ice cover develops a unique, layered structure of ice and slush. Slush layers provide a productive habitat with nutrient input from atmosphere and lake catchment, reaching 6 x 10^5 microbial cells per ml, compared with up to 4 x 10^5 cells per ml in the warmer lake waters beneath and snow above. Betaproteobacteria dominate the microbial community of Alpine lakes, representing up to 24% of total bacteria counts (Pernthaler et al., 1998; Sommaruga and Casamayor, 2009).

2.1.5. Glaciers

Glaciers cover 15% by area of the total cryospheric habitat, but constitute most of its volume and simultaneously most of the freshwater supply of the Earth (Edwards et al., 2013b; Boetius et al., 2015). Glaciers comprise distinct microbial habitats – subglacial, englacial and supraglacial – varying in physical and geochemical characteristics (Fig. 2.1).

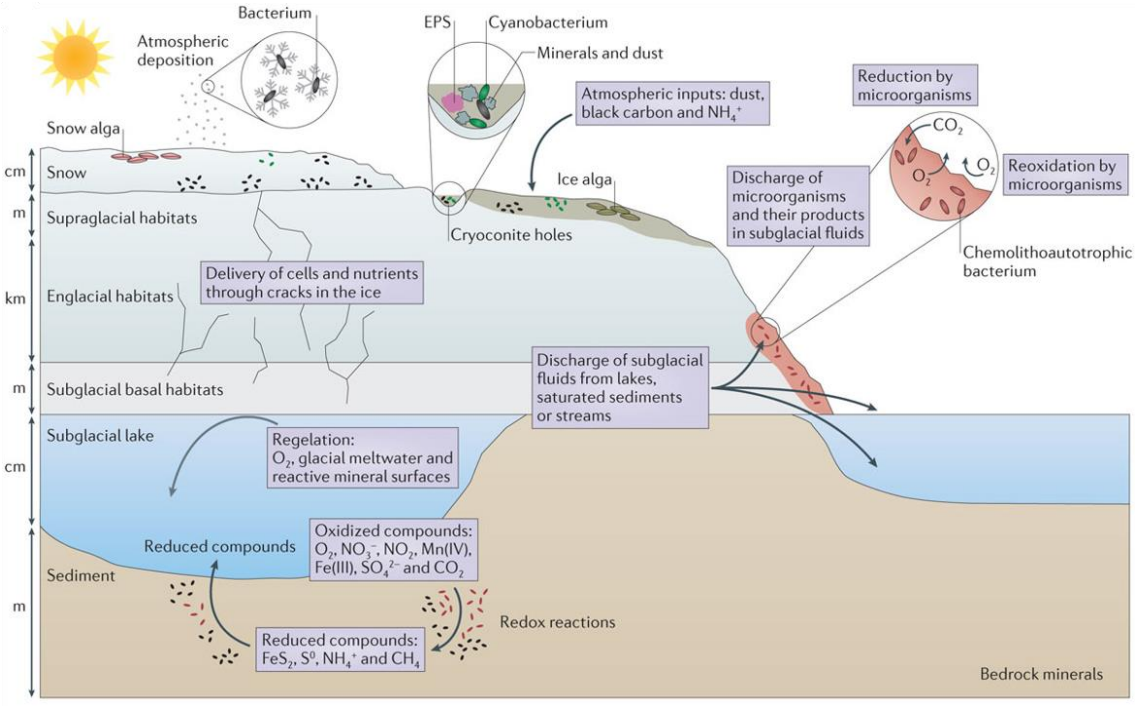

Figure 2.1.Glacial habitable zones and microbial processes (after Boetius et al., 2015).

2.1.5.1. Subglacial environment

The subglacial environment is one of the most extreme on Earth. Despite the lack of light, low nutrients concentrations and high pressure, it supports relatively complex and active microbial communities. Weathering releases solutes into the surroundings (Friedmann and Weed, 1987; Hall et al., 2002) and bacteria were shown to depend on solutes of minerals from deposited dust (Skidmore et al., 2005; Tranter, 2014). Subglacial microorganisms use the minerals and carbonate derived from the bedrock to generate energy through redox reactions (Boyd et al., 2014). Due to challenges associated with sampling (Siegert et al., 2012), subglacial habitats are still poorly understood.

Ice motion creates thin layers of liquid water at the bed of the glacier which allows for microbial activity and biogeochemical processes to occur (Christner et al., 2008). Seasonal input of nutrients from supraglacial melting is delivered through cracks and moulins. Moulins are narrow (0.5 – 10 m wide) vertical channels, sparsely distributed over the ablation zone (only 0.02 to 0.2 per square kilometre), and are crucial for allowing water to flow from the surface to the base of glacier (Banwell et al., 2016). When increased meltwater is delivered to bed, the drainage system evolves, consequently decreasing pressure and speed. The movement of ice and high pressure not only allows for existence of thin liquid layer, but it also grinds up bedrock and sediments. The resulting basal debris has a larger surface area facilitating weathering and allowing microbes to profit from minerals and organic carbon sediment input (Macdonald et al., 2018). The extent of nutrient supply from supraglacial environments by moulins and effects on microbial community are to date poorly understood (Chandler et al., 2013; Lawson et al., 2014).

As no light reaches the bottom of glaciers, the community is supported by primary productivity of chemolithoautotrophs. Chemolithoautotrophs are organisms which oxidise inorganic compounds as an energy source to fix inorganic carbon (CO_2) as opposed to photoautotrophs which use light for this process. Chemolithoautotrophs support a supposedly simplified food web, with heterotrophs deriving organic carbon from their labile exudates, ancient organic matter from basal debris or by recycling fresh OM (Christner et al., 2008; Boetius et al., 2015). As the oxygen supply is often limited, subglacial environments are mostly dominated by anaerobic metabolism (Christner et al., 2008). Active methanogenesis was detected in basal ice and subglacial communities, which is likely coupled with sulphate reduction (Boyd et al., 2010; Lamarche-Gagnon et al., 2019).

Subglacial environments show great variability in geochemical conditions, varying from freshwater with oxygen supply from melting basal ice (Tranter et al. 2005), to anoxic brines

underneath Taylor Glacier (Mikucki et al., 2009). Thus, the most dominant phylotypes depend on the studied site.

2.1.5.2. Englacial – bulk ice

Between productive and diverse top and bottom layers of glaciers, there is bulk, thick, glacial ice. it is a harsh ecosystem with extreme temperatures dropping to -56 °C, high pressure, lack of light and low nutrient input (Margesin and Miteva, 2011). Insights into this environment come from ice core studies, which are a challenging sampling method due to contamination issues. Microbial cells are deposited as airborne propagules with snow and slowly integrated into the bulk ice mass. Consequently, englacial ice shows wide diversity, depending mainly on the source of organisms, which includes proximal and distant seawater aerosols and terrestrial dust. The amount of deposited material, as well as other physical factors like depth, determine the abundance, varying from less than 10^2 up to 10^7 cells per ml (Margesin and Miteva, 2011). Dominant phyla are bacteria from Actinobacteria, Firmicutes, Proteobacteria and Cytophaga-Flavobacterium-Bacteroides (CFB) group.

Bacterial isolates have been recovered from ice cores as old as 750 000 years (Christner et al., 2003b), raising the question if they have remained active, while embedded in ice. Different possible habitats were suggested by Price (2000) – veins between ice crystals, thin, liquid layer on the ice grains or even inside the ice crystals. The exclusion of microbial cells into liquid phase while freezing was shown by Mader et al. (2006). The structure of ice in glaciers and ice sheets contains crystals separated by veins filled with a concentrated water phase. A model laboratory system of polycrystalline ice was created, and fluorescent beads and stained bacteria were detected under microscope (Fig. 2.2). The veins provided a liquid habitat for bacteria to thrive.

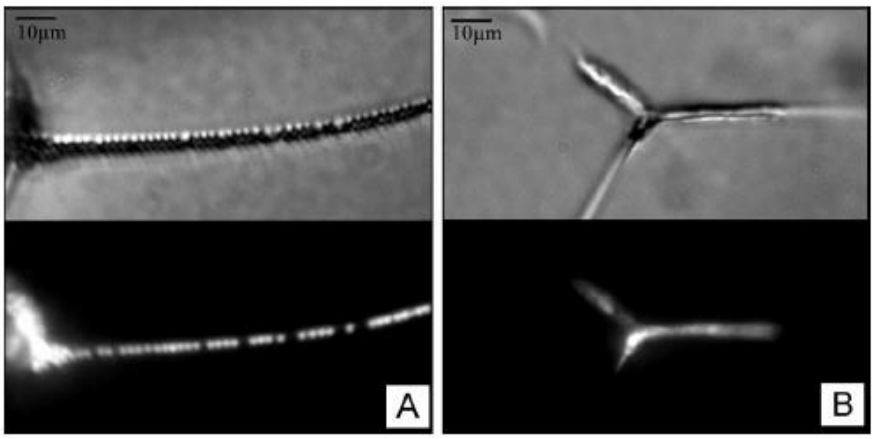

Figure 2.2. Light (top) and fluorescence (bottom) microscopic pictures of fluorescent beads (A) and stained bacteria (B) lined up along the water vein and into node in polycrystalline ice (from Mader et al. (2006)).

Despite the fact that no *in situ* activity has been measured to date, viable bacteria were retrieved from bulk ice. Total bacteria counts in bulk ice typically range from 10^2 to 10^3 cells ml^{-1}. Freezing results in concentration of solutes in the remaining liquid in veins by factor of 10^4 to 10^5. Similar partitioning of bacteria into veins would result in 10^6 - 10^8 cells ml^{-1} (Mader et al., 2006). The concentrated solution contained from 0.35 M of molecules at -1°C to 3.5 M in -10°C, providing a range of chemicals for bacterial metabolism (Mader et al., 2006; Barletta et al., 2012), but also requiring adaptation to high salinities. Bacterial activity at subfreezing temperatures was shown in the literature (Bakermans, 2008; Bakermans and Skidmore, 2011a, 2011b), as well as activity in high-ionic-strength solutions.

2.1.5.3. Supraglacial environments

Supraglacial environments consist of aquatic habitats of streams, lakes and distinct oases for life – cryoconite holes. Streams create a water drainage system and transport nutrients and microorganisms from glaciers into the ocean, connecting the cryosphere to global hydrological cycles (Vincent and Laybourn-Parry, 2009). Supraglacial lakes are similar to cryoconite holes (see Section 2.2), but they are usually bigger and have relatively less sediment in relation to water, as well as lower OM content (Vincent and Laybourn-Parry, 2009).

2.2. Cryoconite holes as hot spots for life in cold surroundings

Cryoconite holes were first described by Arctic explorer Nordenskjöld in 1870 during his expedition on the Greenland Ice Sheet. As the origin of sandy substance in the cylindrical holes on ice was enigmatic, Nordenskjöld named it Kryokonite, from Greek κρύος (krýos) – cold and κόνις (kónis) – powder, dust (Leslie, 1879). Although they were first observed in Greenland, cryoconite holes occur on the glacial surfaces all over the world, including the Antarctic, Arctic – (Svalbard and Greenland) and many temperate glaciers (reviewed in Cook et al., 2016a).

Cryoconite is a matrix of mineral particles and biological material deposited on glaciers by wind and meltwater, most likely of local origin (Porazinska et al., 2004). Having lower albedo than surrounding ice, it absorbs heat and melts downwards, creating a suitable habitat for microbial life in the supraglacial environment (McIntyre, 1984; Wharton, et al., 1985; Tranter et al., 2004; Cook et al., 2016a). The structure of cryoconite holes ensures that the organisms that inhabit them have access to liquid water throughout the ablation season (Fountain et al., 2004; Hodson et al., 2008) and ensures a relative high density of different life forms when compared to other supraglacial habitats (Edwards et al., 2011a). They are typically found in the lower part of the

glaciers, where ablation dominates, rather than upper part which accumulates snow (Porazinska et al., 2004).

'Cryoconite' in the literature refers either to dust particles on the surface; granules with inorganic and organic component; or cryoconite holes. Here, 'cryoconite' will be used as a term describing any dark-coloured material on the glacier surface melting into surrounding ice. This differs slightly to the definition found in the review by Cook et al. (2016a), where they refer to cryoconite as "discrete, aggregated granules of mineral and organic matter, either within cryoconite holes or elsewhere in the supraglacial zone". Antarctic cryoconite holes usually do not contain "discrete, aggregated granules", often the sediment layer is composed of fine-grained sediment, so this distinction from other dust particles on the glacier surface seems insufficient and may be misleading in the Antarctic conditions. Also, the cryoconite in the literature is mainly associated with the holes in the glacier and sometimes used interchangeably, which might add to the confusion. Therefore, it seems reasonable to only use the term 'cryoconite' in connection with the holes, not referring to other dust aggregates in the supraglacial environ, when taking into account the global picture.

Cryoconite holes all over the Earth possess certain similarities and differences. By far, the most important difference described is that cryoconite holes found in the Arctic (Greenland, Svalbard) and on temperate glaciers are predominantly open to the atmosphere during the summer season, allowing gas and water exchange with the surrounding environment. Antarctic holes, by contrast, are often covered with an ice lid, which might isolate the hole from the atmosphere and the surrounding drainage system for years (Tranter et al., 2004; Fountain et al., 2008)

2.2.1. Early research

Cryoconite holes were first noted in the literature in the expedition reports of Adolf Nordenskiold (1875) from Disko Bay, Greenland, and were named for 'ice dust'. The first recorded description of cryoconite from Antarctica was in Griffith Taylor's expedition reports from exploration of the Taylor Valley in 1910-13, where they noted that the dark sediment sunk into the ice (Taylor, 1916). The initial research on cryoconite material focused on its mineralogy and petrology (Leslie, 1879; Mineralogy and Petrography, 1891; Nordenskiöld, 1878; Von Drygalski, 1897). The first assessments of the Greenland cryoconite sediment described its sandy texture and debated the cosmic origin of the dust. Already Nordenskiöld noticed the aggregation of the dust into 'small, round balls'.

Von Drygalski's expedition provided the first systematic observations of cryoconite holes, with measurements taken in the same area in southwest Greenland over a two year period (Von Drygalski, 1897). Over a half of 205 measured holes showed an average width of 5 to 10 cm and depth of 40 to 60 cm. Von Drygalski stated that cryoconite holes were not transient and that the cryoconite material accumulated in the holes over the years. He was also the first to suggest that cryoconite holes can act as refugia for life during colder periods. This idea has developed into hypothesis of cryoconite holes serving as oases, particularly for green algae, during the Cryogenian glaciations known as Snowball Earth (Hoffman, 2016).

The biological studies and importance of cryoconite holes as a microhabitat was recognised by Steinböck (1936). Steinböck also hypothesised that cryoconite holes could act as a refugia for life during episodes of colder climates. These initial studies demonstrate the importance of the GIS in initiating an interest in the microbiology of glacial surface ecosystems. Since then many studies confirmed the importance of cryoconite as an ecological niche on glaciers (De Smet & Van Rompu, 1994; Porazinska et al., 2004; Wharton, et al., 1985; Wharton et al., 1981) as well as its contribution to melting (Gajda, 1958; McIntyre, 1984). Over time scientists described species present in cryoconite from different polar locations, reporting tardigrades and rotifers as the dominant grazers of microscopic algae and cyanobacteria (De Smet & Van Rompu, 1994; Mueller et al., 2001; Steinbock, 1936; Wharton et al., 1981). Similar studies are conducted today with the use of modern tools of molecular biology to identify not only macroinvertebrates and algae, but also bacteria and viruses.

Von Drygalski (1897) was the first one to suggest that cryoconite holes form by melting the dust into the ice. The thermodynamic processes in the holes were later modelled by McIntyre (1984) He estimated the contribution of biological activity to melting to be less than 10% on the basis of comparison of the holes in British Colombia with alive and killed organisms. Most authors conclude that microbial activity produces insignificant amount of heat and the melting properties of cryoconite are attributed to its dark colour (Cook et al., 2016; Musilova et al., 2017; Porazinska et al., 2004; Takeuchi et al., 2001).

2.2.2. Physical evolution of the hole

The size and rate of deepening of the holes depends on air temperature, solar radiation, sublimation rate of the ice surface, as well as snow cover and ice temperature (McIntyre, 1984; Fountain et al., 2008). The rate at which sediment melts into the ice decreases with the depth, as the solar radiation received is diminished by shading by the hole walls and angle of the sun light. Eventually it is coupled to the ablation rate of the glacier and reaches an 'equilibrium state'.

This equilibrium state was first described by McIntyre in 1984 as a steady state when the supply of solar radiation equals heat lost to the surrounding ice (Tranter et al., 2004). This term might be confusing, suggesting that a 'steady' cryoconite hole is not developing. The depth of the hole remains constant, but cryoconite is still melting into the ice, consequently changing its shape, hydrological connections and nutrient and water supply (Cook et al., 2015).

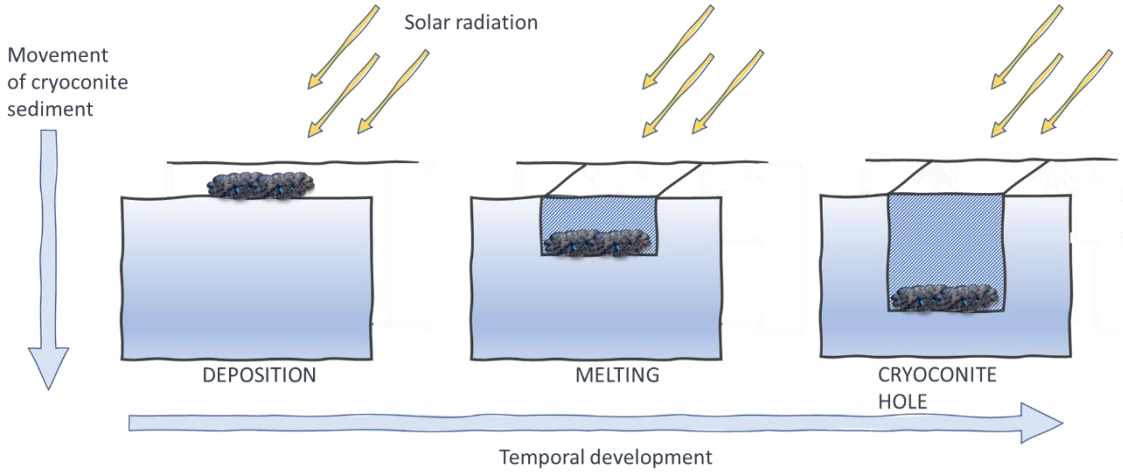

Figure 2.3. The temporal development of cryoconite hole (adapted from Bagshaw et al., 2007)

Most studies and models assume a cylindrical shape of the hole. This is true for the most cases; however, variations can be found. Antarctic holes from Taylor Valley are typically circular to elliptical (Porazinska et al., 2004). Arctic and temperate holes have a greater diversity and dynamics of shapes. Greenland holes often have a "D-shape" with flat edges facing north (McIntyre, 1984).

The cryoconite hole melts during the short summer season and is frozen during most of the year at the poles. Cryoconite holes worldwide have similar evolution, seasonal changes and biological processes (Fountain et al., 2004; Cook et al., 2016a), but do exhibit some physical differences depending on geographical location. Arctic and temperate holes melt every summer, becoming open to the atmosphere, exchanging gases and receiving dust input as well as being flushed with supraglacial streams and mixed. Because the air temperature remains below freezing during the short summer, holes in the Antarctic usually melt internally, but retain an ice lid on the top.

2.2.3. Antarctic cryoconite holes

Antarctic holes are described mainly from Taylor valley in the ecosystem of MCM Dry Valleys. The polar desert of the MCM Dry Valleys is an extreme environment with annual average temperature ranging between -16 °C and -21 °C, precipitation below 10 mm and coastal winds

of 4-5 m s^{-1}, reaching 20 m s^{-1} during winter months (Doran et al., 2002). Local temperature distribution and sediment transport is controlled by katabatic winds which occur primarily during winter months and reach 40 m s^{-1}. The bottom of the valleys are mostly covered by ice and snow-free soils. Despite harsh conditions, microorganisms can be found in soils, as well as in ephemeral streams. The glaciers and sparse, perennially frozen lakes also provide a range of icy environments for life. The glaciers are the primary source of water in an otherwise dry landscape (Porazinska et al., 2004; Tranter et al., 2004).

Cryoconite holes are common on the cold-based glaciers of the MCM, which have mean ice temperatures of approximately -18°C (Fountain et al., 2008). Water freezes in winter and remains frozen until the following summer, when solar energy transmits through the clear ice and heats up the dark sediment. Water in its liquid state might be present within the holes for a couple of weeks or months during austral summer. The holes usually remain isolated from the hydrological system of glacier by 30-40 cm thick ice lids (Fig. 2.4) for extended time periods, which is a unique and distinct feature of Antarctic holes (Tranter et al., 2004). Fountain et al. (2004) proposed a method to calculate the time since a hole was isolated, its "isolation age". This is determined from Cl^- concentrations in the holes. The method makes several assumptions: first, that the initial concentration of Cl^- results from ice melt and was equal to the average of glacier ice; second that the ice lid does not contain Cl^-; and finally that additional Cl^- results from ice melt. The average annual sublimation rate of 8 cm yr^{-1} is also taken into account (Tranter et al. 2004). The calculation of closure duration by Tranter et al. (2004), based on 15 holes on Canada Glacier, gave a range of 0 to 11 years. The isolation time calculated by Bagshaw et al. (2007) on the same glacier in 2006 for 33 ice-lidded holes, corrected for the Cl^- values found in ice lids, varied from 1 to 5 years. Authors argue that this is consistent with the warm summer of 2001. Ice lids were melted, and holes were flushed during exceptionally warm weather. The mean values for 3 Taylor Valley glaciers measured in 2006 range from 0.25 to 11 years (Stanish et al., 2013). To sum up, isolation ages show great variability, but at least part of the glacier holes remain isolated from the surrounding drainage system for multiple years, which may result in the development of unique chemistry and biotic communities.

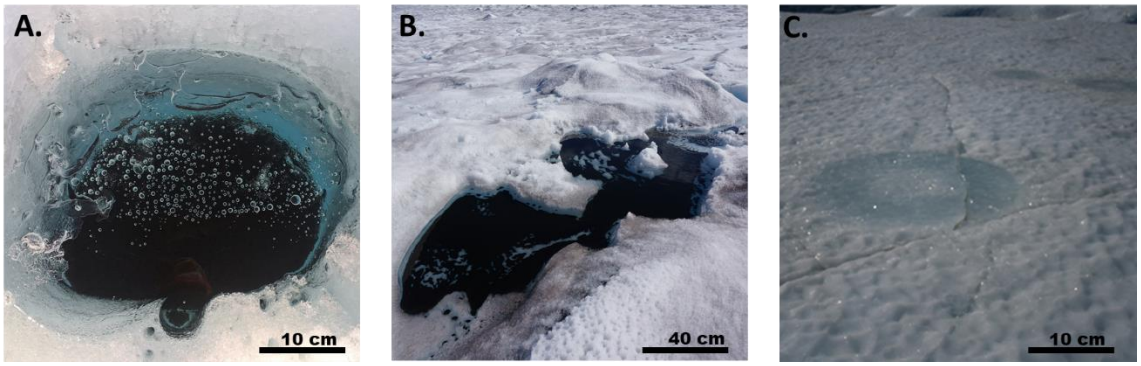

Figure 2.4. Cryoconite holes from Greenland and Antarctica. *A. A typical, temporarily ice-lidded hole (Greenland). B. A thick layer of cryoconite material in the merged holes (Greenland). C. Surface view of an ice-lidded isolated hole (Antarctica, courtesy of Dr Liz Bagshaw).*

The long-term entombment leads to unusual chemistry of water in the holes. The concentrations of major nutrients and ions measured on Canada Glacier differ from the surrounding glacier ice. The holes are enriched in Ca^{2+}, dissolved inorganic carbon (DIC), SO_4^{2-} and Si since dissolution of the dust causes elevated concentrations of major ions (Lyons et al., 2003; Tranter et al., 2004). They are depleted in NO_3^-, NH_4^+, PO_4^{3-}. The pH values average 9.6, ranging from ~ 6.7 to 11. The increasing pH generates low pCO_2 values and oxygen saturation reaches extreme values of ~160% (Tranter et al., 2004). The exceptional alkaline pH values may result from hydrolysis of $CaCO_3$, photosynthesis and freezing. Stable alkaline environments are uncommon and depend on poor buffering capacity, which in this case may be caused by isolating the environment from atmospheric CO_2, meaning that the cryoconite water is unable to degas. Very high pH values in aquatic ecosystems were mainly reported in African soda lakes (Grant and Jones, 2000), several other lakes throughout the world (for example Lake Texcoco in Mexico (Fernandez-Buces et al., 2006)), the perennially ice-covered Lake Untersee in interior Antarctica (Wand et al., 1997) or Sonic Lake in East Antarctica (Sattler and Storrie-Lombardi, 2009).

There are active microbial communities in the MCM DV cryoconite holes. The ratio of DIC to dissolved organic carbon (DOC) measured in the holes was ~ 2:1 (Tranter et al., 2004). It has been suggested that the DOC might be maintaining the DIC pool by inhibiting $CaCO_3$ precipitation, linking decomposition with the potential for photosynthesis (Tranter et al., 2004; Bagshaw et al., 2016a). The decomposition of organic matter occurs via heterotrophs, and although the relationships between photosynthesis, microbial decomposition, DOC, DIC and pH are complex, even in this simple ecosystem (Tranter et al. 2004), the heterotrophs seem crucial for the maintenance of biogeochemical processes. The net ecosystem production (NEP) of cryoconite holes ecosystem is still poorly understood. Studies show differences between Arctic and

Antarctic holes, with much higher rates of production in Arctic (Anesio et al., 2009; Telling et al., 2012a). A long-term study by Bagshaw et al. (2016a) showed that the system is net autotrophic after the net heterotrophic period of 40 days for Antarctic cryoconite incubations.

Phosphorus and nitrogen species concentrations are lower than in surrounding ice, implying biological uptake (Tranter et al., 2004). Particulate organic matter is suggested as a source of the DOC and dissolved organic nitrogen in the holes, as their ratio of ~0.09 is consistent with values measured in other water bodies (Tranter et al., 2004). Thus, the role of decomposition by heterotrophs is further highlighted. The dissolution of rocks and ice to obtain nutrients and substrates is suggested by Tranter et al. (2004), together with necessary recycling to allow biological activity to thrive. The abiotic generation of hydrogen from crushed rocks was showed to provide sufficient energy to support chemolithotrophic microbial activity in subglacial environments (Telling et al., 2015; Macdonald et al., 2018). However, the complex linkages between photosynthetic and heterotrophic bacteria, as well as ongoing chemical and psychical processes in cryoconite holes, are yet to be determined.

2.2.4. Arctic cryoconite holes

Arctic cryoconite holes differ from Antarctic ones in aspects of biological, physical and chemical conditions. The most notable difference is the lack of ice lid and regular hydrological connections between the holes and the supraglacial drainage system (Fig. 2.4), which results in the cryoconite community and structure being influenced by surface hydrology. The recently published review on cryoconite by Cook et al. (2016a) concentrates on Arctic in summer, briefly mentioning Antarctic and temperate ones. The article contains a comprehensive summary of the history of cryoconite discovery, its formation, biology and geochemistry with regard to Arctic settings. Drawing from this work the key features of cryoconite material and holes can be summarized:

Cook et al. (2016a) defines cryoconite as “discrete, aggregated granules of mineral and organic matter, either within cryoconite holes or elsewhere in the supraglacial zone”. The inorganic components consist of mineral particles, probably depending on local source, as well as on the distant sources (i.e. high atmospheric suspension). It may also contain black carbon – one of the drivers of darkening of Greenland Ice Sheet. Another factor contributing to lowering the albedo is production and accumulation of organic matter in cryoconite material (Musilova et al., 2016). Organic matter content varies greatly across cryoconite samples worldwide, ranging 1.8 % to 18.3 % (Cook et al., 2016a). It has a strong correlation with the granule size, possibly due to humic substances acting as an adhesive, but also being a proxy for biota abundance. The growth of filamentous cyanobacteria is believed to be crucial for granule growth and integrity. Continuous

hydraulic redistribution of granules over glacier surfaces allows even distribution of cyanobacteria on the surface and a round shape. Photoautotrophs, such as cyanobacteria, dominate on the surface, where the light is available, and the interior is composed of heterotrophs and minerals, giving the distinctive microstructures (Takeuchi et al., 2001; Langford et al., 2010). The processes controlling granule shape and size are not well understood.

The process of forming the hole is identical to that occurring in Antarctic conditions (Fig. 2.3). The cylindrical shape is common, however Cook et al. (2016a) underline the importance of more complex shapes reflecting geophysical dynamics and mostly ignored horizontal development of the holes (Cook et al., 2015). Also, a deeper understanding of cryoconite hole thermodynamics, commenced by McIntyre in 1984, is required for developing a detailed model of hole evolution. Especially striking is the lack of detailed studies on the hole initiation.

In Arctic cryoconite holes, microorganisms were found both in the granules and overlying water. The abundance of bacteria in the granules can be up to 300 times higher than in water, but the water microorganisms allow easier mixing and redistribution through supraglacial hydrological connections. Primary production is often dominated by cyanobacteria and their abundance ranges from 0.25 to 0.8 x 10^9 cell g^{-1} (Cook et al., 2016a). Heterotrophs in the same study in Svalbard ranged 10 – 50 x 10^9 g^{-1}, exceeding the phototrophs numbers. Heterotrophs, dominated by bacteria, are believed to metabolise autochthonous and allochthonous OM allowing recycling of nutrients within the holes. Viruses are concluded to be another important control of recycling, causing the lysis of bacteria (Bellas et al., 2013). Their abundance in the sediment may reach 20 x 10^6 ml^{-1} (Anesio et al., 2007).

2.2.5. Antarctic vs Arctic

The cryoconite holes from polar regions differ between northern and southern hemisphere (Fig. 2.4). The crucial difference is the presence of ice lids in the Antarctic ones, leading to prolonged isolation times. Arctic cryoconite holes, by contrast, do not exhibit a multiyear ice lid but rather form an ice lid on cold nights which then melts during the day. Not only do the lids isolate Antarctic holes from atmosphere, but also around half of the holes are not connected to the supraglacial hydrological system, limiting the gases, solutes and biota exchange with the surroundings. The mixing occurs on an approximately decadal scale during exceptionally warm summers, when excessive melting occurs. It also seems likely that entombment would promote higher endemism and differences between adjacent holes, which was suggested by Porazinska et al. (2004). In contrast, cryoconite holes and aggregates on high Arctic glaciers and ice sheets are continuously redistributed and washed into streams during the summer season, likely leading

to the higher evenness of the microbial communities and chemistry of the holes. For example, values in Arctic holes do not reach the extremes measured in the Antarctic (Table 2.2).

Table 2.2. pH in cryoconite holes in the literature.

Region	Location	Range	Mean	Samples	References
Alps	Austria	5	n.a.	3	(Margesin et al., 2002)
Antarctica	McMurdo Dry Valleys	~6-8	n.a.	n.a.	(Porazinska et al., 2004)
Antarctica	Canada Glacier	~6.5-11	9.6 ± 1.2	46	(Tranter et al., 2004)
Antarctica	Canada Glacier (frozen holes)	n.a.	6.5 ± 0.47	89	(Bagshaw et al., 2007)
Antarctica	Canada Glacier (wet holes)	n.a.	7.22 ± 0.98	35	(Bagshaw et al., 2007)
Antarctica	Commonwealth Glacier	n.a.	7.02 ± 1.06	10	(Stanish et al., 2013)
Antarctica	Canada Glacier	n.a.	6.89 ± 1.3	8	(Stanish et al., 2013)
Antarctica	Taylor Glacier	n.a.	6.17 ± 0.64	8	(Stanish et al., 2013)
Svalbard	Midtre Lovénbreen	~4.7-7.3	~6	n.a.	(Kastovská et al., 2005)
Svalbard	Midtre Lovénbreen	7.1–8.6	8.2 ± 0.7	4	(Singh and Singh, 2012)
Svalbard	n.a.	n.a.	5.6 ± 0.1	n.a.	Arwyn Edwards, unpublished
Greenland	n.a.	n.a.	5.77 ± 0.38	n.a.	Marek Stibal, unpublished
Greenland	Leverett glacier	5.95-6.7	6.39 ± 0.34	n.a.	David Chandler, 2012, unpublished
Greenland	Black and Bloom camp	n.a.	5.14 ± 0.49	6	This study

***** n.a. – *data not available*

The differences in hydrological stresses influences cryoconite morphology. Although some microorganisms that are able to excrete 'sticky' extracellular polymeric substances (EPS) and create biofilms have been detected, the Antarctic fine-grained debris does not form granules (Bagshaw et al., 2016b). The sediment in the Arctic cryoconite is exposed to constant redistribution by meltwater (Irvine-Fynn et al., 2011), which promotes the formation of distinct granules: clusters of sediment particles and microorganisms, bound together by EPS excreted by the microbes (Langford et al., 2010). The persistence of the debris on the glacier surface is

presently unknown, but the granules promote longevity and enable the microbial community to remain intact despite redistribution (Langford et al., 2010; Bagshaw et al., 2016b).

The average value of cryoconite holes net biological production still remains ambiguous, especially in Antarctica. Multiple small scale studies were performed during the summer months and upscaled to the entire glacier, mostly in the Arctic (Stibal et al., 2008; Anesio et al., 2010; Cook et al., 2012; Telling et al., 2012a; Bagshaw et al., 2016a). However, it is hard to give a definite answer from such snapshots and a comprehensive study, upscaled with remote sensing and modelling, is still needed. So far, the question whether glacial biome captures or releases carbon remains unanswered.

The source of biological propagules in both Arctic and Antarctic cryoconite holes is also poorly understood, but there seems to be consensus that they are largely airborne, coming mostly from either local or globally distributed destinations (Porazinska et al., 2004; Darcy et al., 2011). Some microorganisms were reported as unique, but the others were found on both hemispheres (Mueller et al., 2001; Darcy et al., 2018). Geographically distant cryoconite holes had harboured significantly different microbial communities and they were more different with increasing distance (Darcy et al., 2018). Despite the differences in the community structure as well as the geochemical conditions in the holes, the abundance of microorganisms measured in various location was broadly similar in both polar regions (Table 2.3).

Table 2.3. Abundance of bacteria in cryoconite sediments and water in the literature.

Region	Location	Cell abundance range	References
Cryoconite (cells per g of wet sediment)			**sediment**
Svalbard	Midre Lovénbreen, Austre Brøggerbreen, Vestre Brøggerbreen	0.51 - 1.33 × 10^6	(Singh et al., 2014b)
Svalbard	Midre Lovénbreen, Austre Brøggerbreen	0.29 - 2.93 × 10^9	(Anesio et al., 2010)
Svalbard	Werenskioldbreen	10 – 50 × 10^6	(Cook et al., 2016a)
Svalbard	Hansbreen, Werenskioldbreen, Nannbreen, Austre Torellbreen	34.9±24.1 x 10^7 (dry sediment)	(Stibal et al., 2006)
Svalbard	Midre Lovénbreen, Austre Brøggerbreen, Vestre Brøggerbreen	538 – 7525 x 10^{11}	(Kastovská et al., 2005)

Region	Location	Cell abundance range	References
Antarctica	Patriot Hills and McMurdo Dry Valleys	0.02 - 0.29 × 10^9	(Anesio et al., 2010)
Antarctica	McMurdo Dry Valleys (Canada and Hughes glaciers)	~5-20 x10^4 ml^{-1}	(Foreman et al., 2007)
Antarctica	McMurdo Dry Valleys (Canada Glacier)	7.03 × 10^8 ± 1.39 × 10^8	(Telling et al., 2014)
Greenland	Kangerlussuaq area and Kronpris Christians Land	2.08 ± 1.83 - 10.05 ± 3.87 x 10^6	(Hodson et al., 2010)
Greenland	Leverett Glacier	3.6 ± 0.96 - $4.0 \pm 1.0 \times 10^7$	(Musilova et al., 2015)
Austria	Rotmoosferner and Stubacher Sonnblickkees	0.002 - 2.95 × 10^9	(Anesio et al., 2010)
Cryoconite water (cells per ml of cryoconite water)			
Svalbard	Midre Lovénbreen	4.67 - 7.07 ×10^4	(Säwström et al., 2002)
Svalbard	Midre Lovénbreen and Austre Brøggerbreen	2.15 – 6.99 × 10^4	(Anesio et al., 2010)
Antarctica	Patriot Hills and McMurdo Dry Valleys	0.45 - 7.94 × 10^4	(Anesio et al., 2010)
coastal Antarctica,	Larsemann Hills, Amery Ice Shelf and central Dronning Maud Land	0.07 - 11.8 × 10^4	(Sanyal et al., 2018)
Antarctica	McMurdo Dry Valleys (Canada and Hughes glaciers)	~1 – 7 x10^4	(Foreman et al., 2007)
Austria	Rotmoosferner and Stubacher Sonnblickkees	0.77 - 10.06 × 10^4	(Anesio et al., 2010)

2.2.6. Temperate cryoconite holes

Most studies on cryoconite holes concentrate on polar regions. As a consequence, data on low-latitude mountain glaciers is scarce. This trend is surprising as the mountain glaciers were shown to be sensitive to albedo changes and important for water security (Cook et al., 2016a). This may be because polar regions include the majority of ice cover on the Earth and therefore the influence of climate change on temperate glaciers may appear to be of smaller importance.

In general, temperate glaciers are relatively small, fast flowing and exhibit significant diurnal changes in energy balance (Cook et al., 2016a). Variations in day-night temperatures, cloud cover and other atmospheric conditions cause complex melt dynamics, which means the role of cryoconite holes in physical and biogeochemical dynamics seems to be harder to asses than in polar regions. Not only do meteorological and glaciological conditions differ greatly from higher latitudes, but there is also a bigger input of debris and solutes from nearby sources. In consequence, rates of microbial production and area coverage by cryoconite holes is higher, but their life span is shorter due to rapid melting (Cook et al., 2016a). They are strongly influenced by the surrounding environment, hence may be directly impacted by anthropogenic pollution (Lee et al., 2011).

Cryoconite holes on Alpine glaciers are not visible until the snow layer thaws in the summer (Margesin et al., 2002; Pittino et al., 2018). The holes sometimes persist over one summer season, but new holes are constantly being formed and washed away (Franzetti et al., 2017; Pittino et al., 2018). The typical temperature within the holes in the summer is 2 °C and usually the holes freeze every night. Very few measurements of pH were published and showed that the holes were slightly acidic (pH 5 in all three holes studied by Margesin et al., 2002). Total C was 0.8-1.8% of dry mass and total N (dry combustion) 0.02-0.09%, thus the C:N ratio ranged 20:1 to 40:1, which was concluded to be high by Margesin et al. (2002). Alpine glacier surfaces are generally in closer proximity to anthropogenic pollution sources and organic debris, such as pollen, plant or animal litter than polar glaciers. Those can be blown onto glacier surface from surrounding subalpine and alpine zones and serve as an additional source of OM.

Margesin et al. (2002) were the first to analyze the heterotrophs of cryoconites. They isolated heterotrophic aerobic bacteria, yeasts and fungi – hyphnomycetes. Bacteria were dominant over the other groups, with a major proportion of Gram-negative ones. Amongst them genera *Pseudomonas* and *Sphingomonas* were the most numerous. It was concluded that only few taxa are able to cope with extreme, cold conditions. Interestingly it was noted that even in permanently cold environments around 50% of bacteria are not psychrophilic. Today, thanks to molecular methods, we know that the number of taxa and biodiversity existing in cold environments is larger than it was thought in 2002, but still particular groups are believed to be better adapted to extremes. Several groups of bacteria (Cyanobacteria, Bacteroidetes and Proteobacteria) remained dominant over the few years on Alpine glaciers, but the overall structure of the community showed year-to-year variability (Franzetti et al., 2017; Pittino et al., 2018).

2.2.7. Biogeochemistry of cryoconite holes

Products of cryoconite holes metabolism are not only exported via melt water downstream, but they are also recycled within the supraglacial environments and delivered to glacial bed, where they undergo further transformations. Understanding of these biogeochemical interactions has progressed significantly in recent years, yet they are still avenues to be explored.

2.2.7.1. Carbon flux

The importance of glacial biogeochemical processes for global carbon cycle has long been recognised (Smith et al., 2017; Stibal et al., 2012). Glacial microorganisms cycle carbon and nutrients such as phosphorus and nitrogen, particularly in their dissolved organic forms (Holland et al., 2019). The rates of carbon flux on glacial surfaces reported worldwide vary from net respiration of 3 kg C km^{-2} d^{-1} (southwest Greenland) to net carbon production 9 kg C km^{-2} d^{-1} (southwest Greenland, Svalbard) across the globe(Smith et al., 2017; Stibal et al., 2012). The fate of glacier surface carbon can make a significant and detectable contribution to glacier runoff (Lawson et al., 2014). Glacial runoff in turn exports significant amounts of labile carbon to downstream ecosystems (Bhatia et al., 2013; Hood et al., 2009, 2015; Lawson et al., 2014), such as low carbon deglaciated soils (Foreman et al., 2007) or marine environments (Hood et al., 2009). For example, runoff from Alaskan glaciers provides the Gulf of Alaska around 0.13 x 10^9 kg of DOC per year, and most of this organic carbon is readily available for microorganisms (Hood et al., 2009). Globally, total organic carbon export form glaciers equals around 3 Tg per year, with DOC averaging 1.04 ± 0.18 Tg C per year (Hood et al., 2015). The biggest contribution comes from mountain glaciers (including those in Greenland and Antarctica), followed by Greenland Ice Sheet and Antarctic Ice Sheet. Ongoing glacier loss associated with climate changes will result in estimated increase in glacial export of DOC of 13% (Hood et al., 2015).

The highest microbial activity and carbon cycling on glacial surfaces is associated with cryoconite holes (Anesio et al., 2009; Cook et al., 2012; Stibal et al., 2012)**,** and recently algal blooms on ice surface have also been recognised as important contributors to DOC production and export (Holland et al., 2019; Williamson et al., 2020). Photosynthesis is believed to dominate on the surface of ice and in thin cryoconite layers, whereas thicker layers of cryoconite are believed to be net heterotrophic (Telling et al., 2012). Within these thicker layers, both aerobic and anaerobic processes occur, yet no data are available for the rates carbon flux within cryoconite material. Bacterial production in cryoconite holes is comparable to estimates from other polar soils and fresh water habitats. It is estimated to be much lower than primary production and

constitutes ~3.5% of the gross primary production in Antarctic, Arctic and Alpine locations (Anesio et al., 2010). This suggests that autochthonous carbon can accumulate in cryoconite material over time and be released during large melt events. An early study of cryoconite holes from Svalbard, Greenland and the European Alps demonstrated that the surface of glaciers were likely a net autotrophic ecosystems (Anesio et al., 2009), which is in contrast with other cold freshwater ecosystems. Other studies suggest that respiration exceeds primary production in cryoconite holes on GrIS and Svalbard (Hodson et al., 2007, 2010) and the debate whether cryoconite holes demonstrate net respiration or production is still ongoing. It is likely that the NEP status of cryoconite holes depends on the time of the season and they likely become net heterotrophic in the late summer (Hodson et al., 2010).

Cryoconite holes are also a place of microbial modification and decomposition of organic matter, as evidenced by lack of correlation between photosynthetic production and concentrations of non-carbohydrate low molecular weight compounds (Musilova et al., 2017). Cryoconite microorganisms are likely transforming both allochthonous and autochthonous OM. One type of those uncorrelated compounds were volatile fatty acids (VFAs), which can be produced through anaerobic degradation of OM. VFAs constitute around 7.8% of DOC (~13.5 ±1.8 μg C L^{-1}) exported via runoff on Leverett Glacier in Greenland (Musilova et al., 2017).There are some reports suggesting that the deposition of organic carbon on some glaciers can exceed export (Koziol et al., 2019; Stibal et al., 2008). For example on small Foxfonna glacier in Svalbard atmospheric organic C input (averaging 0.40 ± 0.22 Mg a^{-1} of DOC) exceeded organic C export in runoff (0.36 ± 0.03 Mg a^{-1} DOC) and contribution of biological production in cryoconite holes to the export was deemed negligible (Koziol et al., 2019). By contrast, most of the bioavailable supraglacial DOC on GrIS was produced *in situ* by microbial activity, mostly associated with cryoconite holes (Lawson et al., 2014; Musilova et al., 2017). Cryoconite holes were estimated to fix as much as 64 Gg of carbon per year, roughly 10% of which is released downstream as DOC (Anesio et al., 2009). Extrapolated data from the stream export in Antarctica, suggest that export of DOC from Antarctic Ice Sheet equals around 0.69 Gg C per year, but these data need further refinement (Smith et al., 2017). Overall, snapshot studies and modelling indicate that cryoconite holes are an important component of carbon fluxes on glaciers, but the estimates so far are imprecise and depends on geographical settings.

2.2.7.2. Nutrients

Cryoconite holes are regarded as hotspots of biogeochemical processes on glacier surfaces. Because available inorganic nutrients are rapidly scavenged, organic phases must be

decomposed to access bound N and P, likely by heterotrophs. Phosphorus is believed to be limiting in supraglacial environments. Cryoconite holes tend to have low dissolved P (Stibal et al., 2009; Bagshaw et al., 2013; Telling et al., 2014; Hawkings et al., 2016), but the sediment contains appreciable stores, in some cases even higher than that in the surrounding rocks (Stibal et al., 2009), although much of this remains unavailable to microorganisms on biological timescales. Phosphorus in cryoconite holes is speculated to partly come from dissolution of mineral particles (inorganic P) and partly from aeolian transport (organic P). Recycling of organic phases was indicated by phosphatase activity detection in Svalbard (Stibal et al., 2009), suggesting adaptation to low P environment. Such activity was also detected in the Antarctic (Foreman et al., 2007). The relatively high content of P when compared to the surrounding ice means that cryoconite holes can serve as a potential source for ecosystems downstream (Stibal et al., 2009; Telling et al., 2014).

Nitrogen is mainly provided by deposition within snow and rain. However, N fixation was detected in cryoconite holes from Svalbard, Antarctica and the marginal zone of Greenland Ice Sheet, and is believed to contribute to N cycling when there is no atmospheric precipitation in the melt season (Telling et al., 2011, 2012b, 2014; Anesio and Laybourn-Parry, 2012). It was also suggested that remineralization of organic matter in the holes could provide inorganic N. Another source of N is anthropogenic pollution, which has been increasing in industrial times and could reduce the nitrification abilities of supraglacial microorganisms (Telling et al., 2012b). Similarly to P, N from cryoconite holes may be flushed out and support downstream environments.

2.2.7.3. Pollutants

Bacteria from cryoconite holes are capable of degrading certain pollutants. Although glaciers and polar regions are generally perceived as the pristine environments, recent findings suggest that they are not as isolated from anthropogenic influence as once thought. In Greenland, contaminants such as heavy metals (mercury and lead), dichloro-diphenyl-trichloroethan (DDT), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCB) have been detected (Masclet et al., 2000; Grannas et al., 2013; Odland et al., 2016). Atmospheric deposition of pollutants onto snow and ice exposes cryospheric microorganisms to toxic chemicals and increases potential for resistance to and degradation of contaminants (Hauptmann et al., 2017). It has been observed that cultured heterotrophs from an Alpine glacier were able to use diesel oil and aromatic hydrocarbons, such as PCBs, as a sole carbon source (Margesin et al., 2002). Another common pesticide which accumulates on glaciers (chlorpyrifos) was biodegraded by Alpine cryoconite communities in the microcosm experiment (Ferrario et al., 2017). This could

be explained by the proximity of anthropogenic sources of pollution, contrary to polar regions. Metabolomic data from Greenland Ice Sheet (GrIS) indicated decomposition of a herbicide (2,4-dichlorophenoxyacetic acid) (Cook et al., 2016b) and a metagenomic study detected genes responsible for heavy metal resistance (Hauptmann et al., 2017). Moreover, genomes of organisms inhabiting cryoconite holes likely possesses similar decomposition potential as microbial genomes from other contaminated habitats.

Such data indicate the exposure of glacial microorganisms to contaminants, and the development of decomposition potential, are an important sign of a pollution presence in the polar regions, and a warning of potential future release of chemicals into downstream ecosystems with increasing glacial melt. However, they may also serve as a novel pool of organisms with the potential for bioremediation, and applications in biotechnology.

2.2.7.4. Organic matter production and respiration

All living organisms gain the energy for their life functions from cellular respiration. In oxic conditions, respiration results in transfer of electrons from reduced organic matter such as glucose to oxygen. Organic matter produced by phototrophs such as cyanobacteria is subsequently utilised by heterotrophic bacteria (Fallowfield and Daft, 1988; Thomas, 1997; Abed, 2010; Pannard et al., 2016; Tessarolli et al., 2017). Cyanobacterial exudates typically consist of low molecular weight compounds and exopolymers (EPS) including lipids, proteins, nucleic acids and other long polymers (Abed, 2010; Pannard et al., 2016). Polysaccharides are the most prominent EPS compounds and include labile monomers such as glucose, galactose, mannose or arabinose (Abed, 2010; Decho and Gutierrez, 2017). Cyanobacteria are also capable of fermentation in the dark anoxic conditions and acetate, propionate, lactate and ethanol were reported as their fermentation products (Abed, 2010). Cyanobacteria are commonly found in cryoconite holes, although no studies on their fermentative abilities are available to date. They are known to promote aggregation of cryoconite granules by excreting EPS, one example being *Leptolyngbya* sp. and *Phormidium* sp. species found on Svalbard glaciers (Langford, 2012). EPS could serve as a source of organic matter for fermentation, as heterotrophic bacteria are known to be involved in degradation of EPS (Passow, 2002). Cyanobacteria are typically associated with a diverse community of heterotrophic bacteria embedded in the EPS matrix surrounding the cells, with which they often form a mutualistic relationship (Pannard et al., 2016).

The quantity and quality of exudates such as EPS is highly dependent on nutrient availability. Nutrient limitation and subsequent carbon excess obtained by photosynthesis promotes the overflow of carbon-rich EPS together with storage of intracellular of reserve compounds

(Pannard et al., 2016). The microscale distribution of bacteria and particulate organic nutrients is suggested to influence the rates and type of microbial activity (Azam et al., 1994). Organic carbon influx to heterotrophic bacteria from phototrophs is dynamic and highly variable. Such microscale variations can be especially pronounced in the granular structure of cryoconite sediment (Langford et al., 2010; Uetake et al., 2016). This may also enhance the variation in nutrient availability and augment the role of heterotrophic bacteria in remineralisation.

2.2.7.5. Oxygen

Cryoconite holes are considered to be well-oxygenated habitats (Christner et al., 2003a; Stibal and Tranter, 2007). Oxygen saturation in the water column of Antarctic cryoconite holes ranged from 70 to 160% in MCM Dry Valleys, demonstrating that ice-lidded cryoconite holes could be oversaturated with oxygen in the austral summer (Tranter et al., 2004). The oversaturation is associated with net photosynthesis in some holes and isolation from the atmosphere by the ice lid. Lower oxygen concentrations therefore are likely a result of net heterotrophy in other holes. These first recorded measurements of dissolved oxygen in the cryoconite holes relied on discrete manual sampling, with samples transported to a nearby laboratory to perform Winkler titration (Tranter et al., 2004).

In situ monitoring of oxygen dynamics in a hydrologically connected hole was deployed on Canada Glacier in MCM Dry Valleys with the use of fibre optic minisensors (Bagshaw et al., 2011). Over the four-week measurements of the water, oxygen saturation remained in the range of 50 to 80% (Bagshaw et al., 2011). The undersaturated waters suggest that respiration exceeded photosynthesis. Biological activity in cryoconite holes is associated with the sediment layer (Anesio et al., 2010). Despite this fact, and the evidence that the oxygen is being used up in the cryoconite holes, there are no documented reports on oxygen status of the sediment from Antarctica or elsewhere. Similarly, there is no published record of analysis of oxygen concentrations anywhere but in MCM Dry Valleys.

Although no direct measurements were available, the occurrence of anoxic zones in the cryoconite granules in the Arctic was speculated (Uetake et al., 2016). Segawa et al. (2014) noticed some similarities between cryoconite granules and granules from wastewater treatment. As the centre of such granules is usually anoxic, and they detected genomic transcripts for denitrification, it was an indication that cryoconite granules could also have an anoxic centre. Detailed studies of the structure of cryoconite granules demonstrated that cyanobacteria create a dense biofilm on the granules surface which can restrict oxygen exchange (Takeuchi et al., 2001; Langford et al., 2010). Cryoconite sediments thicker than average could also harbour

potentially anoxic niches (Bagshaw et al., 2007; Telling et al., 2012a), depending on the rates of oxygen being used up and its diffusion through the sediment layers.

Anaerobic metabolism yields less energy than aerobic (Maier and Pepper, 2015), but indirectly leads to additional oxygen consumption through oxidation of reduced substances from anaerobic decay. It also removes nutrients by denitrification, contributes to remineralisation of organic carbon, and changes the alkalinity of the sediment (Werner et al., 2006; Hu and Cai, 2011). Anaerobic microorganisms were detected in the cryoconite holes from Ecology Glacier in Antarctica, but there were no corresponding oxygen measurements (Zdanowski et al., 2017). The availability of oxygen determines the type of biogeochemical reactions that may occur within the sediment, yet even basic measurements of oxygen within sediment layers are as yet absent from studies of cryoconite.

2.2.7.6. Anaerobic metabolism

In anaerobic conditions, there are a variety of pathways leading from organic matter to oxidised inorganic species such as nitrate, sulphate, and ferric iron or organic compounds such as acetyl-phosphate, pyruvate etc. End products of oxic respiration include water and carbon dioxide, whereas anaerobic respiration can produce a variety of reduced inorganic species depending on environmental availability (hydrogen sulphide, nitrogen, reduced iron), simple organic compounds, such as ethanol, acetate, lactate, methane etc. and carbon dioxide or bicarbonate.

Acetate is a common anaerobic metabolite in the microbial world (Wolfe, 2005). It is mostly produced by fermentation in the process of incomplete oxidation of glucose (Wüst et al., 2009) (Equation 1).

$$C_6H_{12}O_6 + 4\ H_2O \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + 4\ H^+ + 4\ H_2 \qquad (1)$$

Various facultative anaerobes and fermenters are capable of synthetizing acetate (Wolfe, 2005). Anaerobic decomposition of organic matter requires interactions of diverse groups of bacteria (Fig. 2.5). Complex organic matter is decomposed to dihydrogen, carbon dioxide or bicarbonate and volatile carboxylic acids, mostly formate and acetate. Higher volatile carboxylic acids are further oxidised by acetogens to acetate and hydrogen or formate. A phylogenetically diverse group of microorganisms is capable of anaerobic respiration and generation of acetate from carbon dioxide and an electron source (H_2, CO, formate etc.) in the process of acetogenesis. In the final step methanogenic microorganisms utilise acetate, and hydrogen or formate resulting in production of methane (Ferry, 1992). Therefore acetate is of particular importance when

compared to other products of fermentation, as it is commonly further used by methanogens to produce methane.

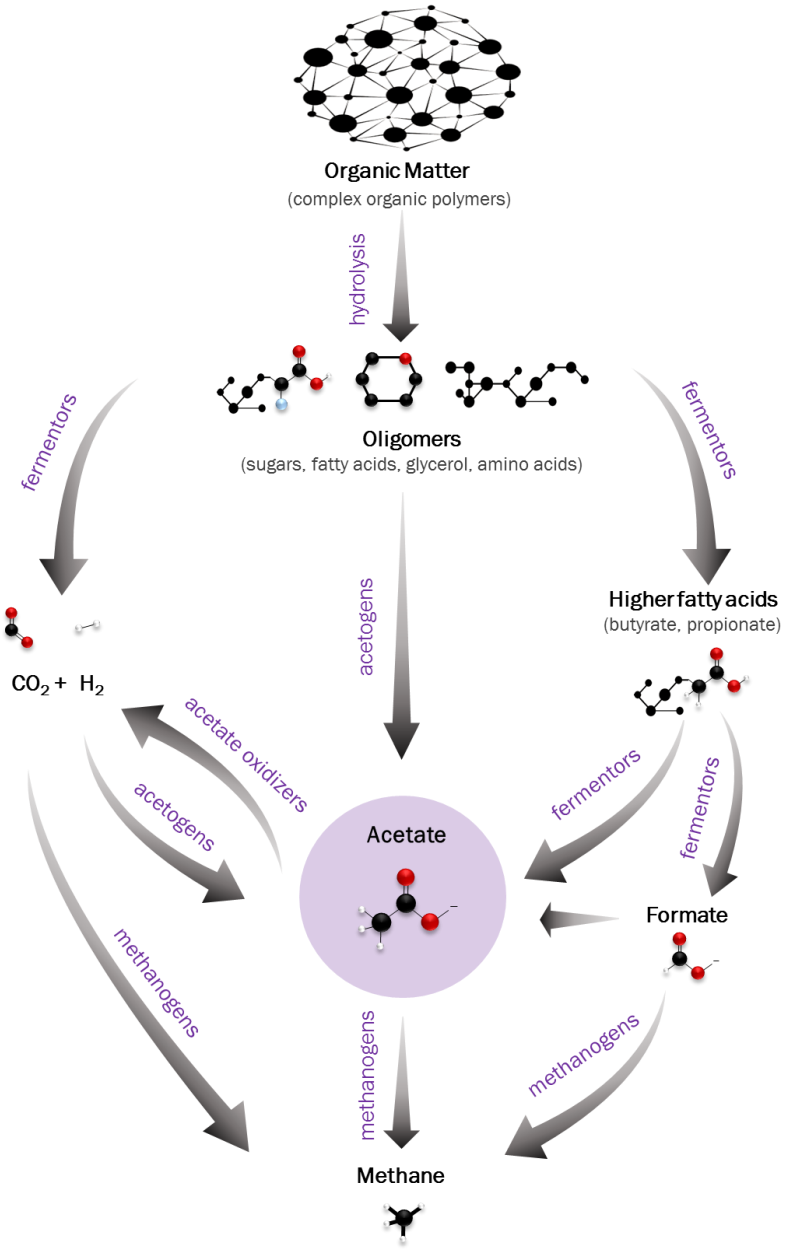

***Figure 2.5. Degradation of organic matter in anoxic conditions**. Initially, complex organic polymers are hydrolysed by excreted enzymes to smaller compounds, which are subsequently fermented, mainly to volatile fatty acids and hydrogen. The most important intermediate is acetate, which can be oxidised to carbon dioxide and hydrogen, which is further utilised in methanogenesis or it undergoes direct methanogenesis.*

Commonly, in anoxic environments such as peatlands, marine sediments or subglacial sediments, acetate is a substrate for methanogenesis (Angle et al., 2017). Methane synthesis is inhibited in the aerobic conditions and with methanogens being sensitive to the oxic conditions, it is generally accepted that methanogenesis is limited to anoxic habitats. This paradigm was recently

shifted as an Archaea capable of efficient methane synthesis in the oxygen-rich soils was found (Angle et al., 2017). However, its methanogenic activity was likely limited to anoxic microniches within soil, as no special oxygen adaptations have been found. Nevertheless, methanogenesis remains the final step of anaerobic decomposition of organic matter (Fig 2.5).

Acetate is a part of major metabolic pathways in the cell. It is an intermediate in the glucose metabolism (Pinhal et al., 2019). In its protonated form it can freely diffuse through the cell membrane. Consequently, an increased concentration outside the cell can influence the internal pool of anions and proton balance through diffusion and following release of protons intracellularly. Excess intracellular acetate inhibits certain metabolic pathways such as methionine synthesis and affects the pool of other important anions such as glutamate (Pinhal et al., 2019). Accumulation of intermediate homocysteine caused by inhibition of methionine synthesis has an inhibitory effect on microbial growth (Roe et al., 2002). Other mechanisms by which weak acids such as acetic acid inhibit growth include the increase in internal pH via diffusion of protonated form and intracellular dissociation of protons, and accumulation of anions (Roe et al., 2002). This in turn has an adverse effect on various cellular processes such as oxidative phosphorylation or photosynthesis (Grime et al., 2008).

2.2.7.7. Acetate on glaciers

Acetate is commonly produced by fermenters and acetogens in the anaerobic degradation of organic matter. It is a labile compound which can be utilised even in sub-zero temperatures. Based on current understanding in glacial habitats, acetate is oxidised by methanogenesis and sulphate reducers in the final step of anaerobic mineralisation in subglacial environments (Lawson, 2012; Fig. 2.5). Rivkina et al. (2000) demonstrated incorporation of radioactively labelled acetate into microbial lipids in the microbial community from Siberian permafrost between 5 and −20 °C. Acetic and formic acids are regarded as simple carbon sources for active bacteria in ice cores and for other polar bacteria (Lawson, 2012). Acetate also serves as a marker of active microbial metabolism in supra- and subglacial environments (Lawson, 2012 and references therein). Musilova et al. (2017) mentions that volatile fatty acids (VFAs) and amino acids detected in supraglacial habitats are likely a product of microbial cycling of organic matter in cryoconite holes. Together with small alcohols, amines, CO_2 and H_2, VFAs are a product of fermentation and an important intermediate metabolite which reflects the conditions of sediment zone (Glombitza et al., 2015). The concentration of these metabolites depends on the amount of organic matter, abundance of electron acceptors, and presence of anoxic zones, which enable fermentation (Glombitza et al., 2015 and references therein). Carboxylic acids are also

commonly present in surface snow samples, where they closely interact with atmosphere, being both readily deposited and easily scavenged (Legrand et al., 2003). Being an important part of DOC pool in snowpack, they serve as carbon sources for microbial community therein (Sanyal et al., 2018). Acetate is commonly found on glaciers and is a large part of labile organic carbon found in supraglacial and subglacial environments (Table 2.4).

O'Donnell et al. (2016) demonstrate acetate as the most common analyte in the studied LMW-DOC (Low Molecular Weight - Dissolved Organic Carbon) pool in the subglacial environment on four different glaciers in Greenland, Antarctica, Norway and Svalbard. Supraglacial LMW-DOC (both streams and cryoconite holes) on Joyce glacier (Antarctica) was dominated by carboxylic acids with a mean of 3300 nM C, however due to the problems with peak resolution the authors could not assign the acetate peak clearly and only estimate its values as high. Acetate and propionate were two most abundant carboxylic acids studied on GrIS, and acetate dominated in supraglacial streams on GRIS with the concentrations ~concentrations ~100 nM C. Moreover, cryoconite holes in GrIS and Joyce Glacier had elevated levels of carbohydrates, particularly fructose, sucrose and glucose, which could be a substrate to produce acetate in anaerobic conditions. Interestingly, the authors notice that supraglacial LMW-DOC concentrations were analogous to concentrations of labile compounds documented in downstream marine and river environments.

DOC was found in low concentrations in cryoconite hole water on GRIS (50±33 µM), but no data are available for cryoconite sediment (Holland et al., 2019a). Another study (Musilova et al., 2017) documented low molecular weight carbon concentrations in supraglacial environments. Volatile fatty acids in cryoconite hole water averaged around 10-15 µg C l^{-1} (~0.8 -1.25 µM) and acetate concentrations in all supraglacial environments (namely streams, ice, snow, ice cores, cryoconite water) averaged at 4±1 µg C/L (0.33 ± 0.08 µM). Acetate is also commonly found in snow samples and ice cores on glaciers over the world. For example Samui et al. (2018) reported values for Antarctic snow from Ice Shelf and Ice Cap in a range of 0-0.73 µM (Table 2.4) and Legrand and Mayewski (1997) found abundant acetate and formic acid in ice cores from Summit (GrIS), in the range of tens of µg/l (around 0.83 µM of acetate).

Studies on organic carbon composition in Antarctic cryoconite holes are limited (Table 2.4). Selected carboxylic acid concentrations were measured in cryoconite hole water in various locations (Samui, 2019), with acetate concentrations ranging 0.08±0.01 – 0.47±0.02 µM (Larsemann Hills), 0.01 – 0.38±0.02 µM (Dronning Maud Land), 0.03 ±0.00 – 0.45±0.02 µM (Amery Ice Shelf). There were no data reported on carboxylic acids or low molecular weight carbon in cryoconite hole sediment. DOC was shown to accumulate over time in cryoconite hole

water in two Antarctic locations (Bagshaw, 2008; Samui, 2019). Therefore measurements of carboxylic acids in cryoconite sediment are required to understand DOC dynamics in cryoconite holes.

Table 2.4. Concentrations of acetate and other notable carboxylic acids detected in published literature.

Location	Environment	Acetate concentrations (μM)	Other carboxylic acids detected	References
Larsemann Hills, Antarctica	cryoconite water	0.08±0.01 – 0.47±0.02	formate, lactate, oxalate	(Samui, 2019)
Central Dronning Maud Land , Antarctica	cryoconite water	0.01 – 0.38±0.02	formate, lactate, oxalate	(Samui, 2019)
Amery Ice Shelf, Antarctica	cryoconite water	0.03 ±0.00 – 0.45±0.02	formate, lactate, oxalate	(Samui, 2019)
GrIS	cryoconite water	<0.83-1.25*	butyrate, formate, oxalate, propionate	(Musilova et al., 2017)
Princess Elizabeth Land, Antarctica	snow	0-0.73	formate	(Samui et al., 2017)
Amery Ice Shelf, Antarctica	snow	0-0.26	formate	(Samui et al., 2017)
Summit, central Greenland	ice cores	abundant	formate	(Legrand and Mayewski, 1997)
Concordia, Antarctica	snow	~ 0.04	formate	(Legrand et al., 2013)
GrIS	supraglacial	> 2	propionate	(Lawson, 2012)
Leverett glacier, GrIS	supraglacial streams	~0.05-0.1		(Lawson, 2012)
GrIS	basal ice	~0.1-2		(Lawson, 2012)

* acetate concentrations calculated assuming that most of total VFAs detected consisted of acetate

2.2.8. Biota

The recognition of cryoconite as an important microbial habitat on glacier surfaces has attracted a lot of attention in recent years (Hodson et al., 2008; Anesio and Laybourn-Parry, 2012). However, Kohshima worked on microbial communities of snow and ice around cryoconite holes

in 1984 and already the expedition of Nordenskjold discovered the “brown polycellular alga” accompanied by “certain other microscopic organisms” in 1875 (Leslie, 1879).

Organisms inhabiting cryoconite holes include bacteria, archaea, fungi, cyanobacteria, algae, Protista, micro-invertebrates (rotifers, tardigrades, nematodes) and viruses (Vincent et al., 2000; Porazinska et al., 2004; Kaczmarek et al., 2016). An extensive list of organisms inhabiting cryoconite holes throughout the world was recently published by Kaczmarek et al. (2016) with micro-invertebrates separately described in Zawierucha et al. (2015). No detailed record of viruses is available, but study of Arctic viral dynamics and abundance was presented by Anesio et al. (2007) and Bellas et al. (Bellas et al., 2013). Microorganisms and microinvertebrates inhabiting cryoconite holes to date comprise 411 reported taxa worldwide (Zawierucha et al., 2015; Kaczmarek et al., 2016) and many unidentified genetic fingerprints, especially bacterial ones 16,17. Importantly, only 40% of taxa were identified to the species level with the highest number (62) of described as algae; and 39 bacteria and Archaea species.

Due to the presence of numerous research stations and extensive ice coverage in Antarctic and Arctic regions most studies of cryoconite biota are based in these regions. Smaller number of taxa were reported from European glaciers and low numbers from North and South American and Asian glaciers. The least studied areas are small glaciers located in Scandinavia and New Zealand, where cryoconite holes have not attracted scientific attention. There is no assessment of cryoconite on tropical glaciers. Consequently, the understanding of the cryoconite microorganisms on the global scale is incomplete (Kaczmarek et al., 2016). On the other hand, given that the polar regions comprise the majority of Earth’s glaciers, it is reasonable to concentrate on these areas and their changes.

2.2.8.1. Adaptations to the cold

The microorganisms of cold environments are divided into two groups depending on their growth temperature range. Psychrophiles have maximal temperature for growth at about 20 °C, with the optimum around 15 °C or lower, whereas psychrotrophs (otherwise known as psychrotolerant) are able to grow at low temperatures, but their optimum is above 15 °C and maximum above 20 °C. The anecdotal reason for this threshold is because the ambient temperature of American laboratories is around 21-22 °C, which is considered to be “not cold” (Moyer and Morita, 2007).

The first psychrophiles were documented in the literature as early as 1884, however most of the discovered individuals where in reality psychrotrophic. The first true psychrophile was described

taxonomically in 1964 - *Vibrio (Moritella gen. nov.) marinus (marina comb. nov.)* MP-1 (Moyer and Morita, 2007). Psychrophiles, and an even greater number of psychrotrophs, are found in the cold environments across the Earth. This includes oceanic water, higher atmosphere and various habitats in the polar regions (Moyer and Morita, 2007).

The lowest temperature at which microbial activity was observed at -20 °C in 2004 in Arctic sea ice. A similar result was obtained by Carpenter et al. (2000) in the Antarctic snow-ice samples. The growth at a temperature of -12 °C (confirmed by growth curve) was recorded for *Psychromonas ingrahamii*, also in the study of Arctic sea ice bacteria in 2004 (Junge et al., 2004). Survival *in situ* was demonstrated at -30 °C and metabolism predicted even at -40 °C. The lowest limits of cryoconite microorganisms were not investigated and to date no true novel psychrophiles have been isolated from cryoconite holes.

Rates of microbial activity in cryoconite sediment are similar to those found in temperate freshwater sediments (Anesio and Laybourn-Parry, 2012). Yet microorganisms in cryoconite holes are subjected to multiple stresses resulting from low temperatures and fluctuating environmental conditions. These include, but are not limited to, freeze-thaw cycles, geochemical extremes (e.g. high pH and low nutrient availability), decrease in diffusion rates, increased viscosity of fluids, osmotic stress, and UV exposure. For microorganisms to adapt to this environment, they need to respond to numerous interacting stresses that are usually unspecific (Anesio and Laybourn-Parry, 2012; Collins and Margesin, 2019), and we need to understand how they interact. Cold-environment constraints often induce cross-protection against other stressors. For example, adaption to freeze-thaw stress will also provide protection against heat and cold shock, oxidative stress, metabolic stress (starvation on C or N sources), and/or osmotic stress (Park et al., 1997; Fonseca et al., 2001; Wilson et al., 2012). Identified mechanisms which allow survival of freezing and accompanying stresses include the increased fluidity of the cell membrane (Fonseca et al., 2001; Meneghel et al., 2017), excretion of antifreeze proteins (Park et al., 1997; Raymond, 2016) or other cryoprotectants (Pegg, 2007; Wilson et al., 2012), as well as the production of stress proteins following exposure (Park et al., 1997; Fonseca et al., 2001).

Metagenomic and molecular studies of Alpine cryoconite hole communities have attempted to characterise the mechanisms of adaptation to these extreme stressors. At Rotmoosferner, Austria, it was demonstrated that microbial community members not only have a large array of stress response genes, but that they also have significant genetic potential for effective nutrient and organic carbon scavenging/recycling (Edwards et al., 2013a). Utilisation of various carbon substrates was also determined in the Austrian Alps (Margesin et al., 2002), Himalaya and Antarctica (Foreman et al., 2007; Sanyal et al., 2018). At Forni, Italy, and Baltoro, Pakistani

Karakoram, a metagenomic study confirmed the presence of versatile and diverse metabolisms in the cryoconite communities (Franzetti et al., 2016). Genes encoding metabolic pathways of heterotrophic anoxygenic phototrophs and anaerobes were found, as well as enzymes for multiple organic carbon sources such as cellulose, chitin and other polysaccharides (e.g. EPS). Yet it still remains mostly unknown which groups of microorganisms are capable of effective recycling; which complement each other; and which are very efficient scavengers.

Much of the research on polar cryoconite holes has focused on geochemistry, net ecosystem productivity and carbon cycling (Bagshaw et al. 2013; Stibal et al. 2008; Cook et al. 2012), whilst the actual functionality of these microbial communities remains largely unidentified and physiological limits are untested. Metagenomes of microbial communities on the Greenland Ice Sheet (GrIS) show the potential for resistance to, and degradation of, anthropogenic contaminants (Hauptmann et al., 2017), but the genetic potential of Antarctic communities has not been investigated. The phenotypic diversity of organisms will affect the robustness of ecosystem and its response to change (Petchey and Gaston, 2006; Srivastava et al., 2019). Ice sheet surfaces are an extreme low temperature environment, but also a very changeable habitat. Cryoconite holes can be saturated with oxygen (Bagshaw et al. 2011) or anoxic (Poniecka et al., 2018); too dark or too light (Perkins et al., 2017); change from hypersaline to low ionic strength (Telling et al., 2014); become acidic or alkaline (Tranter et al., 2004); be frozen and thawed multiple times (Bagshaw et al. 2011); and can be spiked with nutrients or become nutrient limited (Telling et al., 2014; Holland et al., 2019b). Therefore, the microorganisms that inhabit cryoconite holes can tolerate and grow over a wide range of extreme conditions.

2.2.8.2. Molecular analysis of biota

Molecular studies on the organisms inhabiting cryoconite holes began in 2002 (Christner et al., 2003a) by sequencing rDNA obtained from Canada Glacier in McMurdo Dry Valleys, Antarctica (Christner et al., 2003a). Identified bacterial groups included Acidobacteria, Actinobacteria, Cyanobacteria and Proteobacteria. The phylogenetic relatives of isolated species had been previously documented from nearby locations, including lakes, sea ice and glacial ice. Therefore, seeding from the surrounding ecosystems was suggested as the colonization mechanism of cryoconite habitats. Diversity and abundance patterns, as well as soil organic matter gradient, matches the katabatic winds direction in Taylor Valley (Porazinska et al., 2004). However, Porazinska et al. (2004) noted that the community composition was different from the nearest surrounding aquatic environments.

Multiple molecular studies have followed (Cameron et al., 2012; Edwards et al., 2013b; Lutz et al., 2017), attempting to understand what shapes the community structure in the cryoconite holes, and determine the interaction with proximal environments. Current understanding assumes the uniqueness of the cryoconite community compared to adjacent habitats, with input of the biota from both local and long-distance aeolian transport. The communities are likely influenced and selected through unique, extreme physical and geochemical conditions, including freeze-thaw cycles (Stanish et al., 2013). Despite extensive debate, the characteristics of atmospheric redistribution and mixing of biota and questions about their potential cosmopolitan distribution remain unanswered (Cook et al., 2016a; Anesio et al., 2017).

2.2.8.3. Cultivable bacteria

The development of molecular methods in microbiology has allowed to reveal the biodiversity and a potential functional diversity of microorganisms (Chaudhary et al., 2019). A large part of this diversity remains unexplored as most of the bacterial species in the environment are yet to be cultured in the laboratory. It is still believed that around 99% of soil bacteria are unculturable using traditional cultivation techniques, hence the development of new methods to grow bacteria attract a lot of interest (Vartoukian et al., 2010; Pham and Kim, 2012; Chaudhary et al., 2019). Only analyses and manipulations of pure cultures allow a full characterization of a functional potential and ability to produce metabolites by microorganisms. Natural products from bacteria are used in many biotechnological applications, including insecticides, fungicides and pharmaceuticals (Stewart, 2012). Microorganisms living in the cold are considered an unexplored source of cold-active enzymes (Cavicchioli et al., 2011; Collins and Margesin, 2019).

Only a few novel species have been isolated and described from cryoconite sediments. Most of them - 10 bacterial and 4 yeasts species - were published in *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) - the official journal recording novel prokaryotic taxa (Table 2.5). All of those were isolated from Austrian Alps and described by Margesin's group from Institute of Microbiology, University of Innsbruck, Austria. Only one other novel species was described in Cryobiology journal - *Rhodotorula svalbardensis* sp. nov. - isolated from cryoconite holes from Svalbard (Singh et al., 2014c). All of the novel species were aerobic or microaerophilic (required at least some oxygen to grown). Most bacteria were psychrotolerant, whereas all the yeasts were psychrophilic (Table 2.5). None of the tested ones were capable of fermentation or anaerobic growth.

Several culture-based analyses were used to characterise the capabilities of microbial communities of cryoconite holes. Pure bacterial cultures isolated from cryoconite water from

Antarctic and Himalayan holes were tested for biodegradation potential (Sanyal et al., 2018). Retrievable heterotrophs were able to utilize many organic compounds occurring in cryoconite water such as acetate, lactate, formate, oxalate and propionate. Another analysis of bacteria from Antarctic cryoconite hole revealed a few obligately psychrophilic bacteria, but did not characterize any other properties (Christner et al., 2003a).

Cold active enzymes (Singh et al., 2014b) and antifreeze activity (Singh et al., 2014a) was tested for Svalbard bacterial isolates. The strains cultured were psychrophilic and tolerated up to 2-5% salinity. Most of the cultures screened for enzyme production showed catalase activity, followed by amylase, cellulase, lipase, urease and protease. Around half of the tested strains were reported to produce antifreeze proteins. Three strains of yeasts from the same location were psychrophilic and tolerated up to 1.5-3.5% salinity (Singh and Singh, 2012). Like bacteria, all of yeasts had catalase activity, but none showed cellulase activity.

Bacteria isolated from Alpine cryoconite holes (Austria) were psychrotolerant and able to grow at temperatures up to 55 °C, whereas yeasts were not able to grow above 20 °C (Margesin et al., 2002). Most of the microbes could grow on complex natural carbon sources like lignin, cellulose, proteins, carbohydrates, and fat. Some of them utilized xenobiotic substances and pollutants such as aromatic hydrocarbons. A larger scale study comprising 247 microbial cultures from alpine holes demonstrated that most of the isolates were able to grow up to 30 °C as well as showed lipase and protease activity (Lee et al., 2011). Microbial communities exhibited differences in distribution patterns depending on the human activity in the sampling area.

These culture-dependent studies give us a glimpse of physiological characteristics of cryoconite holes communities. Cryoconite microorganisms seem to have a large potential for biodegradation of organic matter, which would be advantageous in the nutrient-poor supraglacial environment. Most of the isolates obtained for the mentioned analyses of cryoconite microorganisms used commercial R2A medium for isolation and culturing. R2A medium is intended for the cultivation of bacteria from water and is considered relatively low in nutrients. Substrate-rich media is believed to promote faster-growing bacteria over slow-growing species which might be better adapted to nutrient-poor environments (Vartoukian et al., 2010). The use of dilute media is therefore crucial to successful cultivation of key players in cryoconite holes. Perhaps use of a medium with even lower nutrient concentrations would increase the recovery of species from cryoconite samples. Another complementing approach to retrieve the most abundant bacteria from a given environment has not yet been employed. The strains isolated and selected in the described studies have been cultured from the bulk of cryoconite water or sediment slurry and randomly selected for further experiments based mostly

on morphological characteristics. Cultivation techniques involving dilution-to-extinction or Most Probable Number method (MPN) would allow to isolate the representatives of the most abundant phylotype in the microbial community and characterise the properties determining the growth success in the cryoconite hole habitat (Martens-Habbena and Sass, 2006; Bartelme et al., 2020).

Table 2.5. Novel microbial species isolated from cryoconite holes.

Novel species	Glacier (Austria*)	Growth temp.	Aerobic/ anaerobic	Gram staining	Spores	References
Bacteria						
Alpinimonas psychrophila	Rettenbach	1-20	aerobic/ micro-aerophilic	positive	N/A	(Schumann et al., 2012)
Nocardioides alpinus	Pitztaler Jöchl	1-25	aerobic	positive	N/A	(Zhang et al., 2012b)
Pedobacter cryoconitis	Stubaier	1-25	aerobic	negative	no	(Margesin et al., 2003)
Sphingomonas glacialis	Stubaier	1-30	aerobic	negative	no	(Zhang et al., 2011a)
Glaciimonas immobilis	Tiefenbachferner	1-25	aerobic/ micro-aerophilic	negative	N/A	(Zhang et al., 2011b)
Arthrobacter cryoconiti	Banker	1-25	aerobic	positive	N/A	(Margesin et al., 2012a)
Devosia psychrophila	Pitztaler Jöchl	1-25	aerobic	negative	N/A	(Zhang et al., 2012a)
Devosia glacialis	Pitztaler Jöchl	1-20	aerobic	negative	N/A	(Zhang et al., 2012a)
Polaromonas glacialis	Pasterze/ Großglockner	1-25	aerobic	negative	dormancy genes	(Darcy et al., 2011; Margesin et al., 2012b)
Polaromonas cryoconiti	Pasterze/ Großglockner	1-25	aerobic	negative	dormancy genes	(Darcy et al., 2011; Margesin et al., 2012b)
Yeasts						
Rhodotorula svalbardensis	Midtre Lovénbreen in Svalbard	1–20	N/A		no	(Singh et al., 2014c)
Rhodotorula psychrophenolica	Stubaier	1-20	aerobic		N/A	(Margesin et al., 2007)

Novel species	Glacier (Austria*)	Growth temp.	Aerobic/ anaerobic	Gram staining	Spores	References
Rhodotorula glacialis	Stubaier	1-20	aerobic		N/A	(Margesin et al., 2007)
Rhodotorula psychrophila	Stubaier	1-15	aerobic		N/A	(Margesin et al., 2007)
Mrakiella cryoconiti	Stubaier	1-20	aerobic		no	(Margesin and Fell, 2008)

2.2.8.4. Photoautotrophs

Cyanobacteria are considered the main primary producer in cryoconite holes (Säwström et al., 2002; Hodson et al., 2008; Anesio et al., 2017), which photosynthesize and accumulate organic matter on glaciers, contributing between 75 and 95% of carbon available in cryoconite (data from Svalbard (Stibal and Tranter, 2007)). They comprise the largest component of the community in many locations, for example in Svalbard (Lutz et al., 2017), in South West Greenland (Cameron et al., 2012), and in large granules in North West Greenland (Uetake et al., 2016). However, some geographical locations exhibit a dominance of algae, for example, in smaller granules from North West Greenland (Uetake et al., 2016); or comparatively low proportions of cyanobacteria, for example, on one Alpine glacier they comprised as little as 2.5% of the microbial community (Edwards et al., 2014). Eukaryotic primary producers, such as *Chlamydomonas nivalis*, and *Ancylonema nordenskiöldii* and *Mesotaenium bergrenii*, dominate in the snow and on the ice surface respectively. During melt season they are washed into cryoconite holes, where they input organic matter (Lutz et al., 2017), and some of them thrive (Säwström et al., 2002; Anesio et al., 2009).

2.2.8.5. Heterotrophs

The largest fraction of the microbial community in cryoconite holes consists of heterotrophic bacteria. In the first metagenomic snapshot of an Alpine cryoconite hole, the dominant group were Proteobacteria (63.3%), followed by Bacteroidetes (14%) and Actinobacteria (11.3%) (Edwards et al., 2013a). Similar proteobacterial dominance was reported from multiple locations in the Arctic and Antarctic (Edwards et al., 2011b, 2013b, 2014; Cameron et al., 2012). Proteobacteria, followed by Bacteroidetes and Cyanobacteria, were the dominating group on a glacier in Sweden, whereas Cyanobacteria were dominant in Svalbard, with Proteobacteria as the second largest group (Lutz et al., 2017). Proteobacterial dominance (21%) was also found in

the Antarctic (Zdanowski et al., 2017), followed by Bacteroidetes (16%) and Actinobacteria (14%). (Uetake et al. (2016) reports abundant Proteobacteria in granules in Greenland, but Acidobacteria dominate in the small granules. Lutz et al. (2017) speculate that Alphaproteobacteria could be so abundant because they can grow at low carbon and nutrient concentrations (Eiler et al., 2003), which means they are well-adapted to the oligotrophic habitat of cryoconite hole. Among a plethora of other bacterial taxa, the genus *Polaromonas* has been identified as abundant and important in cryoconite ecosystem (Segawa et al., 2014; Gawor et al., 2016; Anesio et al., 2017), since they produce EPS and hence can help form granules, by acting as a glue for the mineral debris (Langford et al., 2010). *Polaromonas* strains vary greatly with site and habitat (e.g. depending on the pH of glacial surfaces (Gawor et al., 2016)), and therefore the range of metabolic capabilities is likely connected with adaptations to local environmental conditions.

The spatial variability of the bacterial community can also be affected by multiple factors. Physical characteristics of glaciers, such as surface hydrology, ice temperature and morphology, and the form and function of surrounding habitats (Edwards et al., 2011b; Stibal et al., 2012; Anesio et al., 2017) have been suggested to influence composition of the community, as well as more local factors such as size of the granules (Uetake et al., 2016). The bacterial community seems particularly sensitive to the development of granules, since larger granules likely restricts access to oxygen in the centre (Fig. 2.6), and the larger external surface area increases colonisation by photosynthetic Cyanobacteria. The role of particular bacterial groups in cryoconite holes is still poorly understood, and requires further metabolomic, transcriptomic and physiological studies to unravel their capabilities.

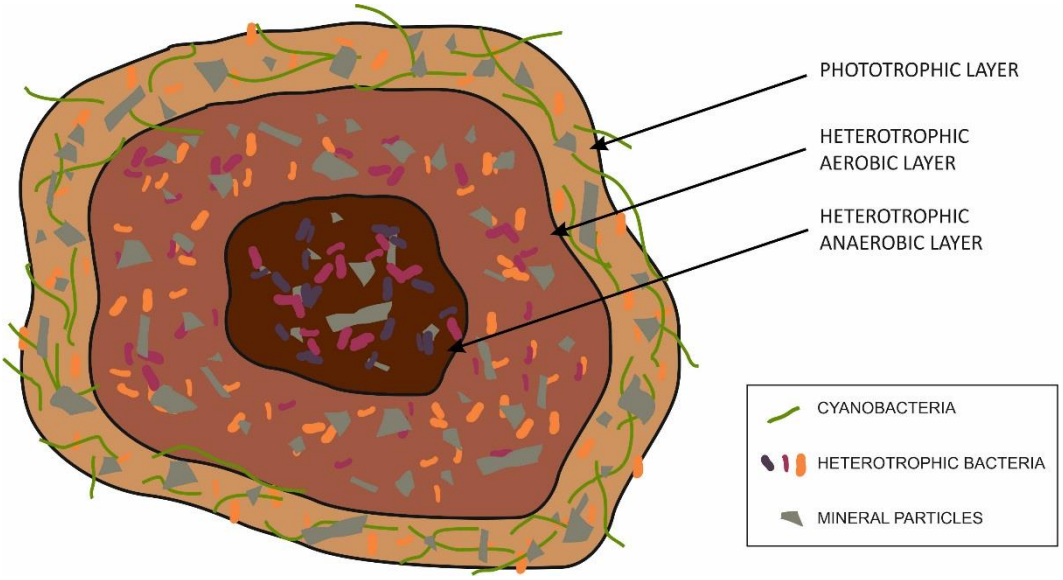

Figure 2.6 Habitable zones in cryoconite granule. *The conceptual model of cryoconite granule, where, with the oxygen decreasing towards the middle of the granule, the community structure changes. The surface – phototrophic layer - is inhabited by phototrophs and heterotrophs. Towards the middle of the granule, in the heterotrophic aerobic layer, the phototrophs disappear and the heterotrophic community structure changes. In the middle of the granule we can potentially find strict anaerobes in the heterotrophic anaerobic layer (From Poniecka and Bagshaw, 2020, submitted).*

Heterotrophs, both bacterial and fungal, are believed to recycle the nutrients from organic matter produced by primary producers (mainly Cyanobacteria) and obtained from melting of the surroundings (Cook et al., 2016a; Lutz et al., 2017), because the cryoconite ecosystem is relatively closed (especially in Antarctic ice-lidded holes) and hence processes are tightly coupled (Porazinska et al., 2004; Tranter et al., 2004). Their potential as recyclers is supported by the variety of genes linked with degradation of organic carbon and nutrient scavenging detected in the metagenome (Edwards et al., 2014). Cultured bacteria from a cryoconite hole were able to use a broad range of carbon substrates at low temperature, which supports their ecological significance (Margesin et al., 2002) and suggests that they could be adapted to utilize broad spectrum of energy sources, depending on temporal availability in the oligotrophic holes. Even though majority of carbon uptake in the holes comes from primary producers and allochthonous sources (Stibal and Tranter, 2007; Stibal et al., 2008; Anesio et al., 2017), bacterial production was estimated to account for up to 7% of inorganic carbon uptake (Anesio et al., 2010). An active viral community has been observed, which can help break down organic matter trapped in the biomass of dead cells via lysis (Bellas et al., 2013).

Whilst the composition of the heterotrophic community is now becoming clear, still little is known about their activity within cryoconite holes (Anesio et al., 2017). One example of such a gap in knowledge is a recent study (Stibal et al., 2015) which showed a discrepancy between the

bulk and potentially active RNA community. This suggests that although holes can act as a sink for various organisms, especially on small temperate glaciers, not every trapped organism is adapted to extreme conditions and thrives in there. The concept of a core active community which ensures the functioning of the ecosystem requires investigation, alongside the impact of granule structure and cryoconite morphology, which causes the development of micro-niches that add additional selection pressures (Takeuchi et al., 2001; Langford et al., 2014; Segawa et al., 2014; Uetake et al., 2016).

2.2.8.6. Anaerobes

A group of microorganisms which likely inhabit cryoconite holes, but have received little attention to date, are anaerobes. An anaerobe-targeted molecular study of Antarctic cryoconite (Zdanowski et al., 2017) detected a small proportion of strict anaerobes. However, when the culture conditions were adapted to promote anaerobic growth, the composition of the community changed drastically. Anaerobic enrichment caused selection of different groups of microorganisms compared to fresh, bulk cryoconite sediment (Zdanowski et al., 2017), eliminating the taxa which dominated before and enriching groups of the Firmicutes (62%) (which were a marginal group of 0.47% within the native samples), Proteobacteria (14%), and Bacteroidetes (13%) with anaerobic genera such as *Clostridium, Psychrosinus, Paludibacter*, and *Acetobacterium* (Zdanowski et al., 2017). Such selection could potentially happen in the centre of cryoconite granules if they were sufficiently large for the anoxic zone to persist. Similar conditions can also develop in thick accumulations of cryoconite, for example, on the side of a supraglacial stream or in a deep, ice-lidded cryoconite hole (Fig. 2.4). Anoxic conditions may promote such species selection and a potential change of community. Anaerobic products which accumulate in these thicker sediments become available for aerobic oxidation after remixing by supraglacial meltwater. Within individual granules, a complete nitrogen cycle was indicated on a glacier in Central Asia (Segawa et al., 2014). This provides evidence that cryoconite granules could potentially operate as largely self-sustainable ecosystems, with tight coupling of anaerobic and aerobic bacterial groups trapped together in relatively nutrient-rich habitat – in contrast to the oligotrophic glacier surface.

Many of the bacteria detected in cryoconite studies do not show any similarities to classified microorganisms (for example, Uetake et al. (2016) in North West Greenland), hence it could be speculated that unique bacteria could have a role in anaerobic processes inside cryoconite granules. Strict anaerobes, such as nitrifiers, were detected in metagenomic studies (Segawa et al., 2014) and Zdanowski et al. (2017) observed indications of the presence of sulphate reducers.

These microorganisms probably do not occur in great abundance, so likely appear as minor players or could be omitted during standard metagenomics. Potentially, targeting micro-niches within cryoconite material for metagenomic, metatranscriptomic and metabolomic studies could reveal the unknown part of biogeochemical cycle within cryoconite holes and in supraglacial ecosystem.

2.3. Summary

Cryosphere provides a range of habitats characterized by extreme physicochemical conditions such as wide range of UV radiation, temperatures and salinities. Microorganisms inhabiting the cryosphere do not seem limited by these conditions and form diverse communities in all cold environments. Among these environments, cryoconite holes are regarded as hot spots of microbial activity thanks to access to liquid water, shading and organic matter available in the sediment. Cryoconite holes vary significantly in physical and biological properties depending on the region. The most prominent difference is the presence of the perennial ice lids on the Antarctic holes which influences the chemistry, but only appears temporarily on the Arctic holes. Antarctic holes also have more fine-grained, sandy sediments whereas the Arctic cryoconite material often forms granules. The physicochemical differences of the holes are reflected in the microbial community structure which varies significantly by location. The concentration of the oxygen within the cryoconite sediment is one of the factors which likely influences microbial community, but to date the presence of anaerobic bacteria was only confirmed in the cryoconite material from Antarctica. Acetate and other compounds which are the common products of anaerobic metabolism have been found in glacier surface habitats including cryoconite holes. The following chapters will explore the oxygen status of cryoconite holes, the physiological capabilities of cryoconite microorganisms including anaerobic metabolism, and the microbial community structure related to anoxic conditions.

2.4. Research hypotheses

This research aims to address the research gaps outlined in the Introduction and Literature Review, via the following hypotheses:

H1 Cryoconite holes are a heterogenous habitat, both worldwide and on a local scale, which harbours diverse niches for microorganisms.

H1a Thick layers of sediment and granule formation restricts oxygen diffusion and allows development of anoxic niches as a consequence of microbial activity within otherwise well-oxygenated supraglacial habitats.

H1b The development and extent of anoxia depends on the structure of the sediment, which varies by location.

H2 Microorganisms inhabiting cryoconite holes are adapted to diverse niches characterized by fluctuating, extreme conditions, such as changing oxygen concentrations; freeze-thaw; varying salinity and pH; fluctuating temperatures; variable availability of carbon sources.

H2a The most abundant microbes are the most successful and demonstrate wide plasticity in a broad range of environmental conditions.

H2b Antarctic isolates are characterized by a higher resistance to harsh environment when compared to their Arctic counterparts.

H3 Part of the microbial community will remain active in the anaerobic niches and switch to anaerobic metabolisms.

H3a Products of these metabolisms, such as fermentation products (e.g. acetate, lactate, formate, propionate) will be detected in the cryoconite pore water.

H3b These products will diffuse to oxic layers and get oxidised, which will result in lower concentrations of metabolic products in the overlying oxygenated water.

H3c Dormant/inactive strict anaerobes such as sulphate reducers or methanogens will become active and consume intermediate anaerobic products (e.g. acetate, lactate) as well as terminal electron acceptors (sulphate) in the process of complete mineralisation of organic matter.

H4 Anoxia promotes the growth of microorganisms adapted to anaerobic conditions.

H4a Over time this will result in a selective pressure and communities from cryoconite holes from different locations will start resembling each other.

H4b Groups capable of anaerobic metabolisms, such as fermenters, sulphate reducers or methanogens, will become enriched.

2.5. The structure of the thesis

The work presented in this thesis consists of four scientific chapters focusing on the discovery of anoxic zones in cryoconite holes and the capabilities of microorganisms inhabiting the holes. Chapter 2 reviews literature specific to the glacial biome and cryoconite holes, including a summary of the hypotheses proposed for the oxygen status of cryoconite sediments. Chapter 3

summarizes the field sites, methodology and instrumentation employed in the analysis of cryoconite holes. Chapter 4 (adapted from Poniecka et al., 2018) explores Hypothesis 1 (H1) and combines in situ measurements on Greenland Ice Sheet with laboratory incubations to reveal the oxygen status of cryoconite sediments from three polar locations – Antarctica, Greenland and Svalbard. Chapter 5 (adapted from Poniecka et al., 2020) evaluates the metabolic capabilities of microorganisms within cryoconite holes to confirm H2. Chapter 6 expands on the work presented in Chapter 4 and 5, employing detailed analysis of water chemistry to assess products of microbial metabolism in order to address H3. Chapter 7 explores H4 and details the microbial community structure of cryoconite material, with particular focus on microbial groups capable of anaerobic metabolism. Chapter 8 summarizes the findings and addresses the research hypotheses outlined in Section 2.4.

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Chapter 3

Materials and Methods

3.1. Introduction

This chapter describes the study sites and methods used to collect and analyse sediment and water samples from cryoconite holes from Antarctica, Svalbard and Greenland. Samples of sediment and water were collected from cryoconite holes on Canada Glacier in Taylor Valley, Antarctica, during the austral summer of 2005/6 by Dr Liz Bagshaw. Samples from Greenland Ice Sheet were collected in the summer season of 2014 (by Dr Liz Bagshaw), and in 2016 and 2017. The *in situ* measurements of cryoconite hole geochemistry as well as the incubations of the sediment under *in situ* conditions took place in Greenland in 2016. Samples from Longyearbreen glacier in Svalbard were collected in 2015 (by Dr Liz Bagshaw) and from Midtre Lovénbreen glacier in 2016. Sampling protocol in the field is detailed, followed by description of measurements in the field. Methodology of laboratory incubations as well as analysis of sediment and water samples is described. The chapter is divided into four main sections, detailing the study sites, sample collection, field methodology and the laboratory methodology. The flowchart of the methodology and interdependence of the samples and incubations (Fig. 3.4) facilitates navigation through the methods used.

3.2. Study sites

The study sites were located in the Arctic and Antarctic, which allowed a bipolar comparison of cryoconite holes. All the study sites were characterized by continual darkness in midwinter and continual sunlight in midsummer. The Arctic samples were collected from Greenland and Svalbard, and the Antarctic samples from McMurdo Dry Valleys.

3.2.1. Arctic:

3.2.1.1. Greenland

The Greenland study sites were located near Kangerlussuaq, in the ablation zone of the southwestern Greenland Ice Sheet (GrIS) and are referred to as 'Point 660' (margin) and 'Black and Bloom' (interior). The site commonly known as Point 660 is located approximately 25 km

[67.0600, -50.1700] east of Kangerlussuaq (Fig. 3.1), on the Russell Glacier at the margin of GrIS. Here, samples for laboratory experiments were collected in the summer melt season of 2014 by Dr Liz Bagshaw. The elevations of Russell Glacier range from 200 m to 800 m a.s.l. with mean annual air temperature of -5.7 °C and mean annual precipitation of 200 mm (Weidick, 1995; Jørgensen and Andreasen, 2007).

The second Greenland study site, 'Black and Bloom', is located 60 km [67.0748, -49.3586] east of Kangerlussuaq (Fig. 3.1), approximately 2 km east of weather station S6 (Utrecht University) which has been used for research for over 20 years (Smeets et al., 2018). It is within dark bands identified on the ice surface by satellite observations (Bøggild et al., 2010; Tedesco et al., 2011; Yallop et al., 2012), and is the focus of an intensive investigation into the processes controlling ice sheet albedo change (blackandbloom.org). Here, *in situ* measurements, incubations and samples for laboratory experiments were collected in the summer melt season of 2016. Water samples were collected in 2017.

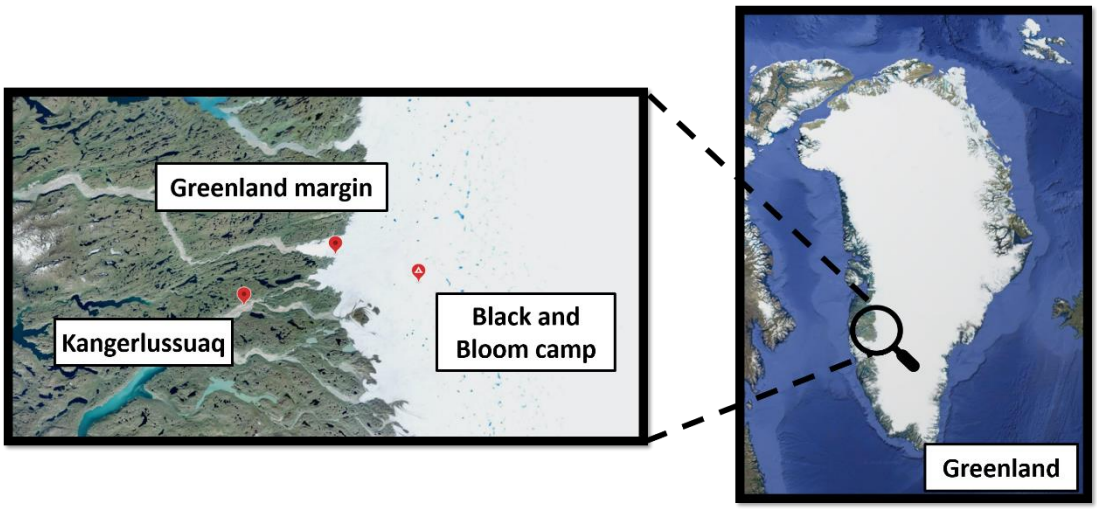

Figure 3.1. Location of Greenland study sites. *Greenland margin site is commonly known as Point 660 on Russell Glacier. Black and Bloom site (Greenland interior) is based near S6 weather station. Source: Google Earth*

3.2.1.2. Svalbard

The first Svalbard study site was located on Longyearbreen glacier (Fig 3.2), approximately 4 km from Longyearbyen [78.1803, 15.5058]. Longyearbreen is situated on the Nordenskiöld Land peninsula of Spitsbergen, the largest island of the Svalbard archipelago. It is a small (2.5 km^2), predominantly cold-based valley glacier extending from 1000 to 250 m a.s.l. (Langford et al., 2014). Here, samples for laboratory experiments were collected in the summer melt season of 2015 by Dr Liz Bagshaw.

The second study site was located on Midtre Lovénbreen glacier (Fig 3.2), approximately 4 km from the Science Centre in Ny-Ålesund [78.8800, 12.0700]. Midtre Lovénbreen is situated in the Kongsfjorden area in the north-western part of Spitsbergen. It is a polythermal valley glacier extending from 650 to 50 m a.s.l. Its surface area is 5.4 km2 (in 2011), with mean annual precipitation of 400 mm and annual mean air temperature around -8 °C (WGMS, 2016). It is a subject of several long-term monitoring programmes with continuous Mass Balance measurements dating back to 1967 (WGMS, 2016). Here, samples for laboratory experiments were collected in the summer melt season of 2016.

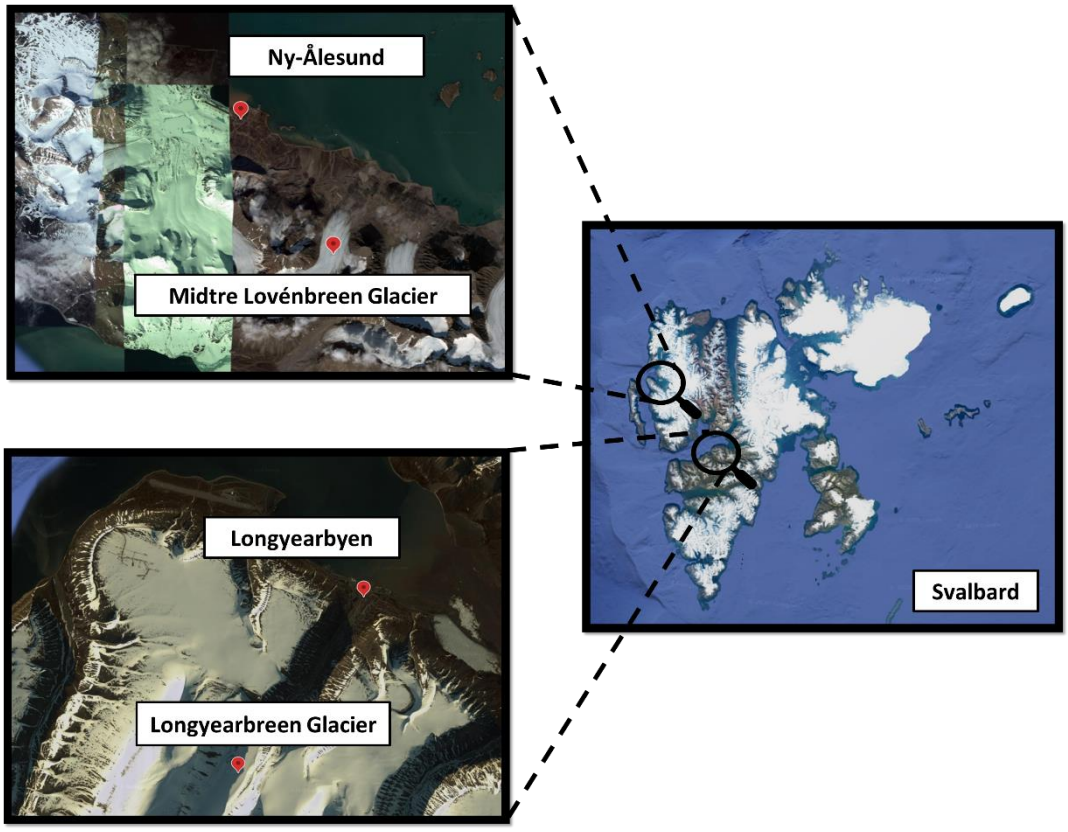

Figure 3.2. Location of Svalbard study sites. *Midtre Lovénbreen glacier is located approximately 4 km from Science Centre in Ny-Ålesund and Longyearbreen glacier is located approximately 4 km from Longyearbyen town. Source: Google Earth*

3.2.2. Antarctica

The Antarctic study site was located on Canada glacier (Fig 3.3) in McMurdo Dry Valleys [-77.6175, 162.9734]. McMurdo Dry Valleys are the Antarctic Specially Managed Area (ASMA) and encompass the largest ice-free region in Antarctica. Its unique landscape contains arid soils, glaciers, ice-covered lakes and unusual communities of plants and microorganisms. Air temperatures in the MCM Dry Valleys average about −17 °C and the annual precipitation consisting of snow does not exceed 100 mm water equivalent (Fountain et al., 1999). Precipitation levels remain very low because of the Trans Antarctic Range which creates a

precipitation shadow (Fountain et al., 2016). Most of snowfall is lost to sublimation (Fountain et al., 2010). Canada Glacier is the second largest glacier in one of the valleys - Taylor Valley. The glacier ranges in elevation from 50 to 1600 m (Fountain et al., 2008). Here, samples for laboratory experiments were collected in the austral summer melt season of 2005/2006.

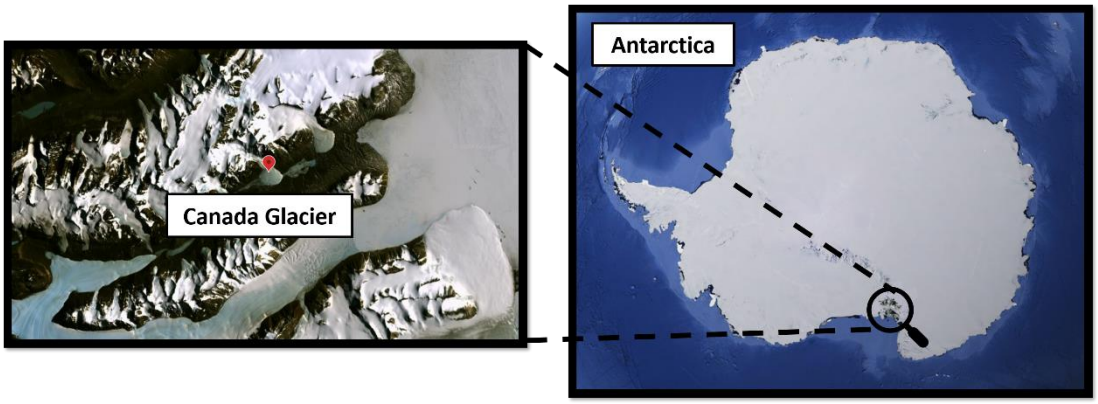

Figure 3.3. Location of Antarctic study site. *Canada glacier is located in Taylor Valley in McMurdo Dry Valleys. Source: Google Earth.*

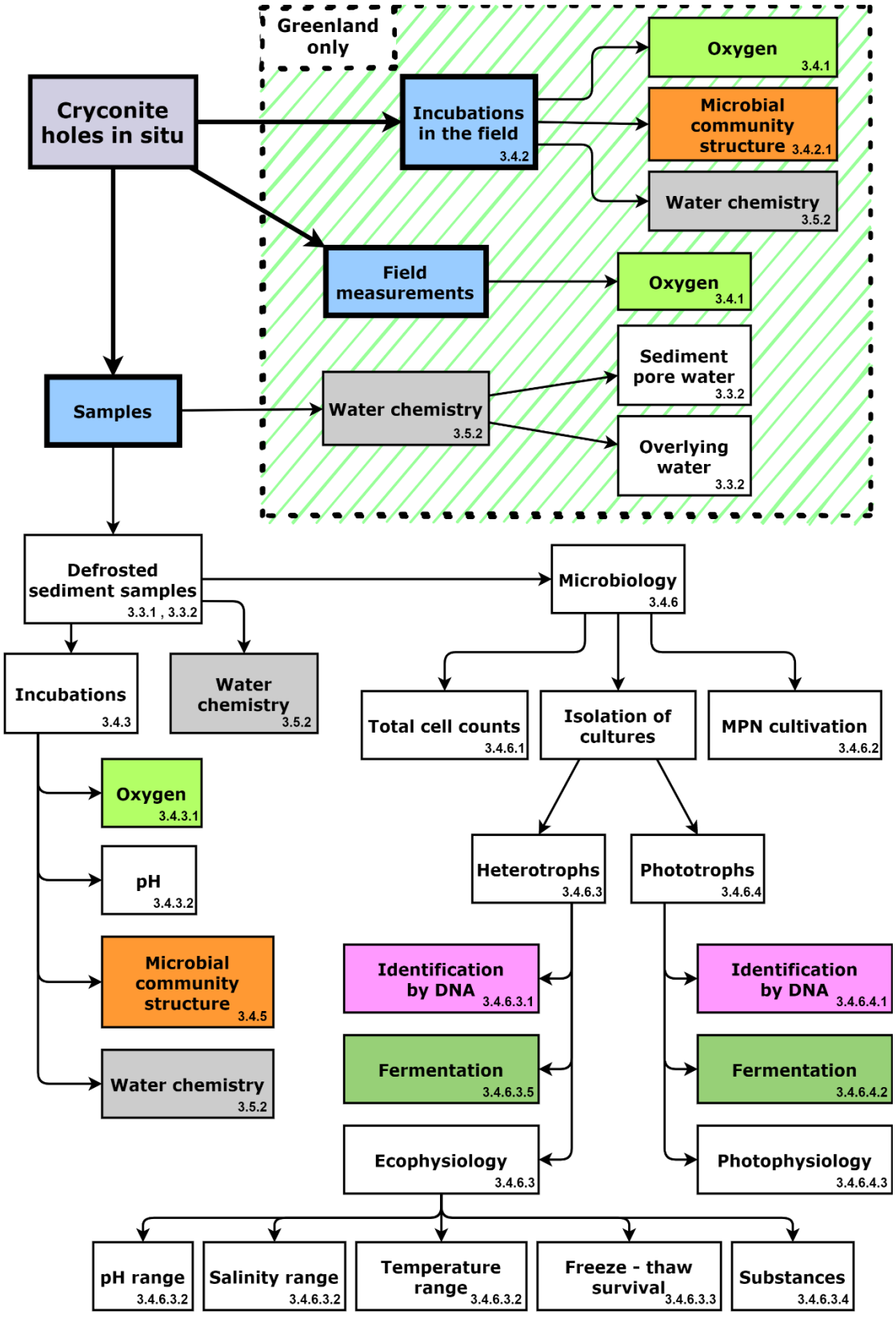

Figure 3.4. Flowchart of methods used to analyse cryoconite holes in situ and cryoconite samples. *Numbers correspond to respective subsections of Chapter 3, where a detailed description can be found.*

3.3. Sample collection

3.3.1. Cryoconite sediment samples

Sediment samples were either i.) scooped from cryoconite holes using clean, disposable nitrile gloves and transferred into Ziploc plastic bags or tubes pre-washed with deionised water (Greenland - site 660, Antarctica, Svalbard), ii.) aspirated with the use of pre-washed turkey baster and transferred into sterile Whirlpack® bags (Greenland – site Black and Bloom). Samples were collected from both isolated and hydrologically connected cryoconite holes, chosen randomly within a 500 m radius (Fig. 3.6). Ice lids were removed from Antarctic cryoconite holes using a Sipre corer prior to sampling (see Bagshaw et al., 2007). Cryoconite samples from Greenland margin were specifically targeted for thick layers of cryoconite material and drained sediments. Greenland interior samples (Black and Bloom) targeted typical circular and semi-circular holes. Additionally, cryoconite material was scooped from the thick accumulations on bottom and side of the streams and from the top layer of the shallow ice cores drilled with Kovacs drill (diameter = 14 cm) in 2017, which were subsequently used for water analysis only (Fig. 3.5). All samples were frozen within six hours, prior to temperature-controlled transport to Cardiff University. Here they were stored in a -20 °C freezer until laboratory experiments commenced. A range of laboratory experiments and incubations with the use of cryoconite sediment is summarised in Figure 3.4 and described in details in the following sections.

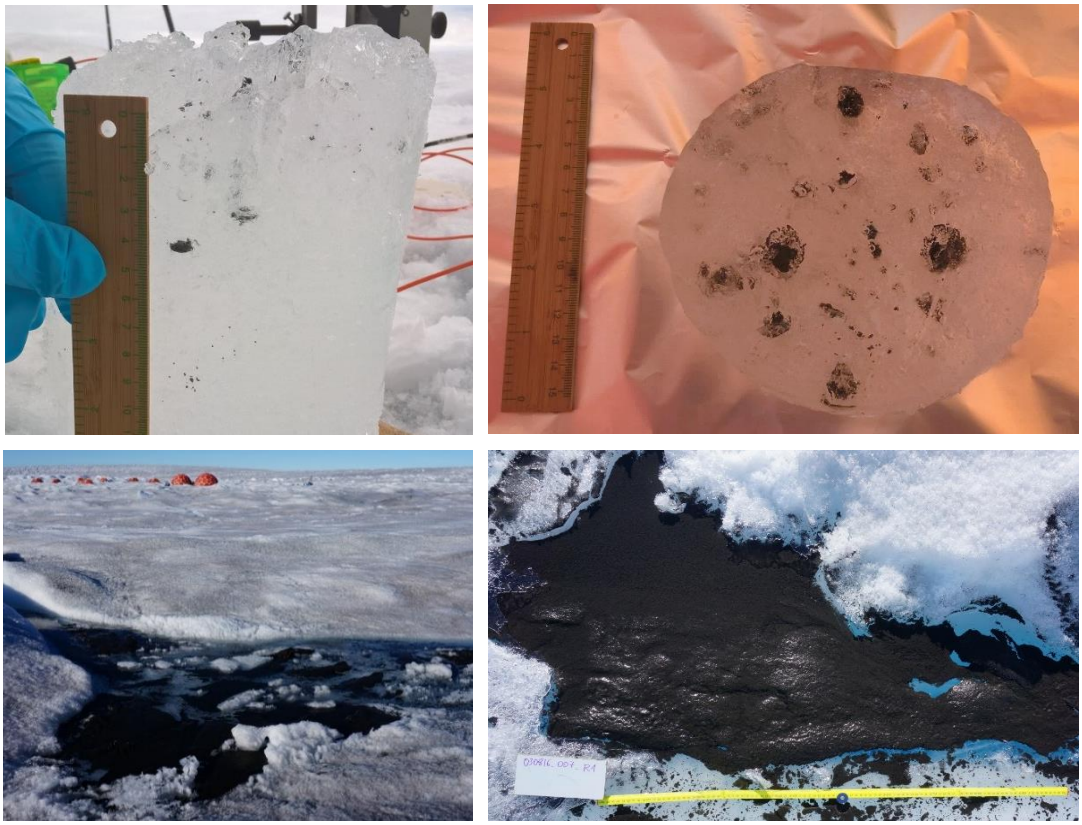

Figure 3. 5. Atypical cryoconite accumulations in Greenland interior. *Cryoconite material in shallow ice cores in the beginning of ablation season (top) and thick layers of partially drained cryoconite material in the middle of ablation season (bottom).*

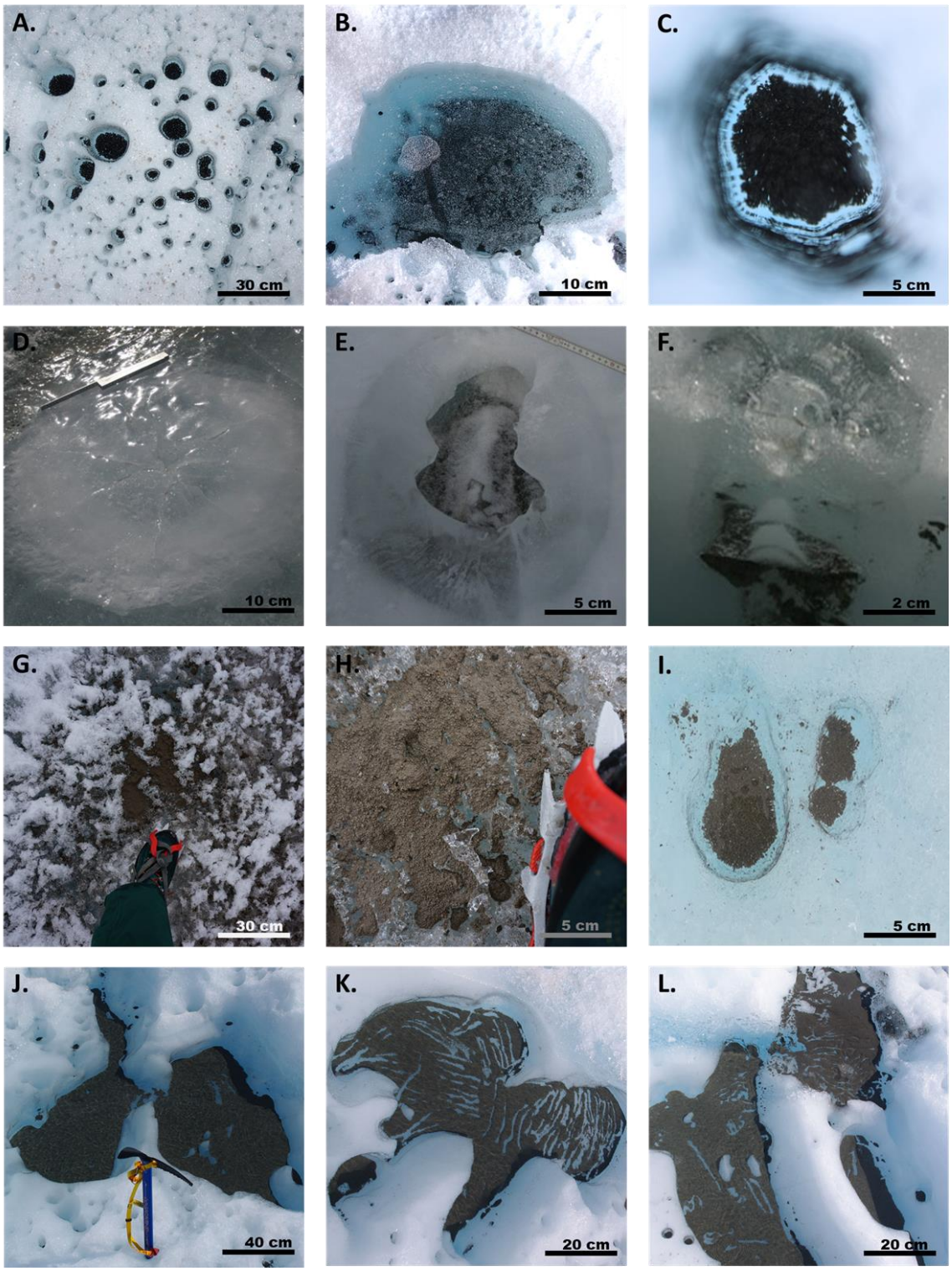

Figure 3. 6. Cryoconite holes from the study sites. *A-C typical circular and semi-circular holes in Greenland interior (Black and Bloom), D-F Antarctic lidded cryoconite holes (courtesy of Dr Liz Bagshaw), G-I Svalbard drained cryoconite holes (I. – courtesy of Jaz Millar), J-L Greenland margin thick layers of cryoconite in the holes (courtesy of Dr Liz Bagshaw).*

3.3.2. Cryoconite water samples

Water was sampled from the cryoconite water column (above the sediment, 'column' in Table 3.1) and from the sediment pore water ('pore') in the cryoconite holes in the beginning of the melt season in 2017 from Greenland Black and Bloom (n= 22) and Point 660 (n=3). Samples were filtered through 0.22 µm Polyvinylidene Fluoride (PVDF) filters (Millipore) to remove particles, frozen in Eppendorf tubes within 2h and transported frozen to Cardiff, where they were kept frozen at -20 °C until further analyses.

Additionally, water was sampled from the defrosted cryoconite sediment samples from all three polar locations in the laboratory (Table 3.1). These water samples were centrifuged (3 min at 14 000 rpm at room temperature), and the supernatant was collected and filtered through 0.22 µm PVDF filters (Millipore) to remove particles ('defrosted' in Table 3.1). Water chemistry was analysed as described in section 3.5.2.

Table 3.1. Water samples from in situ cryoconite holes and defrosted cryoconite samples. *blank = MilliQ deionised water; pore = sediment pore water; column = cryoconite hole water (above sediment); defrosted = water from defrosted cryoconite sample; cryo = cryoconite hole; stream cryo = accumulated cryoconite material connected by stream; ice core = cryoconite material from the top layer of ice cores*

Sample Location	Year	Water sample	Type	Number of samples
Greenland margin	2017	pore	cryo	3
		column	cryo	3
		blank		3
Greenland interior	2017	pore	cryo	10
		above	cryo	9
		blank		3
		defrosted	cryo	5
		defrosted	stream cryo	2
		defrosted	ice core	3
Svalbard	2015	defrosted	cryo	1
	2016	defrosted	cryo	5
Antarctica	2005/6	defrosted	cryo	4

3.4. Field methodology

3.4.1. Oxygen measurements in the field

At Black and Bloom site, *in situ* measurements of oxygen profiles of cryoconite holes were undertaken over several days in July 2016 using the microoptode described below, at 0.5 mm increments measured by a manual micromanipulator (Fig. 3.7). Three different types of holes with different sediment depths and water levels were chosen: 'classic' cryoconite holes, with >10 cm of overlying water and sediment depth of 3-5 mm; 'partly drained and accumulated' holes with 0.5-2 cm of sediment and 1 cm of water; and 'drained and accumulated' cryoconite material deposited on the side of the streams, with >2 cm of sediment and no overlying water. Where possible, measurements of multiple cryoconite holes (n=2 or 3) of the same type were performed ('classic' and 'drained and accumulated'), but n=1 for 'partly drained and accumulated'.

Figure 3.7. Measurement of oxygen profile in situ. *Microoptode positioned with a manual micromanipulator for in situ measurement of an oxygen profile in a cryoconite hole on the surface of the Greenland Ice Sheet.*

Oxygen concentrations in the cryoconite holes were measured using a microsensor. The oxygen microoptode with a tip diameter of 230 µm (Unisense, Denmark) was calibrated using sodium sulphite solution to give a solution with 0 µmol oxygen concentration (as recommended by Presens, www.presens.de), compared to a 100% oxygen measured in humid air just above the water surface. Each measurement lasted 30-60 s until the signal stabilised and were logged every 1 s using Unisense Multimeter datalogger. The oxygen concentration at each depth point was determined by taking a mean of the three last readings for each depth in the cryoconite hole.

3.4.2. Sediment incubations in the field

Incubations of cryoconite material were carried out at Greenland Black and Bloom site (ice sheet interior) over 20 days in July 2016. Several samples from the same sampling location were pooled together to assure sufficient material for incubations. Previous research has suggested that the effect of pooling of neighbouring cryoconite holes in the Arctic is negligible due to similarities of bacterial communities at the small scale (Edwards et al., 2014). Incubating vessels were custom-built using a 3D printer and food-approved ABS plastic (nontoxic), which was sterilised with 70 % ethanol and pre-washed with deionised, autoclaved water. Each incubation vessel, with pre-drilled ports for instrumentation (Fig. 3.8), was filled with ~28 g of cryoconite sediment (± 5%) and 77 ml of stream water, leaving ~10 ml head space. Cryoconite samples were incubated under the following conditions: a) light, open to the atmosphere (n=3); b) dark, wrapped in aluminium foil, but open to the atmosphere, via small, shaded holes (n=3); c) light, closed, where the vessels were sealed with a transparent, acrylic Perspex lid which limited gas exchange with the atmosphere (n=3); and d) dark, closed, with vessels sealed with a lid and wrapped in aluminium foil (n=3). Shortly after the incubation vessels were filled with the sediment, the series of oxygen profiles was measured by the method described above in section 3.4.1.

***Figure 3.8. A custom-made incubation vessel**, manufactured using 3D printing with sampling ports on the sides to enable measurement of oxygen, pH and microbial community changes with depth.*

Incubation vessels were placed in a large (50 cm diameter), hydrologically connected cryoconite hole for the first few days of the incubation (Fig 3.9). After three days, unequal melting of the cryoconite hole caused by the shading effect of the incubated vessels caused the vessels to fall over. These were refilled with water and transferred to a new incubation position on a tray in a small supraglacial stream and re-measured, after stabilising of the profile, several days later. Once on the tray, water exchange with the stream was impeded. In order to replicate this effect in the 'open' incubations, approximately 30 ml of water was removed and replaced by cryoconite hole water every day in each 'open' incubation using a large pipette, pre-washed with stream water.

Figure 3.9. Cryoconite sediment incubations under in situ conditions. *3D-printed vessels incubated in a large cryoconite hole on Greenland Ice Sheet (2016).*

3.4.2.1. Subsamples from the incubation vessels

Subsamples were taken from the initial sediment mix used for incubations for analysis of microbial community structure (t0). Water and sediment samples were collected with a sterile syringe and needle through the dedicated ports (see Figure 3.8) after 20 days of incubation (t20) from the sediment surface, 0.5 cm and 1 cm depths. Samples were frozen in Eppendorf tubes in liquid nitrogen and transported frozen to Cardiff, where they were kept frozen at -80 °C until further analyses. Water chemistry was analysed as described in section 3.5.2. Microbial DNA was isolated from sediment samples and the community structure was analysed as described in section 3.5.3. Details on the type and time of incubation as well as subsampling of the field and laboratory incubations can be found in Table 3.2.

3.5. Laboratory methodology

3.5.1. Incubations of cryoconite sediment in the laboratory

Analogous to incubations in the field (section 3.4.2), cryoconite sediment samples from all three polar locations were incubated in the laboratory (Table 3.2). Additionally, intact cryoconite granules and sediment aggregates from GrIS (margin and interior respectively) were incubated (Fig 3.10).

Community incubations: Prior to experiments, cryoconite sediment samples listed in Table 3.2 were defrosted for 48 h at 4 °C in darkness, pooled together to ensure sufficient material and incubated in the 3D printed vessels covered with acrylic Perspex transparent lids. They were

incubated in Weiss VT LED-illuminated low temperature environmental cabinets at 0.1 °C for 175 days. Samples were incubated in light (n=3, photosynthetically active radiation (PAR) = 145 µmol m^{-2} s^{-1}); and in dark conditions (n=3; wrapped in aluminium foil). Temperature and PAR were monitored continuously using Apogee Quantum and Campbell Scientific 107 sensors, logged with a Campbell Scientific CR7 datalogger.

Granule incubations: Intact cryoconite granules and aggregates (Fig 3.10) were defrosted and placed in freshwater media (without carbon substrates) in sterile petri dishes and incubated for 1h at 0.2 °C under PAR = 145 µmol m^{-2} s^{-1} (to allow time for potential photosynthesis), after which the oxygen profiles were measured as described below. Freshwater media for the cryoconite granule incubation contained the following components: NaCl (0.1 g l^{-1}), $MgCl_2 \cdot 6H_2O$ (0.25 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (0.1 g l^{-1}), KCl (0.1 g l^{-1}), NH_4Cl (0.1 g l^{-1}), and KH_2PO_4 (0.1 g l^{-1}). The medium was supplemented with 1 ml l^{-1} of the trace element solution "SL 10" (Widdel et al., 1983) and 0.2 ml l^{-1} of a selenite and tungstate solution (Sass et al., 1997). It was buffered with HEPES (2.38 g l^{-1}) and the pH was adjusted to 7.2 with 1 M NaOH prior to autoclaving. The granules were incubated for >2 years and the evaporated media was replenished with deionised MiliQ water approximately every three months.

Figure 3.10. Laboratory incubations of cryoconite granules from Greenland Ice Sheet. *The granules were sampled from the margin from Russell glacier (left) and from the inside of the Ice Sheet in the Black and Bloom camp (right).*

Table 3.2. Field and laboratory incubations of cryoconite material. *The type, time and subsampling days of incubations are listed.*

Cryoconite holes location	Cryoconite sampling year	Name	Incubation set	Incubation sampling days	Light	Incubation sampling depths (cm)	n
Greenland interior	2016	BB field	field	20	light	0, 0.5, 1	3
					dark		
	2017	BB lab	I	92	light	1	3
					dark	0, 0.5, 1	3
	2016	BB lab	II	114	light	1	2
					dark	1	2
	2016	BB aggregates	granules	730	light	-	1
Greenland margin	2014	G	I	35; 175	light	0, 0.5, 1	3
					dark	0, 0.5, 1	3
	2014	margin granules	granules	730	light	-	
Svalbard	2015	Sv	I	35; 175*	light	0, 0.5, 1	3
					dark	0, 0.5, 1	3
	2016		II	114	light	1	2
					dark	1	1**
Antarctica	2005/6	Ant	I	35; 175***	light	0, 0.5, 1	3
					dark	0, 0.5, 1	3
			II	114	light	1	2
					dark	1	2

* Only 1 subsample was taken at 175 days from 1 cm (due to technical challenges)
** Only 1 subsample was taken (due to technical challenges)
***Two of the dark incubations were leaking and were excluded from 175 days analysis

3.5.1.1. Oxygen Measurements of the sediment incubations

Oxygen concentrations in the sediments were measured using microsensors, similar to the field measurements (Section 3.4.1). During the laboratory community incubations, a Clark-type oxygen micro-cathode with a tip diameter of 500 µm (Unisense, Denmark) was used to measure

oxygen at 1 mm increments, starting in the water above the sediment. The electrode was positioned using a motor micromanipulator. Each measurement lasted 30-60 s until the signal has stabilised and were logged every 1 s using Unisense Multimeter datalogger. The oxygen concentration at each depth point was determined by first taking a mean of the three last readings for each depth in the individual incubation vessels, and then in triplicates of each type (light and dark). For the first week, measurements were taken every other day; for the next 4 weeks, the measurements were taken weekly; and for the remaining 5 months, monthly. The oxygen microelectrode was calibrated using sodium sulphite solution to give a solution with 0 µmol oxygen concentration (as recommended by Presens, www.presens.de), compared to a solution 100% saturated with oxygen which was created by bubbling ice-cold water with air for 5 min.

Oxygen concentrations in the cryoconite granules were analysed only in Greenland margin incubations and were measured at 0.2 mm increments within the granules using an oxygen microoptode with a tip diameter of 230 µm (Unisense, Denmark). The microoptode was positioned by a manual micromanipulator (Figs 3.7 and 3.11). Measurements were logged every 1 s using Unisense software, and each measurement lasted 20-30 s until the signal stabilized. The oxygen concentration for each depth was determined by taking a mean of the three last readings.

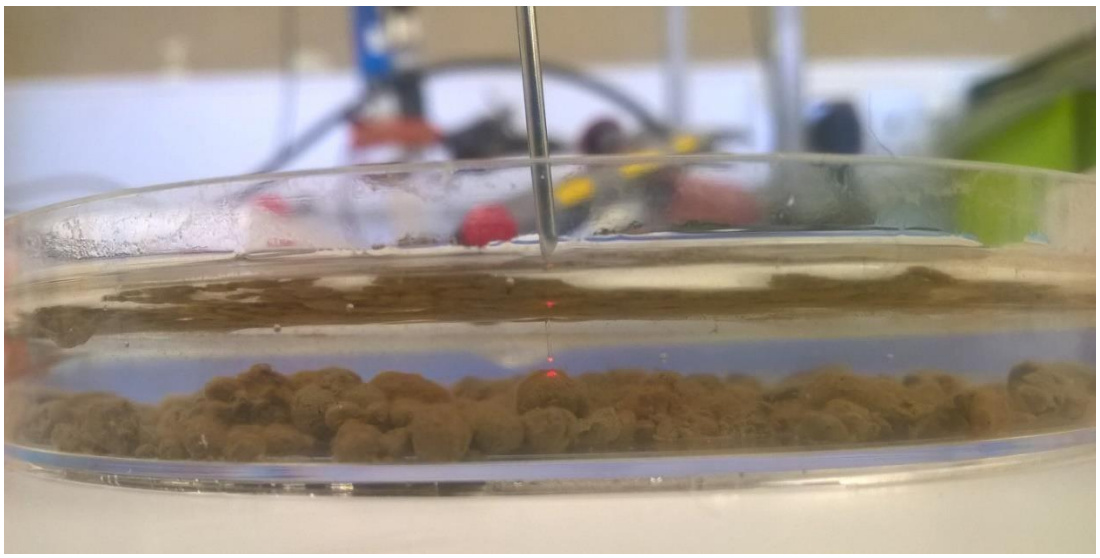

Figure 3.11. Laboratory measurement of oxygen profile of granule*. The tip of microoptode with measuring light (red) is visible just above the surface of the granule.*

3.5.1.2. Oxygen data analysis

NEP (Net Ecosystem Production) as mg C g^{-1} was calculated as the difference between oxygen concentration in light and dark incubations for each sediment depth, multiplied by the molecular mass of carbon , assuming a 1:1 respiratory quotient. The sediment surface was identified in each profile using an in-house Python script (Python Software Foundation) and profile shape comparison, which matched up repeated measurements.

3.5.1.3. pH measurements of the sediment incubations

pH profiles in the laboratory sediment incubations were measured using a Unisense pH microelectrode with a tip diameter of 500 µm. pH was measured in 1 mm increments, beginning in the water above the sediment. The electrode was positioned using a motor micromanipulator. Each measurement lasted 10-20 s until the signal has stabilised and measurements were logged every 1 s using Unisense Multimeter datalogger. The pH value at each depth was determined by first taking a mean of the ten last readings for each depth in the individual incubations, and then in triplicates of each type (light and dark). The pH electrode was calibrated using low ionic strength buffers of pH 4.1 and 6.96 (CamLab, UK). The measurements were taken in the beginning and the end of long-term incubations, where possible. However, breakage of the microsensors prevented obtaining the collection of a complete dataset.

3.5.1.4. Subsamples from the incubation vessels

Analogous to field incubations (Section 3.4.2.1), the initial sediment mix used for incubations was collected for the analysis of microbial community structure (t0). Water and sediment samples were collected with a sterile syringe and needle through the dedicated ports after 35 (t1) and 175 (t2) days of incubation at the sediment surface, 0.5 cm and 1 cm depths. A second set of incubations was sampled after 114 days. Samples were frozen in Eppendorf tubes at -80 °C until further analyses. Water chemistry was analysed as described in section 3.5.2. Microbial DNA was isolated from sediment samples and the community structure was analysed as described in section 3.5.3. Water samples from granule incubations were taken with a sterile syringe and needle after 730 days from the bottom of granule layer were taken and analysed as described in section 3.5.2. Details on the type and time of incubation as well as subsampling of the field and laboratory incubations can be found in the table 3.2.

3.5.2. Water chemistry

Major ions (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}, Mg^{2+}, Ca^{2+}, Na^+ and K^+) and the organic acids (formate, acetate, lactate, and propionate) were quantified using capillary ion chromatography on a ThermoScientific Dionex analytical ICS-5000+ equipped with AS-AP autosampler. Chromatographic separation was conducted on two Ionpac AS15 columns in series and the determination of species was carried out using an Anion Self-Regenerating Suppressor (ASRS 2-mm) unit in combination with a CD conductivity cell. The gradient programme was as followed: 6 mM KOH (31 min), 70 mM (18 min), 6 mM (3 min). The resulting values were compared against previously made standards. The Dionex was operated by the laboratory technician Mrs Xiaohong Tang.

Prior to ion chromatographic analysis, 1 ml of culture or pore water was centrifuged (3 min at 16,000 × *g* at room temperature), and the supernatant was collected and filtered through 0.22 µm PVDF filter (Millipore) to remove the particles.

3.5.3. Microbial community structure

3.5.3.1. DNA isolation from the sediment

Microbial community structure was analysed at the Natural History Museum (NHM, London). A total of 100 sediment subsamples were collected for microbial structure analysis as described in section 3.4.2.1 and 3.5.1.3. Total genomic DNA was extracted from 0.05 - 0.3 g sediment samples with the use of DNeasy PowerSoil isolation kit (Qiagen) according to manufacturer's instructions. Briefly, samples were shaken for 20 min with the beads and lysis buffer to disrupt the cells, and DNA released into the solution was cleaned from proteins and membrane leftovers in the subsequent cleaning steps. The DNA quality and quantity was assessed using a NanoDrop (NanoDrop 3300 Fluorospectrometer, ThermoScientific). DNA extracts were stored frozen (−20°C) before subsequent processing.

3.5.3.2. DNA sequencing

The 16S rDNA fragments were amplified by polymerase chain reaction (PCR) method. Each PCR reaction was performed with 0.5, 1 and 1.5 µl of isolated genomic DNA to ensure the amplification of less abundant and more abundant DNA fragments. Extracted DNA was amplified in the following PCR conditions: initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 50 °C for 60 s and 72 °C for 90 s; with final extension of 72 °C for 10 min. The reaction mix contained an unspecific forward primer 515F (5'-GTG CCA GCM GCC GCG GTA A -3')

and 806R sample-specific reverse primer (5'-GGA CTA CHV GGG TWT CTA AT-3'). The unique sequences of reverse primers were used to barcode individual samples for the bioinformatic analysis (Appendix A). The yield of PCR reaction was visualised on 1% agarose gel to check for contamination and quality of reaction. The PCR products were then cleaned by AxyPrep Mag PCR clean-up kit (Axygen) to remove the short DNA fragments, remaining primers and nucleotides. The triplicates with different initial concentrations of genomic DNA were then pooled together and the concentration double-stranded amplified DNA was checked on Qubit 2.0 fluorometer (Life Technologies). A total of 100 ng of DNA per sample was pooled for the subsequent Next Generation Sequencing (NGS) using a 250 bp paired-end sequencing protocol on Illumina MiSeq platform.

3.5.3.3. Bioinformatic analysis

The QIIME2 software (Bolyen et al., 2019) was used to generate operational taxonomic units (OTUs) following standard pipelines by matching the sequences to SILVA 123 rRNA database (Yilmaz et al., 2014), using a 97% similarity threshold. Before conducting the statistical analyses, samples were rarefied to the minimal sampling depth. All statistical calculations were conducted in R 3.6.3, with the use of phyloseq (McMurdie and Holmes, 2013), ampvis2 (Andersen et al., 2018) and phylosmith (Smith, 2019) packages. The among-location differences in microbial communities were analysed using non-metric multidimensional scaling (NMDS) and Principal Coordinates Analysis (PCoA) also known as classical multidimensional scaling based on Bray–Curtis similarities and results were plotted in two dimensions. SIMPER analysis was performed to determine the contribution of 16S rRNA genotypes to the dissimilarity of community structures between locations. ANOSIM and PCoA analyses were used to test the differences in environmental parameters between selected samples.

3.5.4. Microbiological analysis of the sediments

3.5.4.1. Total cell counts

The total cell count of each of the sediment sample was performed using the epifluorescence microscopy following method of Cragg & Kemp (1995). Briefly, cryoconite sediment from each location (n=9) was diluted 1 to 10 in substrate free medium, then fixed in 1.6 % formaldehyde and stained with acridine orange. Total cell counts were determined in three technical replicates with a Zeiss Axioskop microscope after staining.

3.5.4.2. MPNs and cultivability

The Most Probable Number (MPN) technique was used to enumerate viable cell counts in the cryoconite sediment (n=3 for Greenland and Svalbard, n=2 for Antarctica). Following initial dilution of ~1 g of the sediment in the 9 ml of water, a series of eight 10-fold dilutions in three replicates was prepared on a 96-well plate (Köpke et al., 2005). Samples were grown at temperatures 0.2, 4, 10, 15, 20 and 30 °C under oxic or anoxic conditions. For aerobic microorganisms, a freshwater medium was used (Sass et al., 1997), containing the following components: NaCl (0.1 g l^{-1}), $MgCl_2 \cdot 6H_2O$ (0.25 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (0.1 g l^{-1}), KCl (0.1 g l^{-1}), NH_4Cl (0.1 g l^{-1}), KH_2PO_4 (0.1 g l^{-1}), casamino acids (0.25 g l^{-1}), and yeast extract (0.05 g l^{-1}). The medium was supplemented with 1 ml l^{-1} of the trace element solution SL 10, 0.2 ml l^{-1} of a selenite and tungstate solution (Sass et al., 1997). It was buffered with HEPES (2.38 g l^{-1}) and the pH was adjusted to 7.2 with 1 M NaOH prior to autoclaving. After autoclaving, the medium was supplemented with 2 ml l^{-1} of vitamin solution (Wolin et al., 1963) and glucose (4 mM l^{-1}). For anaerobic microorganisms, a bicarbonate-buffered fermenter medium was used, containing: $MgCl_2 \cdot 6H_2O$ (0.1 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (0.35 g l^{-1}), KCl (0.1 g l^{-1}), NH_4Cl (0.1 g l^{-1}), KH_2PO_4 (0.1 g l^{-1}), casamino acids (0.25 g l^{-1}), vitamin solution (2 ml l^{-1}), glycine betaine (0.5 mM), sodium acetate (0.5 mM), TCA mixture (0.5 mM (Köpke et al., 2005)), choline (0.5 mM), methylamine (0.5 mM), trace element solution SL 10 (1 ml l^{-1}), selenite and tungstate solution (0.2 ml l^{-1}). The medium was reduced with Na_2S (1.25 mM) and $FeCl_2$ (0.25 mM) solutions. Plates were incubated in air-tight bags with AnaeroGen sachet (Oxoid). MPN values were recorded after 71 days of incubation. Oxic growth was scored after visual inspection of the MPN plates. In anoxic incubations, growth was analysed after staining with SYBR green I dye (Martens-Habbena and Sass, 2006) and fluorescence analysis on a plate reader. MPN values with standard error and 95% confidence intervals were calculated according to de Man (1983). Viable cell counts obtained with the MPN technique were related to the total counts to estimate culturability.

3.5.4.3. Heterotrophic microbial isolates

The cultivable microorganisms were isolated from the highest positive MPN dilution, therefore representing the most abundant members of community. A sample of 20 µl from the MPN dilution was streaked on to a 1.5% (w/v) agar plate with freshwater medium or anaerobic medium. Anaerobic cultures were prepared in an anoxic chamber and cultured in air-tight bags with AnaeroGen sachet (Oxoid). At least three subsequent subcultures were streaked to obtain a pure culture. Anaerobic cultures were tested for growth in oxic conditions and for alternative

electron acceptor utilisation (nitrate, thiosulphate, iron, manganese, TMO, DMSO) (Süß et al., 2008).

3.5.4.3.1. Identification of isolates by 16S rDNA sequencing

Genomic DNA of each microbial isolate was extracted by bead beating at speed 5.5 m s^{-1} for 30 s (FastPrep 24 Instrument, MP biomedicals) in Guanidine Isothiocyanate lysis buffer (Invitrogen), and then purified with the use of an automated Maxwell® 16 Instrument and tissue DNA purification kits (Promega), following the manufacturer's instructions. Briefly, DNA cleaning steps were performed with the use of magnetic beads binding to the DNA. Genomic DNA concentrations were then quantified using a Qubit 2.0 fluorometer (Invitrogen), following the manufacturer's instructions.

Extracted DNA was amplified using primers targeting 16S rRNA genes, 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') and 907R (5'-GGT TAC CTT GTT ACG ACT T -3') (Webster et al., 2006). Fungal ITS fragment was amplified using primers ITS1f (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Ihrmark et al., 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) in the following PCR conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30s, 56 °C for 30s and 72 °C for 30s; with final extension of 72 °C for 7 min. The yield of PCR reaction was visualised on 1.2% agarose gel. Amplicons were then sequenced by Sanger sequencing with 27F or ITS1f at Eurofins Genomics (Germany) or DNA Sequencing and Services (University of Dundee). The nucleotide BLAST online tool (blast.ncbi.nlm.nih.gov) was used to determine the closest relative for each isolate. The 16S rRNA gene sequences determined in this study have been deposited in GenBank under the accession numbers MT430950, MT432272- MT432304, MT473233 and MT473713-MT473721.

3.5.4.3.2. Salinity, temperature and pH tolerance

The tolerance of the cryoconite isolates to environmental (temperature) and geochemical (pH and salinity) stresses was tested. Selected isolates were grown in duplicate in liquid freshwater medium at a range of temperatures (1-40 °C), salinities (0.1-10%) and pH (2.5-10.5). Salinities from 0.1% to 10% were achieved by adding saturated MgCl (18.75 g l^{-1}) and NaCl (290 g l^{-1}) solution to the freshwater medium. The pH was adjusted with 1M HCl, with different buffering solutions for pH 5.5-10.5 adopted from Kaksonen et al. (2006). For pH 2.5-5, 100 mM K_2HPO_4 was used. Growth was deemed positive or negative by presence of visible cell pellets when compared to negative control (uninoculated freshwater medium) after 30 days of incubation. Differences between microbial isolates' maximum salinity tolerance according to location, oxic/anoxic

isolation or bacterial/yeasts were compared using the Kruskal-Wallis test. Differences in the range of pH tolerated by the isolates were established by comparing the inoculated pH media with positive growth after 30 days. The number of tubes with positive growth at each pH was then compared between locations, oxic vs anoxic conditions and presence or absence of yeasts using ANOVA, followed by Tukey HSD.

3.5.4.3.3. Freeze-thaw survival

To identify whether cells from the isolated polar microorganisms were susceptible to freezing damage, microbial cultures were subject to alternating freeze-thaw cycles in Weiss VT low-temperature environmental cabinets. All of the bacterial isolates from oxic conditions (16) and representative yeast isolates (7 out of 9) were tested. The isolates were washed with mineral medium (NaCl (0.025 g l^{-1}), $MgCl_2 \cdot 6H_2O$ (0.09 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (0.025 g l^{-1}), KCl (0.025 g l^{-1}), counted and diluted to equal cell numbers in the mineral medium to exclude the potentially protective effect of substrate-rich medium and to minimize growth in between the cycles (Carvalho et al., 2004). Each cycle consisted of 6h at −18°C and 3 h at 0.9°C. Subsamples were taken after 1, 5, 25 and 100 cycles and cell viability was determined using the MPN technique after 30 days of incubation.

3.5.4.3.4. Substrate test

Substrate tests were set up in 96-well plates as described by Süß et al. (2008) to assess the physiological capabilities of microbial isolates. A total of 58 substrates were tested, including carbohydrates, carboxylic acids, amino acids, alcohols and others. The substrates were chosen to cover a wide range of possible substrates typically produced and utilized by microbes, as well as to cover a range of enzymes needed for different substrates (Appendix A). Bacterial isolate inocula were washed three times in substrate free medium (freshwater medium with no casamino acids, yeast extract, glucose or vitamins added) prior to the experiment, to avoid substrate carry-over. Washed cells were resuspended in substrate-free media and 50 µl added to each well containing 200 µl of medium containing a single substrate. Each substrate was tested at least in duplicate. The wells with positive growth were recorded when compared visually to negative controls (substrate-free medium).

Pearson correlation analysis of the response to the experimental conditions was performed in the 'Performance analytics' package in R for each pairwise combination: minimum and maximum pH tolerated, maximum salinity, maximum temperature, average substrate utilisation, and freeze-thaw survival for each isolate.

3.5.4.3.5. Fermentation

Pure microbial cultures of bacteria (35) and yeasts (8) isolated from cryoconite holes were incubated in freshwater medium in air-tight tubes to promote anaerobic conditions for 70 days. Following incubations, water chemistry of culture medium was analysed by DIONEX as described in section 3.5.2 to determine the concentrations of fermentation products (acetate, lactate, propionate, formate).

3.5.4.4. Phototrophic microbial isolates

The cultivable phototrophic microorganisms were isolated from the cryoconite sediment samples. Cells were collected by scraping the frozen cryoconite material from Antarctica, Greenland and Svalbard with a sterile spatula and placed in the 5 ml of ice-cold BG11 medium (Stanier et al., 1971). Additionally, a phototrophic cyanobacteria were isolated from an orange (Fig. 3.12) biofilm from long-term incubations from Greenland margin samples. BG11 medium was prepared by adding 1 ml of the stock solutions of disodium EDTA (1 g l^{-1}), citric acid (6 g l^{-1}), $NaNO_3$ (150 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (75 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (36 g l^{-1}), Na_2CO_3 to 1 l of miliQ water, autoclaving and 1 ml adding sterile-filtered stock solutions of K_2HPO_4 (30.6 g l^{-1}), ferric ammonium citrate (6 g l^{-1}), micronutrients mix: H_3BO_3 (2.86 g l^{-1}), $MnCl_2$ (1.81 g l^{-1}), $ZnSO_4$ (0.22 g l^{-1}), $CuSO_4$ (0.08 g l^{-1}), Na_2MoO_4 (0.39 g l^{-1}), $CoCl_2$ (0.04 g l^{-1}). Samples were incubated under light (photosynthetically active radiation (PAR) = 145 µmol m^{-2} s^{-1}) at 10 °C. Temperature and PAR were monitored continuously using Apogee Quantum and Campbell Scientific 107 sensors, logged with a Campbell Scientific CR7 data logger. Growth of cultures was monitored by light microscopy. Orange or green samples were streaked onto 1.5% agar plates with BG11 medium at least three subsequent times to get unialgal cultures. Obtained cultures were not axenic i.e. they grew with associated heterotrophic bacteria.

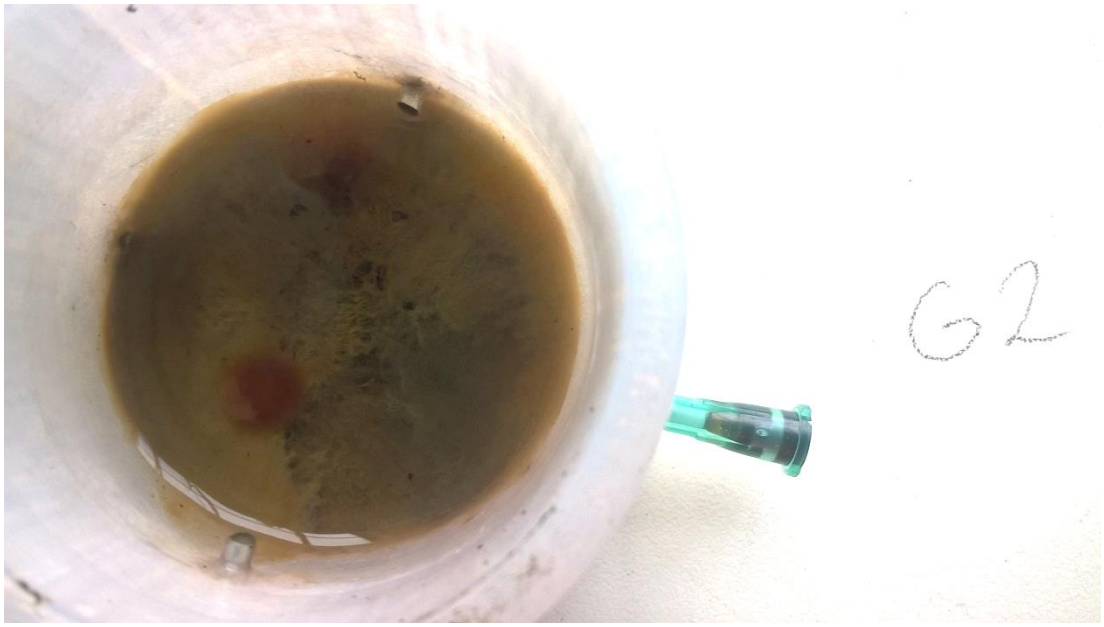

Figure 3.12. Top view of orange biofilm*. This biofilm has developed on the surface of Greenland cryoconite incubation under light conditions.*

3.5.4.4.1. Identification of phototrophic isolates by RuBisCO gene sequencing

Identification of phototrophic isolates took place in School of Biosciences (Cardiff University) by Iain Perry in Prof. Peter Kille's laboratory. Total genomic DNA was isolated from the phototrophic cultures following the modified extraction buffer by Fawley and Fawley (2004) and modified protocol from 'DNA from Blood and Tissue' kit (Qiagen). Briefly, 100 µl of each sample was mixed with 200 µl of extraction buffer (1M NaCl, 70 mM Tris, 30 mM EDTA), 25 µl of 10 % DTAB, some 0.1 mm glass beads and 200 µl of chloroform. The extraction mixture was then shaken on bead beater 2 x 30 s to disrupt the cell walls. Next the sample was centrifuged for 2 min at ~4700 rpm or until the layers clearly separated. Two hundred microliters of top layer (aqueous phase which contains the dissolved DNA) was transferred to a new tube and the QIAGEN protocol to isolate DNA from cultured cells was followed, beginning with step 2. The concentration of DNA was checked on Nanodrop.

The RuBisCO gene fragment was was amplified from isolated genomic DNA by PCR method. Rubisco is a key enzyme in photosynthesis and is found primarily in photoautotrophs and chemolithotrophs (Paul, 2013). Rubisco subunit *rbcL* gene was targeted with primers: Nxt_957F (*TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG* RTG GAT GCG TAT GKS WGG) and (Nxt_1538R *GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G*AA RCA ACC TTG TGT AAG TCT) where fragments in italics are commercial tags (Nextera, Epicentre) used to mark samples for sequencing. The PCR reaction mixture contained per 20 µl: 4 µl of 5x GO Taq buffer, 2 µl of 25mM

MgCl2, 0.4 µl of 10 mM dNTPs, 1 µl of 10mM forward primer (CfD_F), 1 µl of 10 mM reverse primer (DPrbcL7_R), 0.2 µl of Go Taq Polymerase, 11.4 µl of H_2O and 1 µl of isolated DNA. PCR conditions were as follows: initial denaturation 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s, finished with 72 °C for 10 minutes and an infinite hold stage at 4 °C. The PCR reaction product was electrophoresed to check for contamination and quality of reaction. The electrophoresis was performed on the automated QIAxcel Advanced system (Qiagen) using ready-to-run gel cartridges. The PCR reaction was sequenced by the sequencing facility in the School of Biosciences.

3.5.4.4.2. Fermentation of phototrophic organisms

In order to determine the potential of phototrophic community to produce acetate, cultures were subsequently incubated in 6 different treatments: normal light, high light, high light with low nutrients, dark, anaerobic and anaerobic fermentation (Table 3.3). Stress conditions, especially high light with limited nutrients, can induce production of carbon-rich exudates by phototrophic organisms to get rid of excess carbon assimilated by photosynthesis (Grossart and Simon, 2007; Abed, 2010; Pannard et al., 2016).

Table 3.3. Incubations of phototrophic cultures. *Several treatments were applied to promote exudation of acetate or other carbon-rich compounds.*

Treatment	**PAR** (µmol m^{-2} s^{-1})	**Medium**	**Culture vessel**	**Description**
normal light	145	BG11	flask	growth control
high light	550	BG11	flask	stress factor
high light with low nutrients	550	BG11 with 10x less N and P	flask	stress factor
dark	dark	BG11	flask	no photosynthesis
anaerobic	dark	BG11	air-tight tube	promotes heterotrophic growth
anaerobic fermentation	dark	freshwater	air-tight tube	promotes heterotrophic growth

3.5.4.4.3. Fluorescence measurements of photophysiology

Selected phototrophic cultures were transported to the laboratory at Mt Alison University, Sackville, Canada to perform photophysiology measurements. There, cultures were grown in triplicate in glass conical flasks in BG11 medium at 10 °C under PAR = 60 µmol m^{-2} s^{-1} (sample C4 from Greenland interior) or PAR = 135 µmol m^{-2} s^{-1} (sample G2 from Greenland margin), with a

16 h/8 h light/dark photoperiod. The fitness of selected phototrophic cultures was assessed by photophysiology measurements, to determine the impact of ubiquitous fermentation products found in cryoconite incubations and produced by cryoconite isolates. Acetate at 1 mM concentration was chosen as the most abundant fermentation product found in the incubations. The culture from Greenland interior (C4) was incubated with 1 mM of acetate for 1h and 24h. Because of time constraints, Greenland margin culture was incubated for 1h only. Samples were analysed by rapid light curves approach (Perkins et al., 2018; Xu et al., 2018), which provides information not only on the saturation characteristics of electron transport rate (ETR) in the photosynthetic apparatus, but also on the photosynthetic performance of a phototrophic organism. The photophysiology of samples was measured on a Photon Systems Instruments FL3500 fluorometer system (Brno, Czech Republic). Samples were exposed to the 30 s increments of light of increasing intensity to result in a saturating rapid light curve. After each exposure a fast repetition rate (FRR) fluorescence induction curve was induced to measure chlorophyll fluorescence (Kolber et al., 1998; Perkins et al., 2018). A series of 40 red flashlets was applied with a duration of 1.2 µs each of them followed by 1.0 µs of darkness. Cyanobacteria utilise red light more efficiently than blue light commonly used in photophysiology measurements of algae and plants (Luimstra et al., 2018). The intensity of the flashlets was adjusted in the pilot experiment to ensure reaching the fluorescence plateau within around 30 of 40 flashlets (Perkins et al., 2018). The 'psifluo' package in R was used to analyse the data from the fluorometer system: the model was fitted using the equations of Kolber et al. (1998) and ETR values were generated on the default settings, which follows Suggett et al. (2009) equation:

$$\mathrm{ETR}\ (e^{-1}s^{-1}) = \frac{\sigma_{\mathrm{PSII}}}{\frac{\mathrm{F_V}}{\mathrm{F_M}}} \times \mathrm{YPSII} \times \mathrm{I} \times (6.022 \times 10^{17}\mathrm{photons}\mu mol^{-1}) \times (1 \times 10^{-20}\mathrm{m^2A^{-2}})$$

Where σ_{PSII} is effective absorption cross section for PSII photochemistry; F_V/F_M is maximum quantum yield measured in darkness; YPSII is the yield of photochemical electron transport and I is actinic irradiance (µmol photons m^{-2} s^{-1}). All these parameters were generated within 'psifluo' package. The ETR saturation curves were compared between untreated samples and samples incubated with acetate.

3.5.5. Statistical analyses

All statistical analyses of data were performed using Real Statistics Resource Pack software for Excel (Release 7.2) (Zaiontz, 2020) and Python v.2.7 (Python Software Foundation). Plotting of data was performed in Python v.2.7 in chapters 4-6 and in R v.3.6.3 (R Core Team 2014) in chapter 7. Prior to all analyses, normality of data was tested using the Shapiro - Wilk test, and

homogeneity of variance using Levene's test. Differences between more than two levels of independent variable (e.g. three different locations in pH analysis in chapter 5) were examined using ANOVA. Post hoc Tukey's Honest Significant Differences analysis was performed on significant ANOVA results. If the data were not normally distributed, Kruskal-Wallis test was used (e.g. differences in salinity tolerance between locations in chapter 5). Comparison between two levels of a single independent variable was performed by t-test (e.g. differences between acetate concentrations in chapter 7).

Chapter 4

Oxygen in cryoconite holes

4.1. Introduction

Cryoconite holes are a hotspot for this microbial activity on glaciers, yet little is known about the oxygen status of the cryoconite sediment and consequently about the spatial heterogeneity of heterotrophic activity. In this chapter, the oxygen microelectrodes and microoptodes were used to measure oxygen concentrations at the microscale, for the first time revealing a potential niche for anaerobic microorganisms and anaerobic processes. *In situ* measurements on the Greenland Ice Sheet showed a range of oxygen profiles depending on the sediment structure. Long-term incubations of cryoconite sediment from three polar locations showed rapid development of persistent anoxic zones.

4.2. Oxygen profiles in cryoconite holes

4.2.1. *In situ* oxygen profiles of cryoconite holes and cryoconite granules

The shape and size of cryoconite holes seemed to be constrained by local ice surface morphology and hydrology, in common with (Cook et al., 2015). The most abundant types observed on this sector of the GrIS were very small (<5 cm), circular holes (Fig. 4.1A) or 'classic' cryoconite holes with a semi-circular shape (Fig. 4.1A), water depth of 10-30 cm and sediment depth of 0.2-0.5 cm. When several of these holes are connected by melting, they create irregularly shaped, 'partly drained and accumulated' cryoconite holes, with varying water depth of 1-50 cm and sediment depth 0.5-1 cm (Fig. 4.1B). This type is less abundant than the 'classic' and circular holes. Finally, when these bigger holes are drained as a result of hydrological processes, they create thick accumulations of sediment with depths of 1-5 cm, usually not covered with water. These are the least abundant, but are notable features in the landscape (Fig. 4.1C), often found accumulated on a side of small/drained supraglacial streams or old, drained holes.

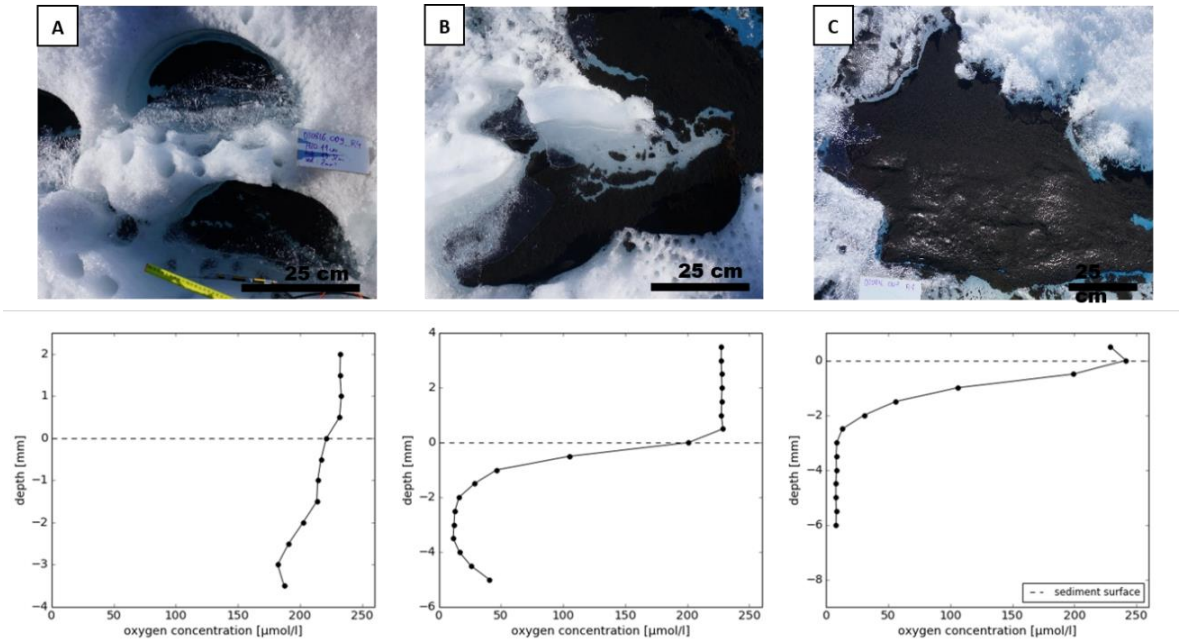

***Figure 4.1. In situ oxygen profiles of varying morphologies of cryoconite sediments**. The profiles were measured with a microoptode in a variety of cryoconite sediment accumulations *note the different vertical scale on the graphs. A. "Classic" cryoconite hole, with a regular, semi-circular shape. Water depth was approximately 11 cm and sediment depth approximately 4 mm. B. Cryoconite sediment in an irregular shape hole, with water depth of 1-7 cm and sediment depth 1 cm. C. Thick layer of cryoconite material (1.5 cm) without a layer of water.*

Each type of cryoconite hole was characterized by different oxygen profiles, shown in Figure 4.1. In the 'classic' type, oxygen decreases steadily with depth, by 90.00 µmol l^{-1} when compared to the overlying water at 3 mm depth, but did not become anoxic (Fig. 4.1A, and Appendix B). Figure 4.1B shows an intermediate stage of cryoconite material accumulation. The 'partly drained and accumulated' hole was connected to the small supraglacial stream and collected additional material, either transported by stream or by the coalescence of several, nearby holes, similar to observations by Fountain et al. (2008). These holes have an irregular shape, resulting in uneven sediment and water depth. The oxygen profiles were characterized by a rapid decrease of oxygen with depth (54.33 ± 63.32 µmol mm^{-1}), becoming almost anoxic at 2 mm depth. Below the anoxic layer of sediment at 2 mm depth, oxygen concentrations increase gradually towards the bottom of the hole, from <10 µmol l^{-1} at 2 mm to 50 µmol l^{-1} at 5 mm depth. The oxygen profile of the 'drained and accumulated' cryoconite (Fig. 4.1C) was marked by a small increase in oxygen concentration at the surface of the sediment (24.87 µmol mm^{-1} in the top 1 mm of the profile), which was not observed in the other types. Below the sediment surface, oxygen rapidly decreased with depth, reaching a state of anoxia at 4 mm. Oxygen profiles of individual cryoconite granules (Fig. 4.2) also showed a rapid decrease of oxygen with depth (42.66± 11.57 µmol mm^{-1}), albeit on a much smaller scale, with the centre of the granule

becoming almost anoxic in all four granules profiled, regardless of their size (2-4 mm, see Appendix B).

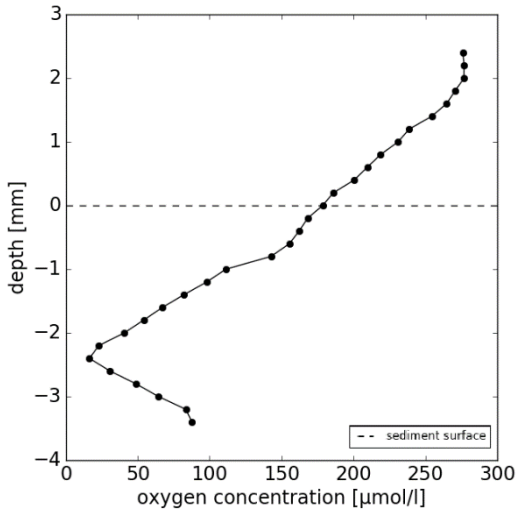

Figure 4.2. Oxygen profile measured using a microsensor within a 4 mm wide, spherical cryoconite granule.

4.2.2. Short-term field incubations of cryoconite sediment

The time series of oxygen profiles and resulting NEP from the field incubations of cryoconite sediment and water in the custom-built 3D printed vessels are shown in Fig. 4.3 and Fig. 4.4. In the open vessels during the first hours of the experiment (Fig. 4.3), the profile changed from oxic to anoxic at 8 mm depth within 90 min. This profile shape remained throughout the course of the incubations, although the oxic-anoxic interface showed vertical migration in some incubations (Fig. 4.4).

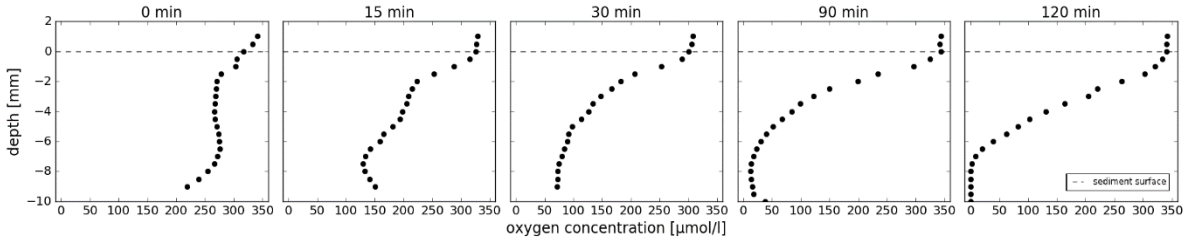

Figure 4.3. Temporal development of a characteristic oxygen profile in 10 mm thick cryoconite sediment after perturbation.

The dark, light, open and closed incubations all developed an anoxic layer within a few hours of mixing (Fig. 4.4), and over time the anoxic layer got thicker – by day 20, there was anoxia at 7 mm, compared with 8 mm on day 6 in the open incubations. The open incubations, which could exchange water with the supraglacial system and gas with the atmosphere, showed greater differences between dark and light incubations than the closed incubations (mean differences: 50.49 ± (st. dev.) 43.70 µmol l^{-1} and 1.81 ± 22.88 µmol l^{-1} in open vessels on day 6;

35.43 ± 31.94 µmol l^{-1} and -17.62 ± 23.86 µmol l^{-1} in closed vessels on day 20). The calculated NEP values in open incubations on the sediment surface increased from 0.5 mg C l^{-1} to 0.69 mg C l^{-1} between day 6 to day 20, whilst in the closed incubations, the calculated NEP values decreased from 0.4 to 0.15 mg C l^{-1} during this time. In general, carbon production decreased with time: the maximum calculated C production in open incubations was lower on day 20 than day 6 (1.62 compared with 0.92 mg C l^{-1}). Moreover, the closed incubations generally show smaller production values, indicating little difference between the dark and light incubations.

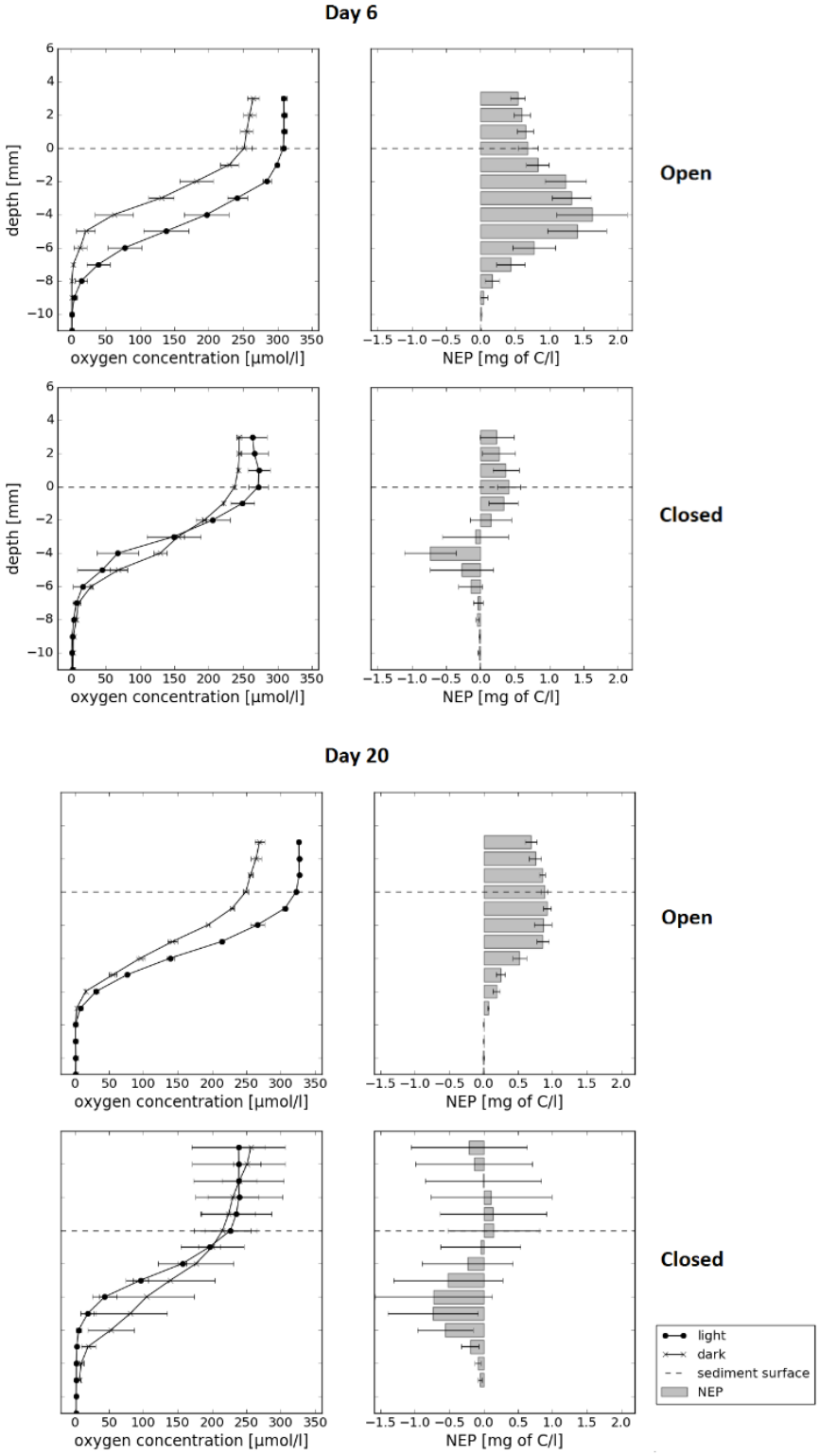

Figure 4.4. Oxygen profiles of 20 day open and closed cryoconite field incubations with calculated NEP. *Error bars show standard deviation of triplicate bottle measurements of oxygen, and standard error of NEP calculations.*

4.2.3. Long-term laboratory incubations of cryoconite sediment

4.2.3.1. Oxygen profiles

The evolution of the oxygen profile and associated NEP of long-term incubations conducted in the lab was tested for Greenland (Fig 4.5), Antarctic (Fig 4.6) and Svalbard (Fig 4.7) samples.

In Greenland samples in the first week, dark and light profiles resembled each other at the sediment surface, with some differences observed in the deeper layer (Fig. 4.5A): oxygen concentrations below the surface are slightly higher in light than in dark incubations (mean: 51.9 ± 15.9 µmol l^{-1}). After forty days (Fig. 4.5B), the greatest differences between oxygen concentrations in the light and dark bottles throughout the profile depth were observed (up to 144.75 µmol l^{-1}, mean: 100.3 ± 46.9 µmol l^{-1}), as well as a difference of 3 mm depth between the location of anoxic layers in the light and dark incubations. By day 116 (Fig. 4.5C) the profiles become similarly shaped again, although oxygen concentrations in the light incubations are lower on the surface (33.5 ± 5.27 µmol l^{-1}) and higher within the sediment (32.7 ± 25.3 µmol l^{-1}) than in the dark incubations. The depth of the anoxic zone at the end of the incubations is approximately the same as on day 8, at 5 mm.

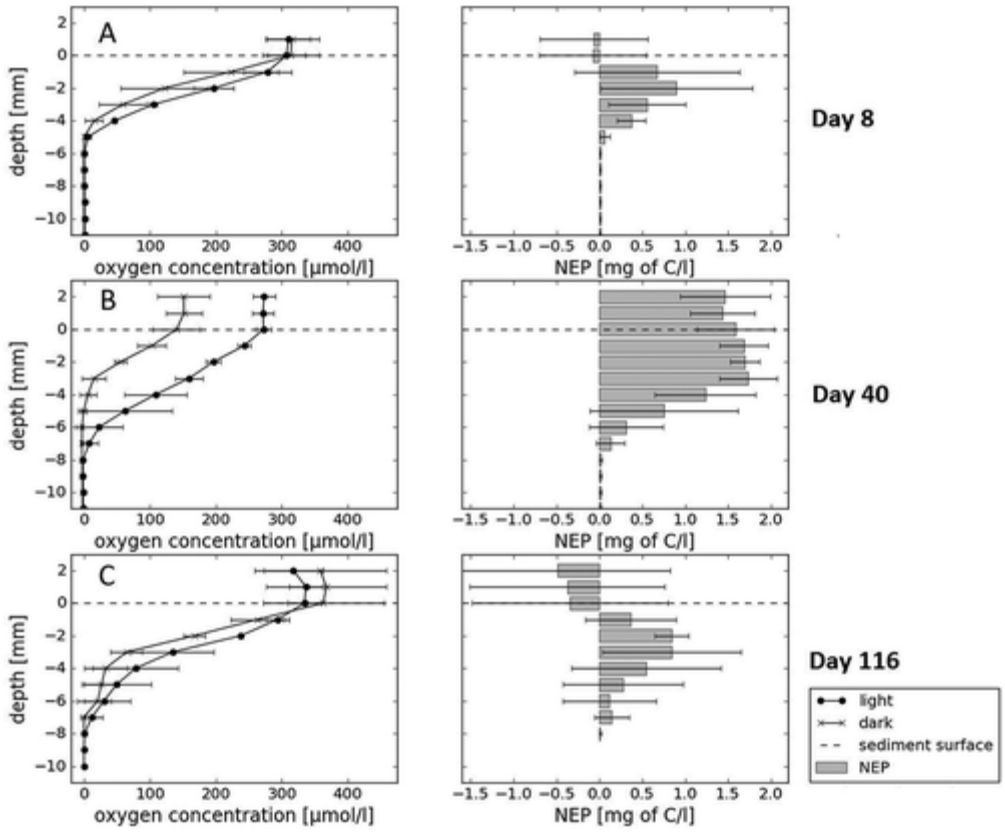

Figure 4.5. Oxygen profiles of long-term, closed laboratory incubations of cryoconite material from Greenland at 0.2 °C for more than four months with calculated NEP. *Error bars show standard deviation of triplicate bottle measurements of oxygen, and standard error of NEP calculations*

Evolution of oxygen profiles in Antarctic cryoconite sediments follows slightly different pattern than Greenland ones. The biggest differences in NEP between dark and light samples are observed in the first two weeks of incubations (up to 0.37, mean of significant differences 0.32 ± 0.06), when primary production is clearly visible at the sediment surface (Fig 4.6A). Below the sediment surface, anoxic zones forms in both treatments at the same depth (11 mm). After 31 days of incubations, the significant differences between light and dark incubations disappeared and anoxic zone levelled off at 7 mm (Fig 4.6B). No significant NEP was observed, in contrast to Greenland samples around the same time. Similarly, no significant primary production was detected at the sediment surface in the end of incubations (Fig 4.6C). Primary production was visible in the individual profile of sample A1 in the light conditions (Fig. 4.7), but not apparent when averaged with other samples. In the deeper layers of sediment, oxygen consumption was greater in the light treatment (-0.95 ± 0.15 NEP), where anoxic layer stabilised at 8 mm. In the dark incubations, oxygen concentrations decreased less with depth and anoxia was reached around 15 mm.

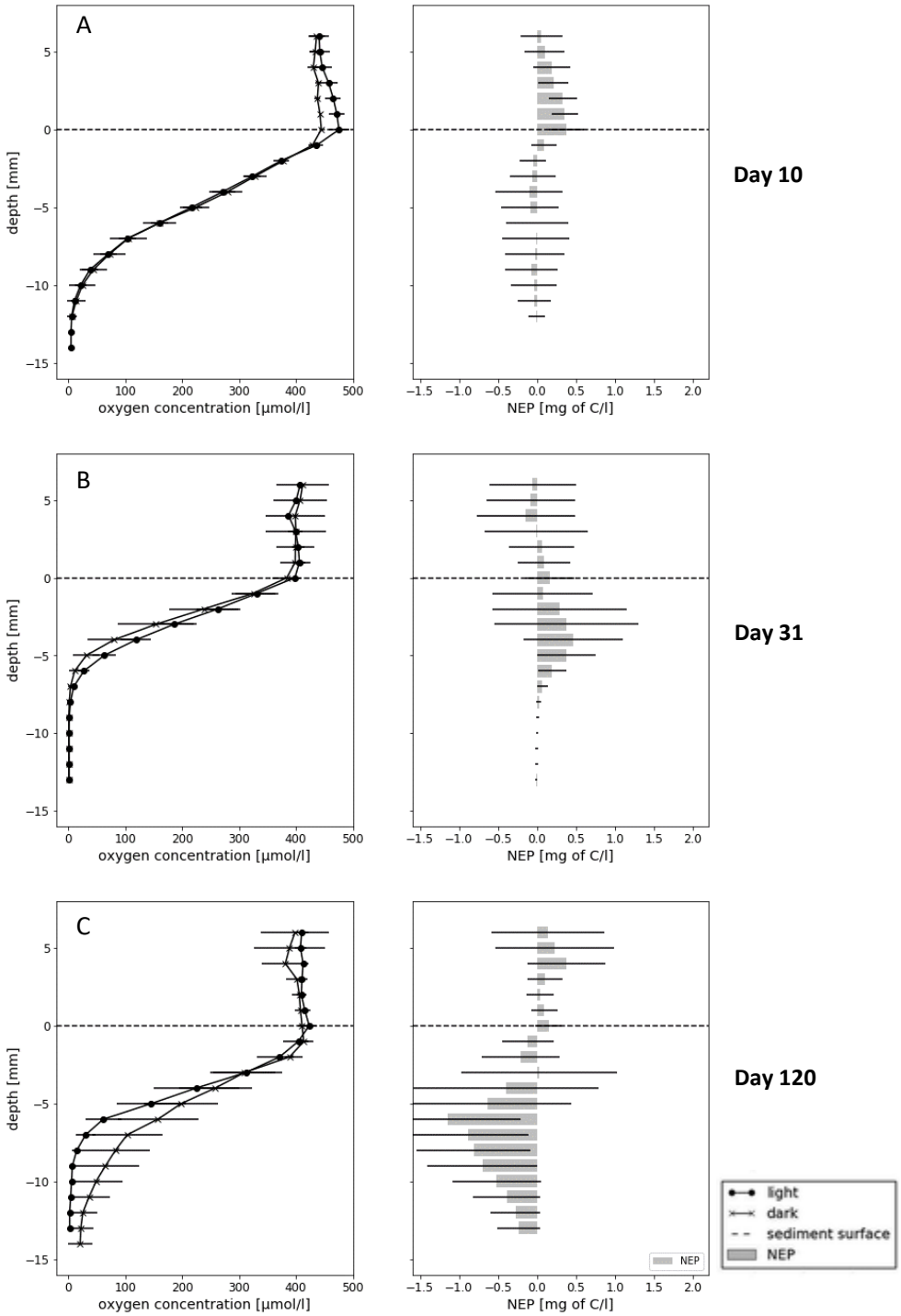

Figure 4.6. Oxygen profiles of long-term, closed laboratory incubations of cryoconite material from Antarctica at 0.2 °C for more than four months with calculated NEP*. Error bars show standard deviation of triplicate bottle measurements of oxygen, and standard error of NEP calculations.*

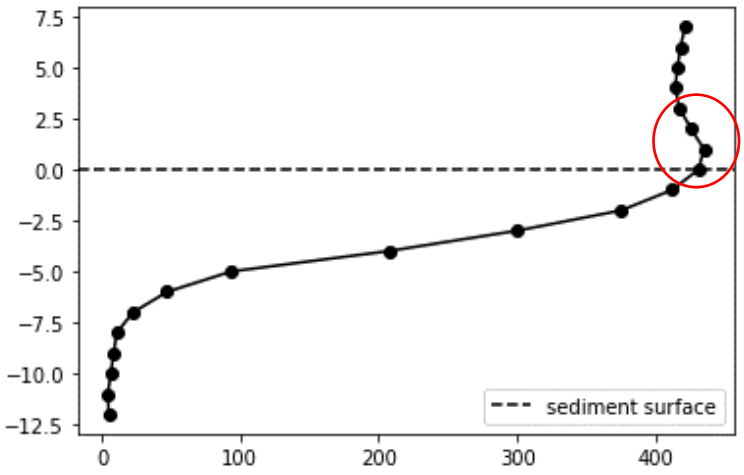

Figure 4.7. Oxygen profile of long-term, closed laboratory incubation of Antarctic cryoconite sample incubated in light conditions. *Red circle marks the oxygen production.*

Svalbard samples were characterized by the high variability of oxygen profiles between them. Similar to Antarctic samples, the cryoconite sediments from Svalbard had the highest NEP after 9 day (0.054 to 1.51, mean NEP 0.76 ± 0.53 between 0 and 4 mm) (Fig 4.8A). After 42 days, dark and light incubations had no significant differences and anoxic zone settled at 4mm (Fig 4.7B). Anoxic zone remained at 4 mm until at least 117 days (Fig 4.8C). At this time just a single depth of the sediment had a significant difference between light and dark treatment (one NEP value below the surface: 0.76 ± 0.61). Oxygen profiles above the surface of the sediment had a different shape in Svalbard samples when compared to Antarctic and Greenland until around 4 months. By the end of incubations, some Svalbard samples developed an orange biofilm on the surface.

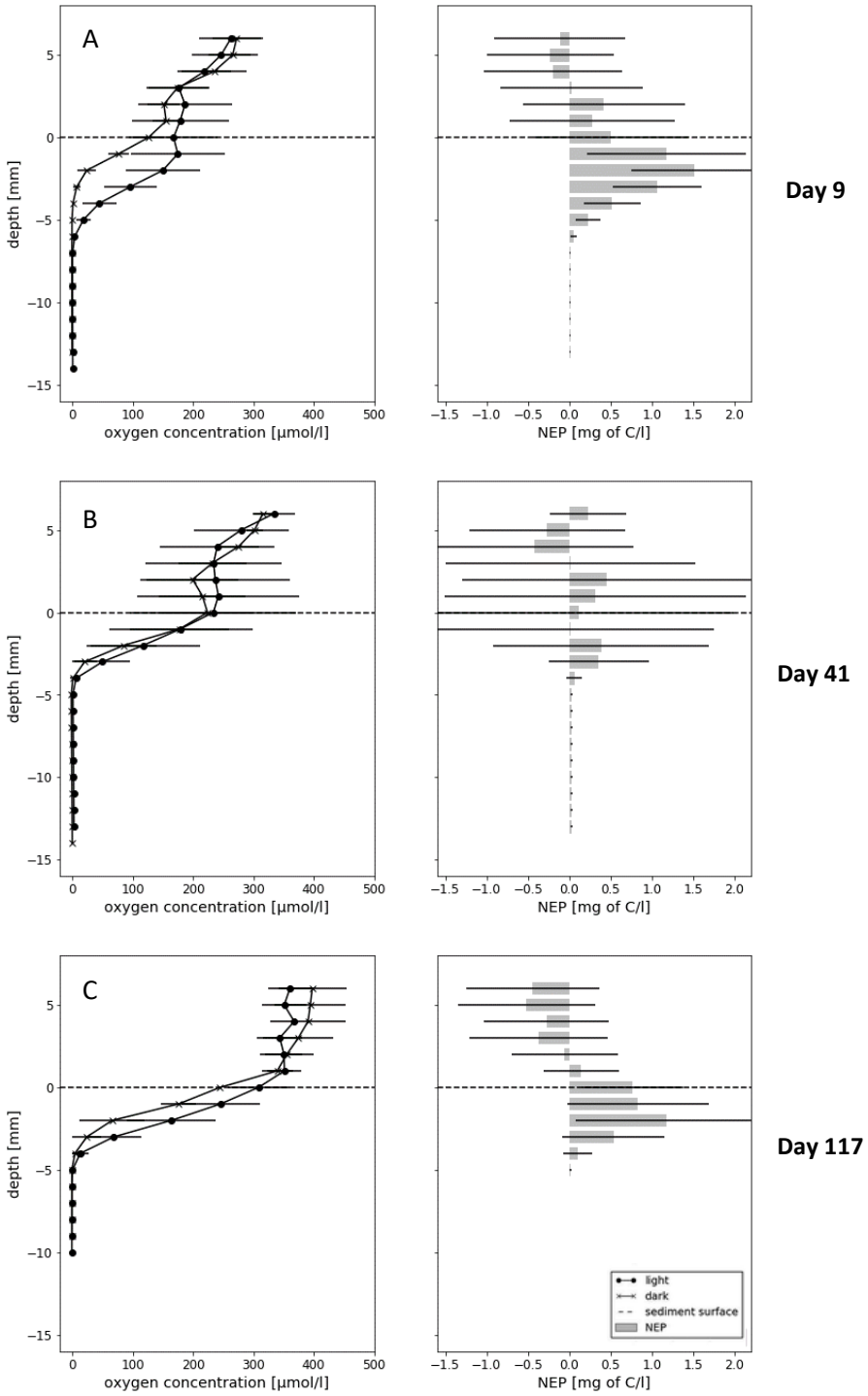

Figure 4.8. Oxygen profiles of long-term, closed laboratory incubations of cryoconite material from Svalbard at 0.2 °C for more than four months with calculated NEP*. Error bars show standard deviation of triplicate bottle measurements of oxygen, and standard error of NEP calculations.*

4.2.3.2. pH profiles

The initial pH of cryoconite sediment from Greenland, measured after defrosting and mixing of the sediment, was 5.58 and was consistent throughout the profile (Fig. 4.9). The pH profiles measured after 116 days (Fig. 4.9) showed little variation with depth (±0.25 units), unlike the oxygen profiles. The mean pH increased by 0.25 in the dark bottles, and 0.8 in the light after 116 days.. In the dark bottles pH increased by 0.5 towards the bottom of the profile when compared to the surface.

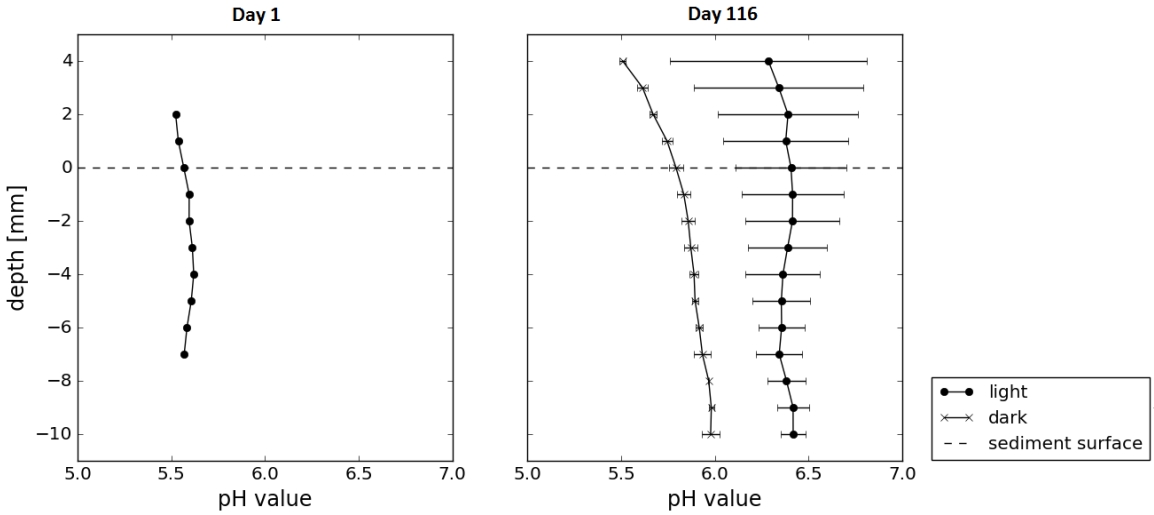

***Figure 4.9. The pH profiles in the beginning and end of the long-term laboratory incubations of Greenland samples**, measured on the first day of incubations (one vessel) and then in dark and light vessels on day 116.*

Patterns in pH changes of Antarctic samples were similar to Greenland. At the start of the experiment, the pH of Antarctic sediment was 7.27 ± 0.09 with little variation with depth (Fig 4.10). After 121 days, the mean pH of dark bottles increased by 0.16 and light samples by 0.43 units. Unlike Greenland bottles, the pH decreased with depth. Dark samples had pH 7.52 on the surface and 7.26 at 7mm, and light samples had pH 7.72 on the surface and 6.76 at 11 mm. Light bottles had greater variability in pH than dark ones.

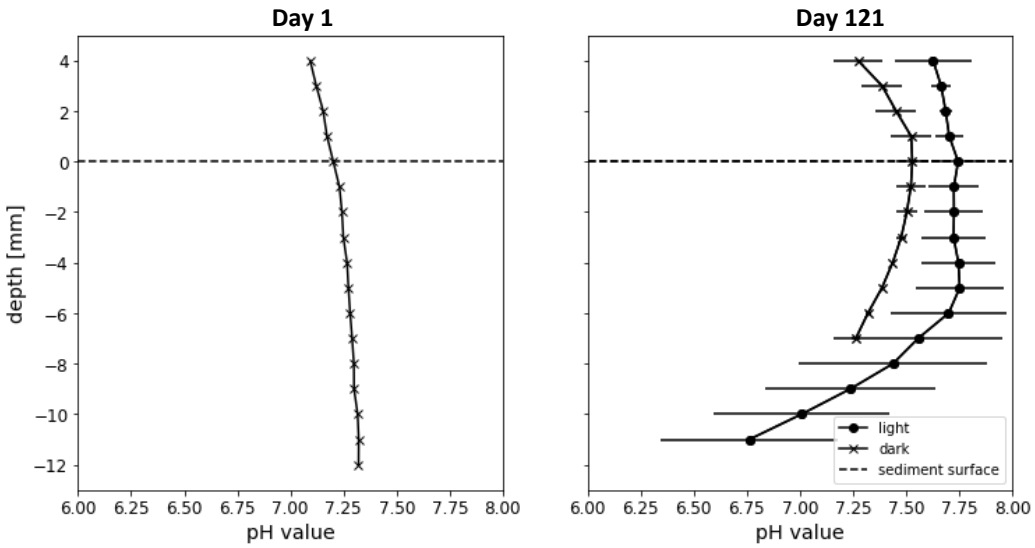

Figure 4.10. The pH profiles in the beginning and end of the long-term laboratory incubations of Antarctic samples, *measured on the first day of incubations (one vessel only) and then in dark and light vessels on day 121.*

At the end of incubations, the pH of Svalbard samples established around 7 (Fig 4.11). Similar to Greenland samples and unlike Antarctic samples, there was not much variation with depth. Light bottles spanned pH 6.82 to 7.04 and dark bottles from 7.00 to 7.22. Contrary to incubations from other locations, the pH of light and dark bottles did not diverge significantly after 118 days. There was no measurement on day 1 so temporal changes could not be assessed.

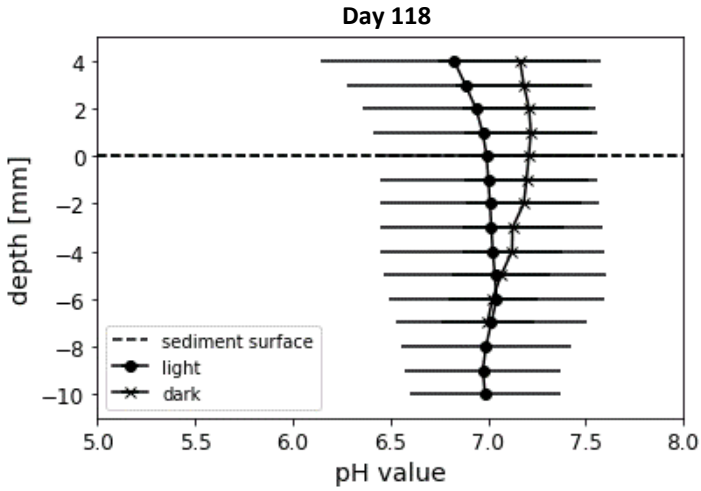

Figure 4.11. The pH profiles in the end of the long-term laboratory incubations of Svalbard samples*, measured in dark and light vessels on day 118.*

4.3. Discussion

4.3.1. Morphology impacts biology

The accumulation of cryoconite on the surface of the GrIS depends primarily on local ice morphology, ice-microbe interactions and hydrology, termed 'biocryomorphology' (Cook et al., 2015). These differences are expressed by the variety of shapes and depths sediment, and of melted holes, which further results in a range of *in situ* oxygen profiles. This provides evidence that physical properties of cryoconite holes can have an impact on the biological relationships between heterotrophic and autotrophic processes in these ecosystems. These results also demonstrate that dramatically different microhabitats may form very rapidly after sediment disturbance, and can persist for prolonged periods. Sediment depth is the primary control of the location of anoxic zones (Fig. 4.1), which corroborates studies previously conducted on cryoconite holes in Svalbard (Telling et al., 2012) and elsewhere in Greenland (Cook et al., 2010). These demonstrated that NEP of bulk cryoconite was strongly controlled by sediment thickness, since net autotrophy was only detected when sediment was thinner than 4 mm, while net heterotrophy clearly developed in thicker sediments. Since chemotrophic processes (e.g. by chemoorganotrophs and chemolithotrophs) use up the oxygen available within sediment, sediments with net oxygen consumption would be expected to exhibit anoxic zones. These experiments demonstrate, for the first time, that low oxygen and anoxic zones exist in laboratory, field experimental and *in situ* cryoconite holes.

The presence of overlying water also influences the oxygen profile, since the water layer restricts diffusion of oxygen from the atmosphere into the sediment. Anoxic zones in thick sediment covered with water are present at a shallower depth than in the sediment that is directly exposed to the atmosphere (Fig. 4.1A, B). In thin sediments composed of relatively large granules, there was a decline in oxygen concentrations through the bulk sediment, but no anoxia until the granules themselves are penetrated by the microsensors (Fig. 4.2).

The cryoconite therefore hosts several niches for anaerobic bacteria: anoxic zones in thick cryoconite sediment, and micro-niches within cryoconite granules (Fig. 4.2). The oxygen is quickly consumed within cryoconite granules and the interior becomes completely anoxic. This is supported by the detection of denitrification genes and transcripts in cryoconite granules in Central Asia (Segawa et al., 2014), and from similar oxygen profiles with micro-niches have been shown in aerated activated sludge granules (flocs), with anoxic niches coinciding with denitrification (Schramm et al., 1999). The presence of an anaerobic floc centre depends on

microbial activity and the size of the granule. Therefore, only the biggest granules are likely to serve as refugia for anaerobic bacteria in the long-term (Ploug et al., 1997).

The structure of the sediment (size of granules, thickness, depth of overlying water) and microbial activity (oxygen consumption) influence the oxygen profile, which consequently may impact the community structure, since the anoxic zones are niches for anaerobic activity. The potential for development of distinct community and nutrients cycling within granules was recognised by Uetake et al. (2016), but more analyses are required to link specific sections of the oxygen profiles with the metabolic groups that thrive there, particularly to detect anaerobic processes that occur only in the absence of oxygen. Sulphate reducing bacteria and methanogenic archaea have been detected in environments with fluctuating oxic/anoxic conditions, such as sandy littoral sediments and soils (Peters and Conrad, 1995; Sass et al., 1997), where they become active as soon as oxygen was depleted. However, these habitats could be seeded by permanently anoxic sediments and soils in close proximity. Cryoconite holes are far more isolated and whether processes occurring in other environments are relevant here requires further investigation.

4.3.2. Impacts of isolation and stability

An unexpected observation in this dataset was the small increase in oxygen at the base of the profile in Fig. 4a and 4b. The likely source of this oxygen is debatable. It could be from *in situ* photosynthesis in moderately shaded microhabitats, which would not occur in the thickest sediments (Fig. 4c). It could also be from the release of trapped bubbles of air during downward melting of the cryoconite hole into glacial ice, which was proposed as a source of oxygen by Bagshaw et al., (2011). Nutrient flux estimates, including inorganic carbon, suggest that resupply of nutrients from the melting ice is crucial to maintain ecosystem productivity (Telling et al., in preparation). These observations suggest that this is one mechanism which prevents the build-up of anoxia in granule-rich 'classic' cryoconite holes: oxygen is delivered by bubble release and water flow in between the granules (Fig. 4.1C), which limits anaerobic activity.

An increase in oxygen concentration at the sediment surface, compared to the water above, was only observed in the thickest sediment accumulations (Fig. 4.1A). The long residence time of thick sediment (the accumulation was present on the ice surface for at least > 14 days of the field campaign) allows the development of a well-structured microbial mat. Filamentous mats were previously observed by Bagshaw et al. (2016a) in Antarctic cryoconite incubations and by Cook et al. (2015b) in cryoconite from the Greenland Ice Sheet (also near Kangerlussuaq).

The development of a large mat and consequently high rates of photosynthesis are not often possible in hydrologically connected cryoconite holes, where the sediment is frequently redistributed (Irvine-Fynn et al., 2011). Although the data demonstrate that the microbial community can rapidly self-organise and begin production very soon after redistribution (Fig. 4.3), the formation of mats, knitted together by cyanobacteria emitting extracellular polymeric substances (Langford et al., 2010), requires sediment stability which is only possible in larger cryoconite accumulations. These generally form on the edge of supraglacial stream or hydrologically abandoned cryoconite holes, and as such are not submerged by meltwater (Fig. 4.1C).

Biogeochemical conditions within the cryoconite change extremely rapidly after the sediment is disturbed (Fig. 4.3), but the system seems to quickly stabilise, as demonstrated by the stable oxygen profiles measured only 2 hours after perturbation. As the perturbation occurs on a daily basis on a glacier, due to melting and changes in local hydrology (Irvine-Fynn et al., 2011), this suggests that microbes must be versatile. They need to respond quickly to oxygen changes in their environment which would be highly selective for facultative anaerobes, as strictly anaerobic organisms must be able to tolerate exposure to oxygen, and strict aerobes must be able to survive sudden oxygen deprivation. Characterisation of anaerobes in the Antarctic cryoconite community showed that only a small percentage were strict anaerobes (Zdanowski et al., 2016). However, vertical migration of strictly anaerobic sulphate reducers along changing oxygen profiles to prevent prolonged oxygen exposure has been described (Taylor et al., 1999; Sass et al., 2002), and observation of our samples under a phase contrast microscope showed an abundance of motile cells. In addition, anoxic enrichment cultures generally yielded facultative anaerobes growing usually even better in the presence of oxygen [data not shown]. The cryoconite microbial community must therefore be extremely versatile, and able to thrive under a range of both physical and biogeochemical conditions.

4.3.3. Controls on productivity

The field incubations mimic the ‘partly drained and accumulated’ type of cryoconite hole, with deeper sediments where oxygen rapidly decreases with depth (Fig. 4.4). The open incubations reached steady state, with a constant difference between light and dark bottles between day 6 and day 20, likely showing that heterotrophic and phototrophic activity rates are in equilibrium with diffusion rates. A similar situation is likely to be observed *in situ*, where thick accumulations persisting over prolonged period of time develop a stable oxygen profile and distinct communities thrive until perturbation occurs.

The prolonged (116 - 121 days) closed laboratory incubations aimed to mimic the thickest cryoconite sediment deposits observed in Greenland (Fig. 4.1) and Svalbard (data not shown, Hodson 2008), some of which are covered with meltwater and some in direct contact with the atmosphere. Thick sediment layers can also be found in Antarctic cryoconite holes, which often are sealed from atmosphere (Tranter et al., 2004; Bagshaw et al., 2007).

Closed incubations showed smaller differences between light and dark incubations than open incubations (Fig 4.4). In samples from Greenland, the light vessels remained relatively stable, albeit with lower concentrations of oxygen than the open counterparts, and there was very little discernible difference between light and dark vessels at the beginning and end of the experiment. A similar pattern was observed in Svalbard and Antarctic samples, where both light and dark treatments did not change much with time and most of the differences were not significant. Oxygen did not accumulate long-term in light vessels, contrary to previously reported extreme oxygen values from natural perennially isolated cryoconite holes in Antarctica (Bagshaw et al., 2016b).

Overall, both closed incubations from the field and from the laboratory demonstrate that majority of phototrophic activity happens in the first few weeks and the vessels later become dominated by heterotrophic activity. Such activity remains in equilibrium with diffusion rates, as the shape of the oxygen profile and the depth of anoxic zone remains relatively stable. This could be because the heterotrophs in the dark, closed vessels, deprived of organic carbon input from phototrophs and input of nutrients from melting of the surrounding ice and/or flushing of meltwater, may be reaching a dormant state or slowly dying. It may also relate to the lower PAR in the laboratory vs. the field (145 vs. peak of 1617 µmol m^{-2} s^{-1}, see Appendix B), which is likely further restricted by the lids in closed vessels. Low light and no nutrient input could result in weakened phototrophic community and consequent dominance of heterotrophic activity in closed light vessels, similarly to the dark ones. A similar shift from phototrophic to heterotrophic community was observed over the ablation season in Alpine cryoconite holes (Pittino et al., 2018).

Individual patterns of the oxygen consumption and production differ in the samples from all locations. The peak of activity and the biggest difference between light and dark vessels was observed earlier in Antarctic (9 days) and Svalbard (10 days) samples than Greenland samples, where there was a spike of activity around day 40. Antarctic sediments had visible primary production on the surface (up to 0.37 mg of C l^{-1}), whereas in Svalbard and Greenland the differences reached deeper layers as well. In the Arctic samples, dark vessels became anoxic, or

had very low concentrations of oxygen throughout the profile, and the light vessels were elevated compared to dark vessels (±140 µmol at the sediment surface in Greenland ones and ±97 µmol at 3 mm in Svalbard ones). In the first few weeks, the anoxic layer is shallower in the Arctic samples than Antarctic. Difference between Arctic and Antarctic samples can be explained by higher initial heterotrophic activity in the Arctic samples and consequent faster oxygen consumption in the shallower depths. Lower activity in Antarctic samples is likely a consequence of more nutrient-deprived source environment (Bagshaw et al., 2013). The microbial community composition also differs between locations (Darcy et al., 2018) and likely scavenges oxygen with different efficiency (Zdanowski et al., 2017).

The later peak of activity of Greenland samples when compared to the Svalbard and Antarctic incubations can be explained by different period needed for the establishment of a stable community (Bagshaw et al., 2016b), and also be attributed to disturbance of the sediment during the collection of microbial subsamples. The addition of Milli-Q water allowed for redistribution of nutrients and a temporary boost of activity (Day 40 in Greenland). In Antarctic sediments which generally have more sandy, porous structure (Fountain et al., 2004), diffusion might be a very important component of oxygen profile development from almost the beginning, as the water oxygen remains at relatively steady concentration after day 31.

Finally, heterotrophic activity is detected in all of the samples until at least 116 days. However dark samples after 121 days in Antarctica show decline in activity, as the curve is more linear and anoxia is found at deeper layers, which indicates more diffusion of oxygen and less consumption by microorganisms. The closed light vessels cannot maintain high activity rates (Day 116-121), and instead decline to a steady state, similar to that observed in field incubations after 20 days. Overall, these results are different to other studies showing accumulation of oxygen over time in closed cryoconite sediment incubations (Cook et al., 2012; Bagshaw et al., 2016a, 2016b), and support those that show balance between light and dark bottles (Hodson et al., 2010; Telling et al., 2012).

The closed laboratory incubations imitate communities isolated by ice lid which have restricted nutrient input, although the influx of nutrients and oxygen from meltwater flushing and ice melt below the hole is not simulated by bottle incubations. The importance of nutrients acquired from the supraglacial water in stimulating microbial communities in cryoconite holes is still under investigation, with a recent study (Cameron et al., 2016) showing no effect of N additions on the community structure and cell abundance. However, Cameron et al. (2016) only demonstrated that there was no nitrate limitation on a short time scale, and did not test other nutrients, co-

limitations or long-term effects. P limitation of cryoconite microorganisms was suggested previously by Stibal et al. (2009). The possibility that isolation from supraglacial water and the ice below restricts the activity of microorganisms or even initiate their survival/dormant stage requires further investigation.

The pH in Greenland and Antarctic vessels increased during the long-term incubations (Fig. 9). The increase in pH in both dark and light vessels is attributed to the dissolution of minerals (Tranter et al., 2002) and anaerobic mineralization (Hu and Cai, 2011). Unlike the oxygen concentration profile, there was little variation in pH with depth in Greenland and Svalbard samples, and in the initial Antarctic sample. Contrarily, at the end of the experiment, the pH in the Antarctic samples was lower at depth. This could potentially be caused by acidic products of fermentation (Maier and Pepper, 2015). Why such acidity could only be detected in Antarctic samples remains obscure. Perhaps it could be attributed to the differences in the mineral content and consequent variation in dissolution products. Another possibility is the difference in nitrogen cycling, as net denitrification performed by anaerobes could also be a source of alkalinity in the sediment (Hu and Cai, 2011). The elevated pH in the light vessels from Greenland and Antarctica is most likely because of photosynthesis in a closed system, which forces OH^- ions into solution (Tranter et al., 2004).

4.4. Technical limitations

Modern chemical sensors allow *in situ* and laboratory measurements of a number of parameters at a microscale. Three different sensors were used in this study, each characterised by different limitations: pH microelectrode, oxygen optode and oxygen microelectrode. pH electrodes generally perform poorly at very low ionic strength, which is characteristic of glacial meltwater, and exhibit measurement errors of >0.01 pH (Bagshaw et al., 2011b). To minimise the error, special buffering solutions for calibration at low ionic strength were used and measurements of these buffered solutions were reproducible. Furthermore, in this study, the accuracy of individual measurements was of less importance than the detection of differences between samples from different locations. The latter were greater than aforementioned typical errors of >0.01 pH.

According to the specification provided by the manufacturer (Unisense.com), the oxygen sensors used in the study show no interference with CO_2, CH_4, H_2S and any common ionic species as well as change of pH. Although high concentrations of sulphide can affect the sensitivity of the oxygen electrode and chlorine can interfere with optode readings (www.unisense.com), these compounds are unlikely to occur in high concentrations in the glacial environment. Fiber-optic

sensors (optodes) have been used for *in situ* monitoring of dissolved oxygen dynamics in the glacial settings for the past 10 years (Bagshaw et al., 2011b, 2011a). Optodes are sensitive to temperature, which was compensated by in-built software in the data logger and the simultaneous use of temperature sensor. They were also shown to exhibit electronic failure in cold temperatures, but this did not happen during daylight measurements on GrIS. According to manufacturer, fluctuating ambient light conditions can potentially affect the measurements. Therefore, a dark umbrella was used to shade the cryoconite holes during *in situ* measurements. However, no difference was observed between shaded and unshaded measurements. Last limitation is a relatively short lifespan, caused by bleaching of the coating, but this problem was not relevant as none of the optodes were used for prolonged time. Instead, microelectrodes were used for laboratory measurements and their expected lifespan was within the timeframe of experiments.

Clark-type microelectrodes show similar sensitivity and accuracy as fiber-optic sensors and traditional Wilker titration (Bagshaw et al., 2011b). They usually require stirring because of oxygen use-up on the top of the tip, but the use of microsensors allows to overcome this limitation (Bagshaw et al., 2011b). As they are more robust, more affordable than optodes and less sensitive to temperature changes, they were used in repeated laboratory measurements. Optodes were chosen for field experiments, because unlike microelectrodes, they could be operated with a USB-powered data logger, which was smaller in size and which could be easily connected to a laptop. Moreover, the optodes did not require pre-polarisation. Oxygen Clark-type electrodes require pre-polarisation of around 3h, which would not be practical in the field.

4.5. Summary

Microscale measurement of oxygen and pH profiles in cryoconite from the Greenland, Svalbard and Antarctica implies that the physical structure of the cryoconite controls the potential for biological activity. Anoxic zones are common within cryoconite, and can establish rapidly after perturbation of sediment. This means that anaerobic microorganisms, as well as more widely studied aerobic species, are likely important taxa in the cryoconite ecosystem which contribute to biological production in the glacial biome. Production is dependent on supply of gases and nutrients, from meltwater flushing, via diffusion, and from melting of the ice below the cryoconite sediment. Cryoconite microbial communities are extremely tolerant to a wide range of biogeochemical conditions, including complete anoxia, and can rapidly adapt via motile or facultative mechanisms to maximise productivity during the short summer melt season. The limits of microbial communities will be discussed in the following chapter.

Chapter 5

Microbial physiology

5.1. Introduction

The microbial community of cryoconite holes is exposed to the extreme conditions of the supraglacial environment, including fluctuating temperatures, extreme and varying geochemical conditions and limited nutrients. The previous chapter (chapter 4) described anoxic microniches detected in the cryoconite holes *in situ* and in laboratory incubations. This chapter discusses the physiological capabilities of microbial isolates from cryoconite holes from Antarctica, Greenland and Svalbard grown in aerobic and anaerobic conditions. Their physiological limits were tested under selected environmental conditions: extreme pH, salinity, freeze-thaw and limited carbon sources. The results suggest that heterotrophic microorganisms in cryoconite holes are well adapted to fast-changing environmental conditions.

5.2. Microbiology of cryoconite holes

5.2.1. Total cell counts

Total microbial cells were counted in each of the cryoconite sediment samples, which were subsequently used for MPNs. The cell numbers are uniform across the samples, with no significant differences between Antarctica, Svalbard and Greenland (Table 5.1). Mean total cell counts in Antarctic cryoconite holes were 5.85 x 10^8 cells g^{-1}, compared with 9.22 x 10^8 cells g^{-1} in Svalbard and 5.83 x 10^8 cells g^{-1} in Greenland.

Table 5.1. Abundance of microorganisms in cryoconite sediments.

Location	Sample	Total cell counts per g of wet sediment	95% confidence interval
Svalbard	O1	4.85×10^8	0.04
	O2	1.82×10^9	0.36
	O3	4.61×10^8	0.18
Greenland interior	O4	4.33×10^8	0.20
	O5	8.86×10^8	0.22
	O6	4.30×10^8	0.02
Antarctica	O7	5.27×10^8	0.21
	O8	4.62×10^8	0.04
	O9	7.68×10^8	0.26

5.2.2. MPN counts and cultivability

Freshwater medium and fermenter medium inoculated with cryoconite sediment yielded viable cells under oxic and anoxic conditions, and all temperatures tested. Oxic conditions yielded higher numbers of cultivable microorganisms: mean counts of $4.60 \times 10^8\ g^{-1}$, $1.30 \times 10^7\ g^{-1}$, $1.69 \times 10^7\ g^{-1}$ for Svalbard, Greenland and Antarctic cryoconite respectively (Fig. 5.1), compared with $3.98 \times 10^5\ g^{-1}$, $9.61 \times 10^3\ g^{-1}$, $8.04 \times 10^5\ g^{-1}$ for Svalbard, Greenland and Antarctic cryoconite in anoxic conditions (Fig. 5.2). The number of cultivable cells in oxic conditions was in the same order of magnitude at each location after 71 days of incubation between 0.2 and 20 °C (Fig. 5.1). At 30 °C, viable counts were on average 100 times lower than those at 20 °C, but these samples unfortunately dried out after one month (marked on Fig. 5.1 with stripes). When incubated in an anoxic atmosphere, viability peaked at the coldest temperatures tested (0.2 °C) and the number of cultivable cells was comparable between the temperatures from 4 to 30 °C (Fig. 5.2).

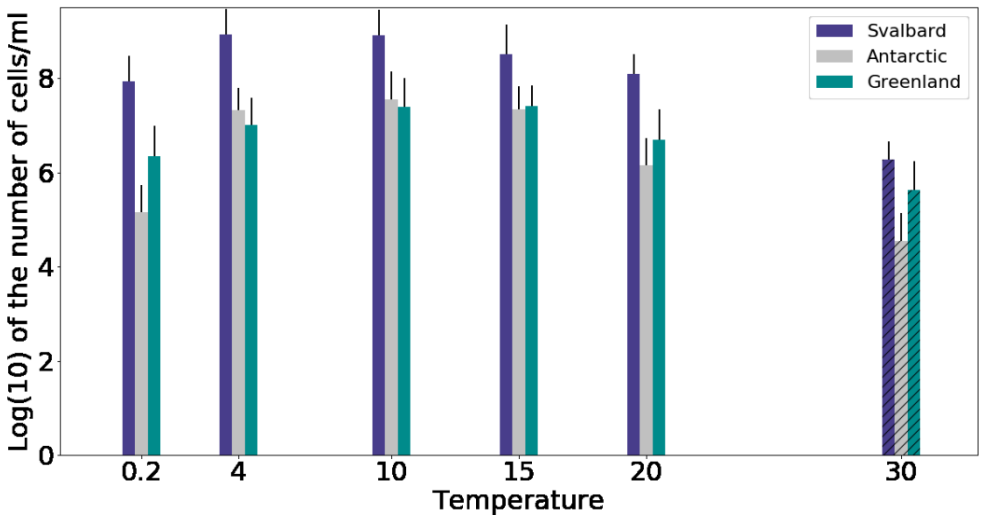

Figure 5.1. MPN counts of aerobic (oxic) microbial community of cryoconite holes in freshwater medium. *Microbial growth was measured by MPN counts as the (average of 3 different cryoconite holes sediments for each location after 71 days of incubation. Samples incubated at 30 °C dried out after 30 days, but they are included on the graph for comparison (marked with stripes).*

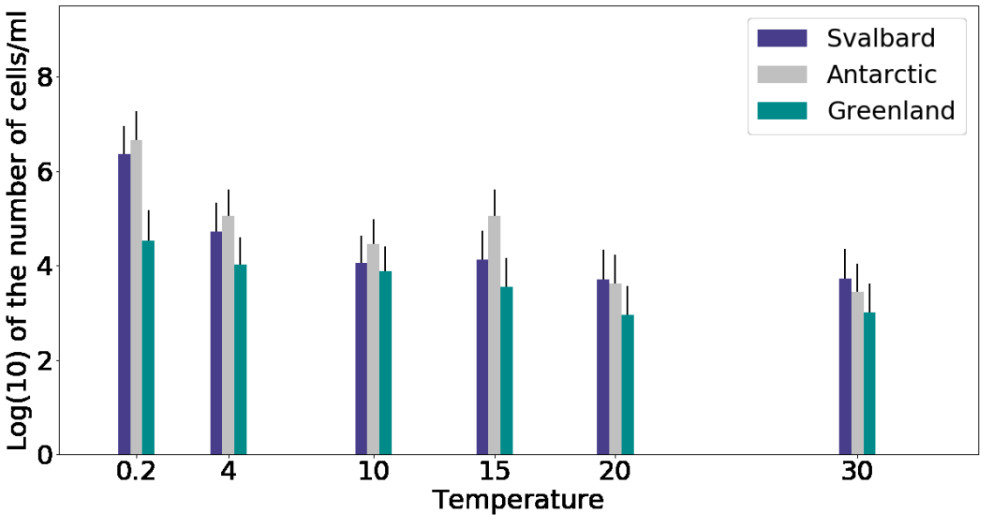

Figure 5.2. MPN counts of anaerobic (anoxic) microbial community of cryoconite holes in fermenter medium. *Microbial growth was measured by MPN counts as the average of 3 different cryoconite holes sediments for each location after 71 days of incubation.*

The cultivability of cryoconite microorganisms was estimated based on total cell counts and MPN counts after 71 days of cultivation. Cultivability is expressed as the percentage of the total cell counts which can be cultivated in the laboratory conditions. Cryoconite microorganisms yielded very high viable counts under the conditions tested (Table 5.2). Under oxic conditions, the culturable bacterial count of Svalbard microorganisms was an order of magnitude higher than for Greenland and Antarctic (p=0.00). There were no statistically significant differences between the locations under anoxic conditions. However, it is notable that Antarctic and Svalbard samples have especially high culturability in the lowest temperature tested (0.2 °C).

Table 5.2. Cultivability of microorganisms from cryoconite holes. *Cultivability is expressed as % of total cell counts which can be cultured by MPN technique in the aerobic and anaerobic conditions.*

Temp (°C)	Anoxic conditions			Oxic conditions		
	Svalbard	Greenland	Antarctica	Svalbard	Greenland	Antarctica
0.2	0.28 ±0.19	0.01 ±0.01	0.96 ±0.79	7.28 ±0.47	0.48 ±0.35	0.03 ±0.01
4	0.01 ±0.01	0.00 ±0.00	0.02 ±0.03	48.38 ±46.97	1.79 ±1.25	4.37 ±3.81
10	0.00 ±0.00	0.00 ±0.00	0.01 ±0.01	53.62 ±8.79	3.70 ±2.12	7.65 ±5.16
15	0.00 ±0.00	0.00 ±0.00	0.02 ±0.03	27.05 ±2.86	5.98 ±9.69	5.13 ±0.07
20	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	13.16 ±0.15	1.06 ±1.46	0.31 ±0.13
30	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.17* ±0.11	0.10* ±0.15	0.01* ±0.00

**In the oxic conditions at 30°C samples were measured after 23 days.*

At all temperatures, the cultivability in anoxic conditions was several orders of magnitude lower than in oxic conditions, with values up to 15200, 3400 and 400 times lower for Svalbard, Greenland and Antarctica, respectively. However, at 0.2°C, the differences between oxic and anoxic incubations were less pronounced (Table 5.2).

None of the anoxic samples had statistically significant differences in cultivability. ANOVA, followed by Tukey HSD, revealed that oxic samples from Svalbard and Greenland were statistically different at 0.2, 10, 15 and 20 degrees (p= 0.00, p=0.02, p=0.04 and p=0.00 respectively), whereas Svalbard and Antarctic samples were different at 0.2 and 20 degrees (p=0.00 and p=0.00 respectively). There were no statistically significant differences between Greenland and Antarctic samples' cultivability.

5.2.3. Microbial isolates

The highest positive dilution of MPNs was used to inoculate solid agar plates with freshwater medium and isolate the most abundant culturable microbes. A total of 44 isolates were isolated and identified by 16S rRNA gene sequencing (Table 5.3). Svalbard cryoconite samples yielded 13 bacterial isolates and 4 fungi (yeast) isolates, Greenland 12 bacteria and 5 fungi, and Antarctica 10 bacteria. No yeasts were isolated from Antarctic cryoconite. Most bacterial isolates affiliated with the Actinobacteria (79%), followed by Bacteroidetes (18%) and Proteobacteria (3%). All isolates were capable of fermentation, but did not utilise alternative electron acceptors.

Table 5.3. Physiology of microbial isolates of cryoconite holes. *The closest relative of isolates is based on rRNA gene sequencing and similarity is expressed in percent (%). Isolation was done in oxic (ox) or anoxic (anox) conditions. Carbon substrates utilisation (substr) is expressed as the percent used of tested. 'Salinity' is the maximum salinity and 'pH range' is the range of media pH in which positive growth was recorded. All the isolates were able to grow at lowest tested salinity of 0.1%. Freeze-thaw survival is the number of cycles after which a culture was viable. Tmax is the maximum temperature that growth could be detected. All tested isolates grew at the lowest tested temp (1°C). Phyla detected include Actinobacteria (Actino), Bacteroidetes (Bacte) and Proteobacteria (Proteo). Sampling sites are Antarctica (Ant), Greenland (Gr) and Svalbard (Sv).*

Isolate	Closest relative	%	Phylum	Site	Isolation	Salinity (%)	pH range	Substr (%)	Freeze - thaw	Colony colour	T_{max} (°C)
Bacteria											
An02O7	Flavobacterium sp. R-36976	99	Bacte	Ant	ox	10	6.5-10	69	1	orange	
An4O7	Flavobacterium sp. R-36976	99	Bacte	Ant	ox	7	6.5-10	66	1	orange	
An15O7	Arthrobacter agilis strain LV7	99	Actino	Ant	ox	6	7-10.5	44	100	yellow	32
An15A7	Tessaracoccus sp. strain AU I5	99	Actino	Ant	anox		7-10.5			yellow	
An15A8	Tessaracoccus sp. strain AU I5	99	Actino	Ant	anox		7-10.5			orange-yellow	
An4A7	Bacterium CS117	99	Actino	Ant	anox	10	4.0-10	74		yellow	36
An4A8	Bacterium CS117	99	Actino	Ant	anox	8	6.5-10.5	38		orange	
An4O8	Marisediminicola sp. N26	99	Actino	Ant	ox	10	6.5-10.5	63	100	orange	
An15O8	Marisediminicola sp. N26	99	Actino	Ant	ox	6.5	6.5-10.5	28	100	orange	
An02O8	Cryobacterium sp. 1021	99	Actino	Ant	ox	8	5-10.5	43	25	red	24
Gr15O6	Frigoribacterium sp. MP117	99	Actino	Gr	ox	8	4-8.5	38	25	orange	
Gr15O5	Frigoribacterium sp. MP117	99	Actino	Gr	ox	2.5	4-8.5	17	25	orange	
Gr02A4	Antarctic bacterium 2CA	99	Actino	Gr	anox	10	4-8.5	28		pale yellow	
Gr15O4	Glaciihabitans tibetensis strain TGC-6	99	Actino	Gr	ox	2	4-8.5	16	25	orange-yellow	
Gr4O4P	Uncultured Bacteroidetes clone IC4058	99	Bacte	Gr	ox				5	pink	
Gr4O6	Rugamonas rubra strain HCR18a	99	Proteo	Gr	ox	0.75	4.5-8	50	25	white	31
Gr4A5	Cryobacterium sp. MDB2-A-1	99	Actino	Gr	anox	10	4-8.5	72		pale yellow	24
Gr02O4	Cryobacterium psychrotolerans MLB-34	99	Actino	Gr	ox	8	4.0-10	59	5	yellow	
Gr02A6	Cryobacterium psychrotolerans ZS14-85	99	Actino	Gr	anox	10	4.0-10	41		pale yellow	
Gr02A5	Cryobacterium sp. MDB1-44	99	Actino	Gr	anox	10	4-9.5	50		pale yellow	
Gr4A4	Cryobacterium sp. MDB1-44	99	Actino	Gr	anox	10	4-8.5	42		pale yellow	
Gr4A6	Cryobacterium sp. MDB1-44	99	Actino	Gr	anox	10	4-8.5	70		pale yellow	

Table 5.3. Continuation.

Isolate	Closest relative	%	Phylum	Site	Isolation	Salinity (%)	pH range	Substr (%)	Freeze - thaw	Colony colour	T_{max} (°C)
Bacteria											
Sv4A3	Cryobacterium sp. MDB1-44	99	Actino	Sv	anox	10	4-8.5	67		pale yellow	
Sv4A2	Cryobacterium sp. MDB2-A-1	99	Actino	Sv	anox	8	4-8.5	48		pale yellow	25
Sv15A2	Cryobacterium sp. MDB2-A-1	99	Actino	Sv	anox	5.5	4-8.5	23		yellow	
Sv02A1	Antarctic bacterium 2CA	99	Actino	Sv	anox	5.5	4-8.5	23		yellow	
Sv4A1	Antarctic bacterium 2CA	99	Actino	Sv	anox	8	4-8.5	66		pale yellow	34
Sv02A3	Antarctic bacterium 2CA	99	Actino	Sv	anox	8	4-8.5	21		yellow	
Sv4O2	Uncultured bacterium clone LE201D02	99	Bacte	Sv	ox	10	6.0-10	7	25	orange	
Sv02O2	Flavobacterium sp. KJF4-15	99	Bacte	Sv	ox	8	6.0-10	47	5	orange	25
Sv02A2	Flavobacterium sp. TMS1-10 16S	99	Bacte	Sv	anox	8	4-9.5	34		yellow	
Sv15A1	Cellulomonas cellasea strain WB102	99	Actino	Sv	anox		2.5-8			pale yellow	
Sv15O1	Frigoribacterium sp. Ha8	99	Actino	Sv	ox	2	5.0-10	54	5	yellow	31
Sv15A3	Actinobacterium Muzt-D93	99	Actino	Sv	anox		4-8.5			pale yellow	
Sv15O3	Glaciihabitans tibetensis strain SD-70	99	Actino	Sv	ox	2	4-8.5	41	25	orange	
Fungi											
Gr02O5	Basidiomycota sp. TP-Snow-Y1	91	Basidio	Gr	ox	10	3-8.5	41	100	pale pink	
Gr4O5	Basidiomycota sp. TP-Snow-Y1	91	Basidio	Gr	ox	8	5.5-8.5	27	100	pale pink	19
Gr4O4	Basidiomycota sp. TP-Snow-Y1	91	Basidio	Gr	ox	10	3-8.5	24	100	pale pink	
Gr02O4w	Basidiomycota sp. TP-Snow-Y1	91	Basidio	Gr	ox	10	3-8.5	33		white	18
Gr02O6	Basidiomycota sp. TP-Snow-Y1	91	Basidio	Gr	ox	7	3-8.5	34	100	pale pink	
Sv02O1	Basidiomycota sp. TP-Snow-Y1	92	Basidio	Sv	ox	5	3.0-10	48	100	pale pink	
Sv4O1	Basidiomycota sp. TP-Snow-Y1	92	Basidio	Sv	ox	8	3-8.5	23		pale pink	
Sv02O3	Mrakia sp. isolate J-36	99	Basidio	Sv	ox	10	3.0-10	52	100	white	22
Sv4O3	Mrakia robertii isolate J-127	100	Basidio	Sv	ox	10	3.0-10	66	100	orange	

5.2.4. pH tolerance

Antarctic isolates grew in medium with pH ranging from 4 to 10.5, whereas those from Svalbard grew in 2.5 to 10, and those from Greenland, 3 to 10 (Table 5.3). Comparison of the differences in these pH ranges was analysed by ANOVA followed by Tukey HSD and showed that the pH range tolerated by Antarctic isolates was significantly different from Svalbard and from Greenland (p=0.00 and p=0.00, respectively) (Fig. 5.3). The mean pH of the successful growth media was highest in the Antarctic samples; the mean pH of media with detectable growth was 8.2, compared to 6.2 for Greenland and 6.5 for Svalbard. There was no statistical difference between oxic and anoxic conditions, or between yeasts and bacteria. Most yeasts grew at pH ≥3; Svalbard yeasts were able to grow from pH 3 to 10, although interestingly yeasts from Greenland samples that were assigned to the same OTUs could only grow at pH 3 to 8.5 (Table 5.3). One bacterial isolate grew at pH 2.5, but the remainder did not tolerate pH ≤4.

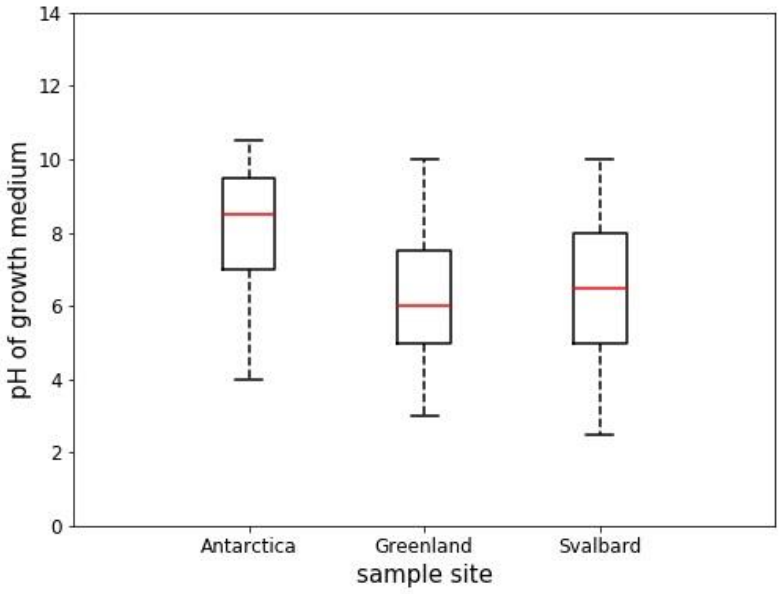

Figure 5.3. Boxplots showing the median pH tolerance of all microbial isolates from cryoconite holes from Greenland, Svalbard and Antarctica. *The pH of all incubations with positive growth after 30 days (n = 43) was noted, and compared between the locations. The red line depicts the median pH, the box envelops an interquartile range and the whiskers mark the 97th centile. There were no outliers.*

5.2.5. Salinity tolerance

The microbial isolates from cryoconite holes were able to grow in a surprisingly wide salinity range (Table 5.3). Most (34 out of 39) were able to grow in up to 5% salinity (~ 42000 µS cm^{-1}). The highest tested salinity of growth medium was 10 % (~ 77000 µS cm^{-1}), where 16 isolates tested positive for growth (Fig 5.4). Interestingly, there was no significant difference of maximum salinity tolerance between yeasts and bacteria from Svalbard and Greenland, nor between isolates from the different sites of origin, or those isolated under anoxic or oxic conditions.

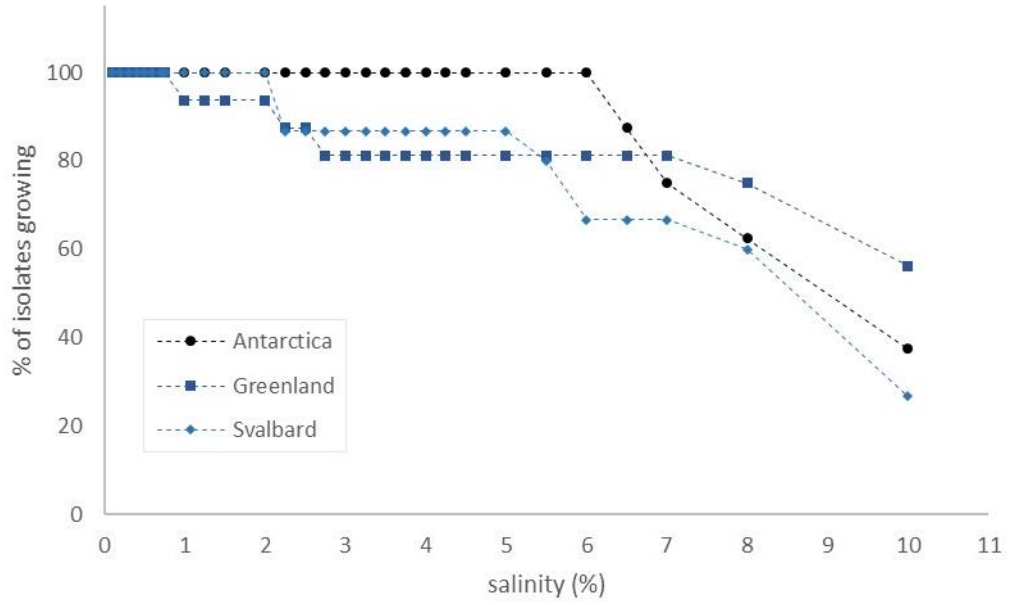

Figure 5.4. Positive growth of microbial isolates at increasing salinity of the medium. *The salinity of all incubations with positive growth was noted, and compared between the locations.*

5.2.6. Temperature range

Twelve isolates were tested to determine the temperature range in which they were able to grow (Table 5.3). All tested isolates grew at the lowest tested temp (1°C). All of the bacterial isolates were able to grow above 22 °C, with Antarctic isolate AN4A7 having a maximum growth temperature of 36 °C, whereas yeasts were limited to 22 °C.

5.2.7. Freeze-thaw survival

Isolates from all sample locations had a mixed response to freezing: some isolates survived multiple freeze-thaw cycles without losing viability (e.g. Antarctic isolate An15O7), whereas others did not (e.g. Antarctic isolate An02O7) (Fig. 5.5). All the yeast strains survived numerous (>100x) freeze and thaw cycles without a significant decrease in viability, when assessed by MPN. In some yeast strains cell counts increased after 100 cycles, whereas others showed a slight decrease, but none were completely inviable (Fig. 5.5a). Two of the yeast isolates (Gr4O5 and Gr4O4) increased in viable cell counts after a single cycle, which might indicate an adaptation to freeze-thaw stress and cells transitioning from non-culturable to culturable state.

Bacterial isolates showed greater variability in response to freeze-thaw stress. The majority (10 out of 15) of the cultures tested belong taxonomically to Gram-positive bacteria (Actinobacteria). Of these, 8 out of 10 remained viable after 25 cycles, and three (all from Antarctica) were viable after 100 cycles. In contrast, none of the Gram-negative strains survived 100 cycles, and they generally lost culturability more rapidly after freeze-thaw stress. The viability of cultures following treatment was variable, regardless of the sampling site. However, the bacteria isolated from Antarctic cryoconite differed from the Arctic (Svalbard, Greenland): the only three bacterial isolates (An15O7, An4O8, An15O8) able to survive 100 cycles of freezing and thawing came from Antarctic samples, although it should also be noted that some Antarctic isolates survived only 1-5 cycles (Fig. 5.5b). Svalbard bacteria viability decayed rapidly, with only one able to survive 25 freeze-thaw cycles (Fig. 5.5c). Greenland bacteria ceased to be viable between 25 and 100 cycles (Fig. 5.5d). Freeze-thaw survival appears to follow phylogeny and hence cellular structure, as isolates belonging to the same genus but obtained from different sites showed a similar response. For example, *Cryobacterium* sp. from Antarctica and from Greenland survived well during the initial cycles and collapsed after 25 cycles. *Flavobacterium* sp. from Svalbard and Antarctica did not cope well and survived 1 to 5 cycles.

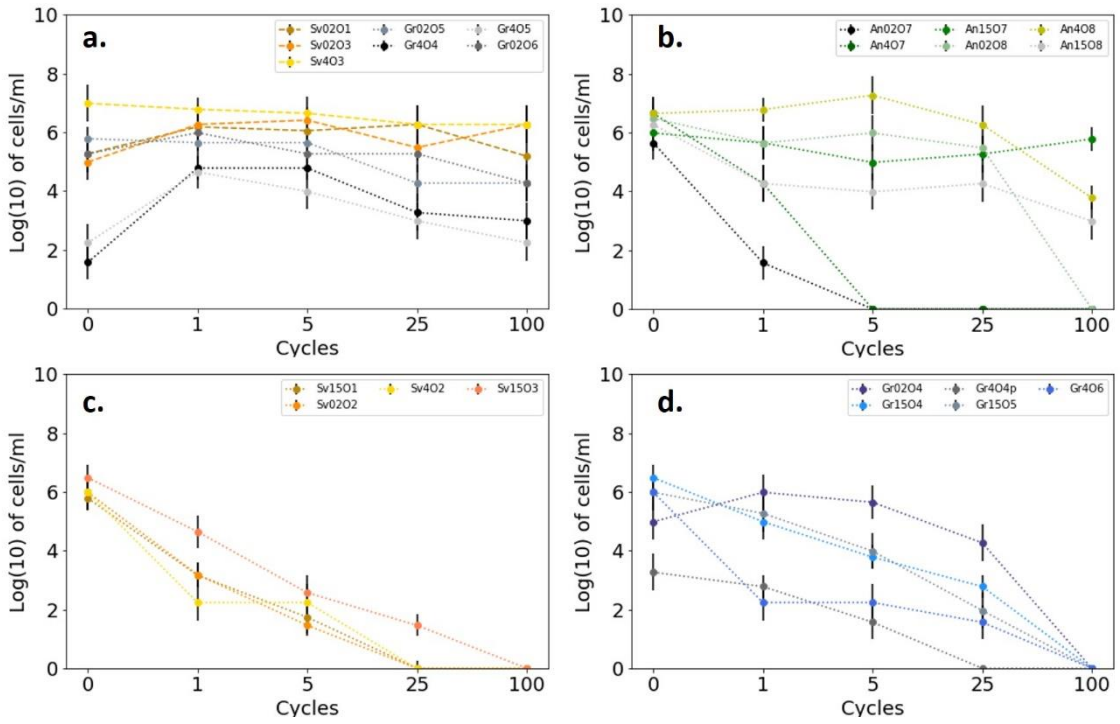

Figure 5.5. Survival of cryoconite isolates subjected to multiple freeze-thaw cycles as measured by MPN technique. *Error bars depicts 95% confidence levels calculated for MPNs. a.) Greenland and Svalbard yeasts, b.) Antarctic bacteria, c.) Svalbard bacteria, d.) Greenland bacteria*

There was no significant correlation between the pigmentation of the strains, resistance to salinity and/or extreme pH and freeze-thaw survival (Table 5.3). Among the isolates with the highest freeze-thaw resistance (25 -100 cycles), there were cultures which survived only salinities up to 0.75% (isolate Gr4O6), but also up to 10% (An4O8); dark pigmented isolates (orange) which survived one cycle (A02O7) and 25 cycles (Gr15O6); and strains able to survive extreme pH 10 with both minimal (1 cycle only, An02O7) and good freeze-thaw tolerance (25 cycles, Sv4O2). The three isolates most resistant to freezing (Antarctic isolates An15O8, An4O8, An15O7) were pigmented, resistant to pH 10.5 and able to cope with high salinities (6.5, 10 and 6% respectively).

5.2.8. Substrate test

The cultures tested utilised a wide range of carbon sources from different groups (carboxylic acids, simple and complex carbohydrates, amino acids, alcohols and polyalcohol and other complex substances).There was no strong preference for one single type of carbon source such as carboxylic acids, carbohydrates or amino acids (Table 5.4), and some isolates were able to live on almost all substrates tested (e.g. genera *Flavobacterium* sp. or *Cryobacterium* sp.). There are some exceptions, for example most isolates belonging to *Antarctic bacterium* species only utilised a small selection of carboxylic acids.

Table 5.4. Mean utilisation of carbon substrates by cryoconite microorganisms expressed as proportion of all carbon substrates tested in percent. *All the isolates are divided into groups based on isolation or location. Colours indicate a heatmap of substrates' utilization, with red hues representing a higher proportion (>50%) of substrates being utilized by selected isolates and blue hues representing a lower proportion (<40%) to zero substrates being utilised.*

Mean substrate utilisation (%)	Carboxylic Acids	Carbohydrates	Amino Acids	Polyhydric Alcohols	Alcohols	Other
Isolates						
Anaerobic bacteria	44	68	53	44	14	4
Aerobic bacteria	47	46	56	34	19	12
Yeasts	46	45	29	37	41	0
Locations						
Antarctica	57	65	62	42	15	31
Greenland	40	50	48	38	22	0
Svalbard	45	53	42	39	26	0

Antarctic isolates used a bigger pool of substrates (45% of those tested) when compared to Svalbard (34%) and Greenland isolates (32%), however the differences were not statistically significant. Interestingly, Antarctic isolates were the only ones able to utilise the 'other' substrates (Appendix C) which include 'non-competitive' choline, betaine and methylamine. Yeasts had a greater capability to use alcohols and were less likely to use amino acids when compared to bacteria. Microbes isolated in anoxic conditions decomposed more types of carbohydrates than those obtained under oxic conditions.

5.3. Discussion

5.3.1. Microbial abundance and growth

The spread of cell numbers reported in the literature is astonishingly wide. After excluding the very extreme values of 0.5-7.5 x 10^{14} cells g^{-1} in Svalbard (Kastovská et al., 2005), the cell numbers reported in cryoconite holes worldwide are between 10^{6} and 10^{9} cells g^{-1} (Anesio et al., 2010; Cook et al., 2015; Hodson et al., 2010; Musilova et al., 2015; Singh et al., 2014; Stibal et al., 2006; Telling et al., 2014) and our data are within this range. Svalbard, Greenland and Antarctic samples showed no significant differences in the cell counts (mean counts of 9.2, 5.8 and 5.8 x 10^{8} cells g^{-1} respectively).

The microbial diversity and community structure of cryoconite holes is often based on only 'snapshots' of the community measured at a given place and time (Edwards et al., 2013), so there is clearly variability in reported results. However, many of the microorganisms isolated in this study have been identified elsewhere (Appendix C), for example, Flavobacteria class is often dominant in freshwater polar environments (Michaud et al., 2012) and the Cytophaga-Flavobacteria group, to which 18% of isolates in this study belong, was found to be dominant (87.2%) in Canada Glacier cryoconite holes from Antarctica (Foreman et al., 2007). *Flavobacterium, Cryobacterium* and *Arthrobacter* spp. were also isolated in Antarctic cryoconite holes from Canada Glacier (Christner et al., 2003). *Cryobacterium* spp. was also common in Svalbard soils (Hansen et al., 2007) and basidiomycetous yeasts were predominant in Svalbard sea and glacial ice (Gunde-Cimerman et al., 2003). Lutz et al. (2019) found Cyanobacteria, Proteobacteria and Actinobacteria phyla to be the most abundant in Antarctic cryoconite holes from Queen Maud Land. The dominance of Actinobacteria, in this study, followed by Bacteroidetes and Proteobacteria, is therefore consistent with these previous findings.

The experiments showed that oxic conditions yielded higher numbers of viable, cultivable microorganisms than anoxic. Cryoconite holes are largely oxygen rich environments, with aerobic metabolisms and consequent dominance of aerobic microorganisms, but there are anoxic niches (Poniecka et al., 2018) where a thriving anaerobic community can be found. Oxic microorganisms have quite uniform viable counts across the temperatures of 0.2 to 20 °C, with visible decline at 30 °C, whereas the highest cultivability in anoxic conditions was observed at 0.2 °C and the majority of the anaerobic community was still growing at 30 °C. It is interesting to speculate the cause of the maximal cultivability at lower temperatures. The anaerobic part of the community could be specifically adapted and active at the times of lower melt. During initial spring melt, there is a lack of mixing by meltwater that can lead to anoxic zones and an ionic pulse (Telling et al., 2014) likely supports higher metabolic activity. Facultative anaerobic heterotrophs may therefore be important in the reactivation of the community after the polar night (Vick-Majors et al., 2014).

5.3.2. Limits of cryoconite microorganisms

Cryoconite microorganisms were able to grow in a wide range of pH values. Reported pH of cryoconite holes in Antarctica was pH ~6-11 (Bagshaw et al., 2007; Porazinska et al., 2004; Stanish et al., 2013; Tranter et al., 2004), in Svalbard ~4.7-8.6 (Kastovská et al., 2005; Singh & Singh, 2012), in Greenland ~ 4.35 to 6.7 (Chandler, 2012, unpublished; Stibal, unpublished; Black and Bloom Team, 2016, unpublished), and in the Alps ~5 (Margesin et al., 2002). To some extent, this

growth range reflects the physical differences between Arctic and Antarctic holes, with Antarctic microorganisms growing in the highest pH in the laboratory and values up to pH 11 being measured in the field (Tranter et al., 2004). Most of Antarctic cryoconite isolates (7 out of 10) were able to grow in pH 10.5, suggesting that some of them could perhaps withstand even more alkaline conditions. At the other end of the spectrum are the yeast isolates, which seem to be more acidophilic than bacteria, tolerating pH of 3. The individual strains had specific tolerances that were similar to those previously published; for example, *Arthrobacter agilis* strain L77 from a lake in Himalayas (water pH 8.7 to 9.1) had a pH range of 6–9 and tolerated 5 % salinity (Singh et al., 2014), whereas *Arthrobacter agilis* strain LV7 in this study from Antarctic cryoconite hole had a pH range of 7–10.5 and tolerated 6 % salinity.

Tolerance of high salinity was a universal trait regardless of the sampling site, with all the isolated microorganisms able to grow outside the salinities typically found in cryoconite holes and on the glacier surfaces. Electrical conductivity (EC) of cryoconite holes in southwest Greenland was 2.2-3 µS cm^{-1} (Chandler, 2012, unpublished) and in Antarctica 5-20 µS cm^{-1} on Canada Glacier (Bagshaw et al., 2011) and ~60 – 110 µS cm^{-1} on Taylor Glacier (Porazinska et al., 2004). This compares with adjacent habitats which frequently have extreme EC: Lake Hoare and Lake Bonney (Taylor Valley) were 65 – 7798 µS cm^{-1} (Courtright et al., 2001); Fresh, Orange and Salt Ponds on the McMurdo Ice Shelf were 158, 937 and 52900 µS cm^{-1} respectively (Jungblut et al., 2005) and soils in Wright Valley > 1500 µS cm^{-1} (Courtright et al., 2001). In Svalbard, in the forefield of Midtre Lovenbreen, a 2347-year-old permafrost soil was ~ 8200 µS cm^{-1} (Hansen et al., 2007). These habitats are likely important inoculum for cryoconite holes (Bagshaw et al., 2013; Porazinska et al., 2004). Salinity tolerance may also assist in freeze-thaw protection: cryoconite holes undergo multiple freeze-thaw events in their lifetime, so microorganisms must either survive freezing, or avoid it by persisting in high salinity brine veins within the ice crystal structure (Mader et al., 2006; Telling et al., 2014). Fungi isolated from Svalbard sea ice and glacial ice grew better on halotolerant media than on the traditional media, with fungal growth up to 24% salinity, indicating that a high number of halophilic species can be found on glaciers and sea ice (Gunde-Cimerman et al., 2003). Another possibility is that resistance to salinity is the by-product of resilience to other environmental conditions such as high UV, dehydration or freezing (Poli et al., 2010). Cyanobacteria from hypersaline ponds on McMurdo Ice Shelf use organic osmolytes as a protection from osmotic stress (Jungblut et al., 2005) and large quantities of EPS were found in the brine channels in sea ice (Nichols et al., 2005). Mechanical damage to cell walls during freezing results either from intracellular ice crystals formation or recrystallization of extracellular small ice crystals into large grains, or by osmotic stress caused by dehydration

following extracellular freezing and electrolyte concentration in the remaining liquid phase (Fonseca et al., 2001; Pegg, 2007; Raymond, 2016). The similar mechanisms of cell damage by dehydration, freezing and hypersaline solution often results in cross-protection, however there was no correlation between high salinity resistance and other variables such as freeze-thaw survival in this study.

Physical damage sustained to the cell during freezing depends on its shape, structure and membrane rigidity (Jordan et al., 2008; Mai-Prochnow et al., 2016), hence it might explain some of the differences between the isolates. Yeasts have a thick cell wall composed of polysaccharides. Gram-positive bacteria have a thick (20–80 nm), rigid cell wall built of peptidoglycan, and Gram-negative bacteria have a thin wall. Gram-positive bacteria were classically considered to be typical for soil ecosystem and consequently adapted to dry conditions (Barka et al., 2016). In this study, all the yeasts survived well, and typically Gram-positive bacteria generally survived better (almost all survived 25 cycles and some survived 100) than Gram-negative (none survived 100 cycles). The structure of cell envelope has a major impact, but additional protection against concomitant damaging factors such as reactive oxygen species generated during thawing can be achieved by carotenoids – pigments which protect from photosensitization and from reactive oxygen species (Dahl et al., 1989; Park et al., 1997; Dieser et al., 2010; Mai-Prochnow et al., 2016). Most of the isolated cryoconite hole microorganisms are pigmented (Table 5.3). Another protective strategy is excreting protective antifreeze proteins and/or EPS (Poli et al., 2010; Sathiyanarayanan et al., 2015; Raymond, 2016; Perkins et al., 2017). Many cryophilic genera found also in cryoconite holes were shown to produce EPS, such as *Flavobacterium sp.* (Nichols et al., 2005; Sathiyanarayanan et al., 2015) or *Arthrobacter sp.* (Singh et al., 2014). The *Arthtobacter* genus was reported to survive multiple freeze thaw cycles (Muñoz et al., 2017). Moreover, numerous cryosphere bacteria show antifreeze proteins activity, including Actinobacteria and Bacteroidetes from Antarctic moss (Raymond, 2016), *Sphingomonas, Plantibacter, Pseudomonas* and *Arthrobacter* sp. from Antarcic ice and sediments (Muñoz et al., 2017), or *Cryobacterium, Pseudomonas* and *Subtercola* sp. among Arctic cryoconite bacteria (Singh et al., 2014). Most of the isolated bacteria in this study belong to Actinobacteria, and there are also several isolates of *Cryobacterium* from all locations.

Several isolates were tested to estimate the temperature range in which they were able to grow. Bacterial isolates were psychrotolerant, all being able to grow above the arbitrary threshold for psychrophilic growth of 22 °C (Cavicchioli, 2016). Yeasts, which only grew in the lower incubation temperatures and were able to better withstand freezing-thawing cycles, were psychrophiles. Although the sample size is small, it is interesting to debate the difference between bacteria and

yeasts isolated form these cold environments. The results suggest that yeasts are better adapted to lower temperatures and stresses encountered in glacial environments such as freeze-thaw. Conversely, bacteria present a greater variability in their physiology, with some species adapted as well as yeasts to freeze-thaw and with similarly good salinity tolerance and broad pH range.

Survival of bacteria is therefore determined by multiple factors, including the structure of the cell envelope, internal pigments, excreted antifreeze proteins and compatible solutes. As the detailed mechanisms by which each species survives freeze-thawing were not the aim of this study, we can only speculate the cause of the differences. It was notable that yeasts are very resistant to freezing, yet they are not found as the dominant group in other studies of cryoconite community. The most abundant retrievable microorganisms of cryoconite holes are resistant to a wide range of fluctuating environmental conditions and stressors. Such conditions, including freeze-thaw cycles, high salinities, temporary anaerobic conditions and pH variability are typically encountered in arid polar soil habitats (Bagshaw et al., 2013; Courtright et al., 2001; Paul, 2006; Poage et al., 2008; White, 2006), which are likely to be seeding grounds for cryoconite holes.

5.3.3. Organic carbon utilisation and metabolic capabilities

It is well known that organic matter is plentiful in cryoconite holes (Tranter et al., 2004; Musilova et al., 2016) and that there are genes present for biodegradation (Edwards et al., 2013) that are able to decompose organic matter (Sanyal et al., 2018). Thus, unsurprisingly, strains were detected which are known to decompose organic matter (e.g. *Flavobacterium* sp. (Williams et al., 2013), *Cryobacterium* sp. (Sanyal et al., 2018)). Overall, cryoconite holes microorganisms produce a variety of enzymes for different groups of carbon substrates, suggesting they are effective in scavenging carbon substrates when and if they become available. Isolates obtained under anoxic conditions tend to utilise a higher proportion of carbohydrates when compared to those obtained aerobically. Facultatively anaerobic microorganisms have a different type of metabolism, depending on the availability of oxygen, and will commonly use monomeric sugars as their electron acceptor, hence it is unsurprising that they specialise in carbohydrates. Antarctic bacteria have the capacity to use a bigger pool of substrates, including 'non-competitive' organic matter such as choline and betaine, when compared to the Arctic microorganisms. This can be explained by a smaller input of carbon sources due to entombment of Antarctic cryoconite holes (Telling et al., 2014; Webster-Brown et al., 2015), whereas the Arctic holes are frequently flushed with melt water during the melt season. Whilst the differences are not statistically significant, due to a very high variability among isolates, none of the Arctic isolates utilise 'non-competitive' carbon substrates.

Some closely related isolates assigned to the same OTU showed striking differences in their metabolic capabilities. Without further studies it is impossible to pinpoint the source of the differences, but possible reasons include the initial incubation temperature, fitness of the culture and the metabolic impact of genome rearrangement in stressed and starving microorganisms. Such differences are quite common, for example *Cellulomonas* strains can have differences in their physiology (Hatayama et al., 2013), even when they are closely related (~ 98% sequence similarity, which is enough to assign them to the same OTU). Another example is *Flavobacterium* genus, which is also widespread in cryoconite holes, where two distinct species had 98.9% 16S rRNA gene sequence similarity (Peeters and Willems, 2011). The resolution of 16S rRNA gene at species level is often limited, as it is a highly conserved gene (Peeters and Willems, 2011). Isolates which share the same OTU are not necessarily the same bacterium, it only means that they have a high degree of similarity in one gene. Even if they have highly similar 16S rRNA genes, their entire genome is not likely to be the same (Woese, 1987; Rosselló-Mora and Amann, 2001). Finally, culturing is a selective process, so it is possible that a single bacterium which gave a pure isolate was devoid of an enzymatic pathway. Therefore, while it is useful to investigate the capabilities of the organisms, it cannot be excluded that in the environment their metabolic capabilities could differ, depending on overall fitness and competition. Regardless of these differences, microorganisms of cryoconite holes clearly complement each other and partly specialise in the types of substrates used. It may depend on the microniches within the cryoconite holes and the relationships with other microorganisms, as well as the availability of particular substrates, but there is not enough data in the literature on the composition of cryoconite organic matter to draw clearer conclusions. What can be concluded from this experiment is that isolates in pure culture were able to utilise a broad range of carbon substrates and as a community, they can scavenge almost all substrates tested. They seem well adapted to the extremely low organic matter content, which is typically encountered in glacial environments (Anesio and Laybourn-Parry, 2012) and barren Antarctic soils (Poage et al., 2008).

5.4. Summary

Microorganisms inhabiting cryoconite holes are exposed to a wide range of extreme conditions, and this chapter demonstrates their broad tolerance. The generally oxygen-rich cryoconite holes harbour an active, culturable anaerobic community. Anaerobic cultivability is better in the coldest conditions tested, which suggests their adaptation to and dominance of the beginning or end of the melt season, when anoxic conditions are likely to occur. Apart from anoxia, cultured microorganisms can withstand a wide range of other physical stresses, including extreme pH and

salinity. As pH tolerance broadly reflected the values found in the samples locations, this may indicate that extreme salinities could also be found in cryoconite holes in certain conditions, for example, during freeze-thaw cycles, or that the microbial community is seeded from nearby saline habitats, such as arid soils or melt ponds. Cryoconite microorganisms use a wide range of substrates and as a community are effective in scavenging limited carbon sources in cryoconite holes. Their metabolic capabilities seem to depend not only on the genetic affiliation, but also, perhaps, fitness of the culture and phenotypic differences between closely related species. Such phenotypic differences are especially likely, as the bacteria with a high 16S rRNA gene similarity show differences in their physiology. Antarctic isolates showed greater resistance to freezing and thawing cycles and greater use of variable carbon sources when compared to Arctic ones, suggesting they might be adapted to the harsher Antarctic environment. However, the same genera were found in both Arctic and Antarctic samples, similar total cell numbers and the same range of salinities withstood, demonstrating that cryoconite hole microorganisms from both poles also share some physiological traits.

Chapter 6

Fermentative potential

6.1. Introduction

Anoxic zones were detected in the cryoconite holes *in situ* and in the incubations of cryoconite material (chapter 4). Microorganisms from cryoconite holes were able to grow both in aerobic and anaerobic conditions (chapter 5). In this chapter, the chemistry of water from cryoconite holes and incubations was analysed to explore microbial metabolism and their fermentative potential. Carboxylic acids were detected in the water from cryoconite holes and in the pore water from long-term incubations of cryoconite material. Two carboxylic acids were particularly elevated in some (propionate) or most of the samples (acetate).

6.2. Acetate in cryoconite holes samples

6.2.1. Black and Bloom samples

Acetate concentrations in water directly sampled from cryoconite holes in Greenland were below detection limits (<0.07 µM) in the majority of early season field samples (20 out of 28). In the water column, only one sample had detectable acetate (0.84 µM), whereas the mean acetate concentration in the sediment pore water was 0.65 µM (n=13, standard deviation (st.dev.) = 1.07). The mean concentration in the water column was 0.07 µM (n=12, st.dev.= 0.23), which was below the MilliQ blank/control (n=3, 0.08 µM, st.dev.=0.11). Overall, the acetate concentrations were very low when compared to incubations or defrosted sediment samples, but there was a significant difference between water column and sediment pore water (p=0.08, t-test).

6.2.2. Defrosted cryoconite sediment samples

Acetate concentrations in the pore water from thawed cryoconite samples (collected in Antarctica, Svalbard and Greenland; stored at -20 °C) were much higher when compared to the water collected *in situ* in the field. The Greenland Black and Bloom water sample collected *in situ* had an acetate concentration of 0.9 µM, but after the sediment from the same cryoconite hole was collected, frozen and defrosted, the concentration in the porewater was 632.9 µM. After a

second freezing-thawing cycle, the concentration was 548.4 µM. Other defrosted samples (Table 6.1) also had elevated acetate, and the majority (6 out of 8) released more acetate into the surrounding water after a second freeze-thaw treatment. Black and Bloom samples consistently showed higher concentrations after the first and second freeze-thaw cycle (197.6 ± 251.5 vs 383 ± 202.1 µM, n=4). Small, dispersed cryoconite sediment samples from shallow ice cores (see Methods 3.5.1) also had high acetate concentrations following defrosting. Antarctic cryoconite defrosted sediment had the lowest concentrations (4.4 ± 2.5 µM, n=2), yet still detectable, and increased to 54.3 ± 16.9 µM (n=2) following a second freeze-thaw. Svalbard samples were very variable (184.2 to 1675.0, st.dev. = 545.0), which was also the case for the incubations of cryoconite sediment (see section 6.3.3).

Table 6.1. Acetate concentrations measured in defrosted cryoconite sediment samples*. cryo – cryoconite, stream cryo – accumulation of cryoconite material in the stream, ice core – cryoconite material from shallow ice core.*

Site	Sample type	Acetate after first defrost (µm)	Acetate after second defrost (µm)
GrIS Black and Bloom	cryo	47.1	194.4
	cryo	632.9	548.4
	cryo	66.2	618.4
	stream cryo	44.2	170.8
	cryo	726.8	
	cryo	816.0	
	stream cryo	691.0	
	ice core	8.7	
	ice core	158.4	
	ice core	996.2	
Svalbard	cryo	1013.5	759.8
	cryo	574.9	1640.6
	cryo	370.7	
	cryo	1675.0	
	cryo	184.2	
Antarctica	cryo	6.9	71.1
	cryo	1.9	37.4
	cryo		24.6
	cryo		9.0

6.3. Acetate in cryoconite sediment incubations

6.3.1. Greenland Ice Sheet samples

6.3.1.1. Greenland Field (Black and Bloom) incubations

After 20 days of incubation of cryoconite material in the field (methods 3.4.2), increased concentrations of acetate were observed in the pore water (Fig. 6.1). Concentrations near the

surface of the sediment were much lower than in the deeper layers (26.0 µM at the surface vs. 269.6 µM and 169.2 µM at 0.5 cm and 1 cm, respectively). Such difference between different sediment depths was only recorded in incubations of Greenland material.

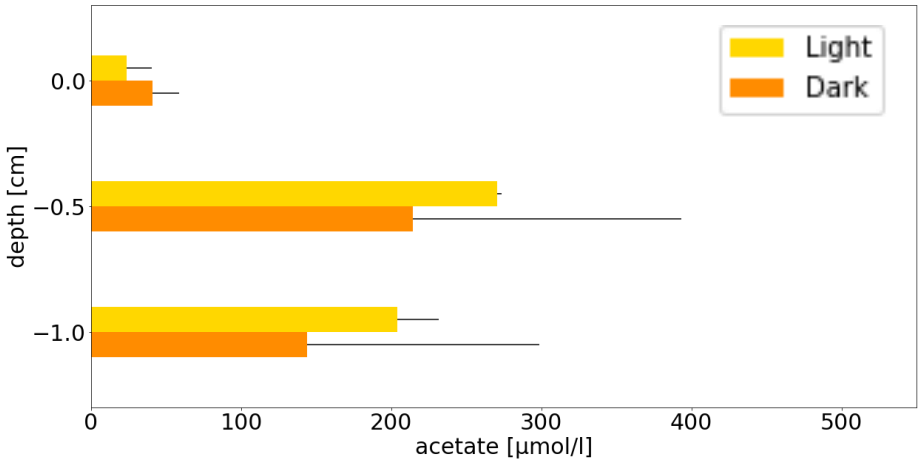

Figure 6.1. Acetate concentration in Black and Bloom field incubations, incubated at in situ temperature for 20 days under light and dark conditions. *Error bars are generated from triplicate samples.*

6.3.1.2. Laboratory incubations of Greenland interior cryoconite

The incubations were repeated in the laboratory in triplicate, using pooled samples from the same sampling location as above, to confirm the accumulation of acetate. After 92 days, acetate accumulation in pore water samples was higher in both light (160.0 µM more, p=0.1) and dark (512.7 µM more, p=0.048) than after 20 days of incubation in the field (Fig 6.2A). However, the high variability and small sample size make it difficult to draw any significant conclusions.

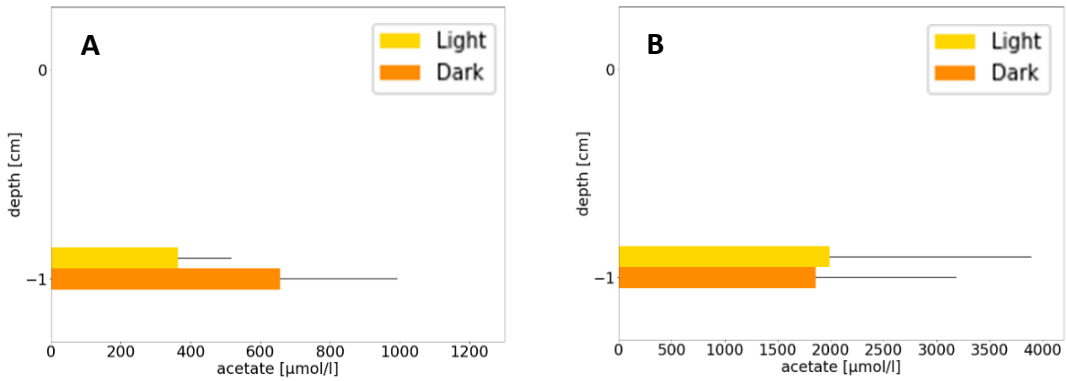

Figure 6.2. Acetate concentration in two sets of Greenland interior (Black and Bloom) laboratory incubations, incubated at 0.2 °C for 92 days (A) and for 114 days (B) under light and dark conditions*. Error bars are generated from standard deviation across triplicate samples. Note different scale on the A and B charts.*

Incubations were repeated in duplicate for 114 days to confirm the production and accumulation of acetate in cryoconite samples (Fig 6.2B). Very high concentrations of acetate were observed, with a great variability (light: 1989.7 ± 1905.6 µM, dark: 1856.8 ± 1332.0 µM). The concentrations were on average 5 times higher (light) and 3 times higher (dark) when compared to the 92-day long incubations, but they were not significantly different because of high variance (p= 0.44 and p=0.43 respectively).

6.3.1.3. Laboratory incubations of Greenland margin cryoconite

After 35 days of incubation, Greenland samples showed similar, elevated levels of acetate to Antarctic samples at all depths when compared. Variability between samples was high, and there were minimal differences between concentrations in dark incubations and light incubations (Fig. 6.3). The concentrations at the Greenland margin were in the same range as the interior samples after 20 days (4.5-137.5 µM vs 10.7-420.4 µM at Black and Bloom). The concentrations remained almost unchanged after 175 days of incubations (Fig. 6.3). There was no difference in concentrations between t1 (35 days) and t2 (175 days) (p=0.41) and the range of concentrations remained the same, suggesting that Greenland margin incubations overall were not very variable with time – the inter quartile range was 82.5 µM vs 85.9 µM for t1 and t2, respectively.

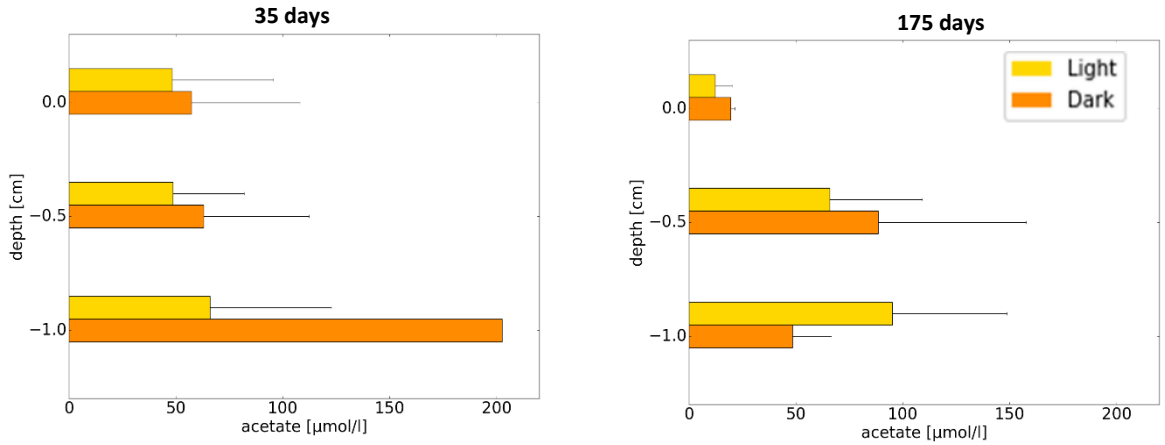

Figure 6.3. Acetate concentrations in Greenland sediment incubated at 0.2 °C for 35 days (left) and 175 days (right) under light and dark conditions. *Error bars generated from standard deviation of triplicate samples*

6.3.1.4. Laboratory incubations of intact cryoconite granules

A single layer of cryoconite granules from margin of GrIS was incubated for 730 days. Sediment from Greenland interior (Black and Bloom) was also incubated in a uniform thin layer (2 mm) which created soft aggregates with time (Fig. 3.10 in Methods). Both types of the sediment released acetate to the surrounding water over time (n=1). The acetate concentration in the water above samples from the ice sheet margin was 122.9 µM and the ice sheet interior was 218.3 µM.

6.3.2. Laboratory incubations of Antarctic cryoconite

After 35 days of incubation in the laboratory, Antarctic samples showed elevated levels of acetate at all sampled depths (0, 0.5 and 1 cm), similar to Greenland margin samples (Fig. 6.4). The highest concentrations of acetate were found at the greatest depth in both light and dark incubations (significantly correlated with depth, Pearson's correlation coefficient = 0.66), however the variability between samples was again noticeable. The concentrations rose 150 times by day 175 of incubations (p=0.00, t-test). Light incubations seem to have more acetate in the shallower depths than dark after 35 days, but this tendency disappears after 175 days of incubation, where all concentrations were equally high (4377 µM on average). However, further conclusions about the dark samples after 175 days cannot be made, as only 1 sample was used for comparison (the other two samples leaked and had to be excluded from further analysis). Incubations were repeated in duplicate for 114 days and high concentrations of acetate were again observed (Fig. 6.5).

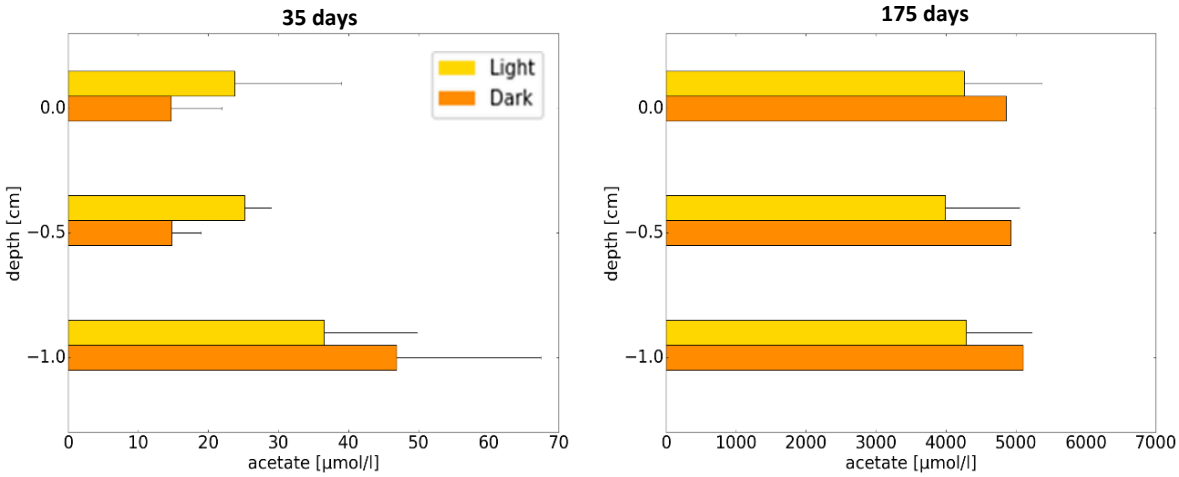

Figure 6.4. Acetate concentrations in Antarctic sediment incubated at 0.2 °C for 35 days (left) and 175 days (right) under light and dark conditions. *Error bars generated from standard deviation of triplicate samples. After 175 days, two dark samples had leaked and the water had drained, thus there are no error bars for the dark samples at the end of the incubation. Note different scale on the charts.*

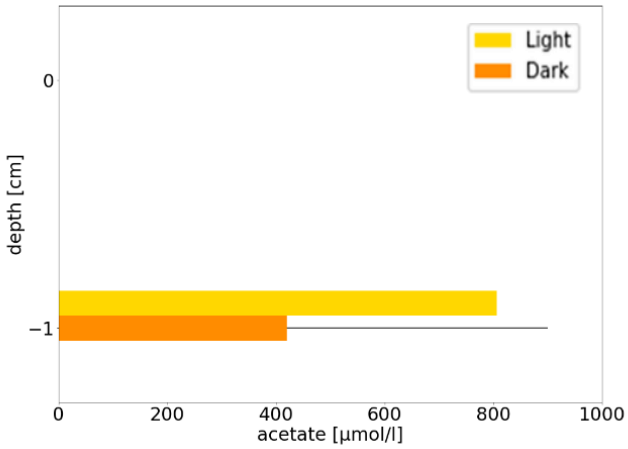

Figure 6.5. Acetate concentrations in a repeated Antarctic sediment incubations, incubated at 0.2 °C for 114 days under light and dark conditions. *Error bars generated from standard deviation of duplicate samples.*

6.3.3. Laboratory incubations of Svalbard cryoconite

After 35 days of incubation, Svalbard samples showed 10-fold higher concentrations of acetate at all depths when compared to Antarctic samples incubated for the same time (Fig. 6.6). The variability between the Svalbard samples was high: the mean concentration at t1 was 780.2 µM and on t2, 1284.5 and the interquartile range was 1166.2 and 3444.6 respectively. The variability was therefore high after 35 days and increased further after 175 days. The change in concentration between 35 and 175 days of incubation was not significant (p=0.38). The light sample from 1 cm depth seemed to have much lower acetate concentration, however no other samples could be taken (due to technical challenges) and the variability of the light samples could not be included, so was likely just an artefact of variability. Overall, there was no significant difference between light and dark samples and the prevailing characteristic of the samples from Svalbard was variability. Incubations were repeated in duplicate for 114 days to confirm the production and accumulation of acetate in cryoconite samples (Fig. 6.7), and very high concentrations of acetate were again observed (mean 4103.4 µM for light samples and 918.7 µM for dark sample - only one dark sample was measured because of the leak).

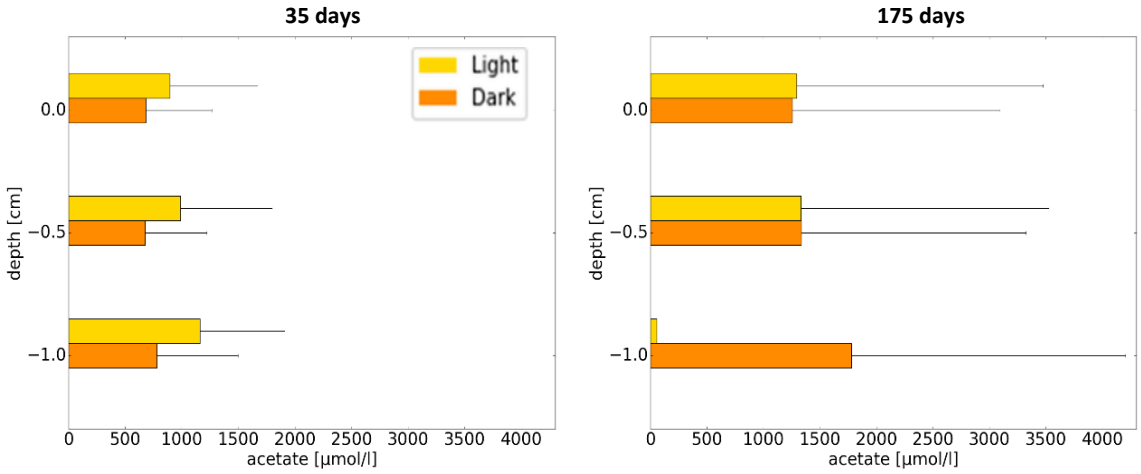

Figure 6.6. Acetate concentrations in Svalbard sediment incubated at 0.2 °C for 35 days (left) and 175 days (right) under light and dark conditions. *Error bars generated from standard deviation of triplicate samples. After 175 days, there are no error bars for the light samples at 1 cm depth, because the sediment blocked the needle and it was impossible to subsample water in 2 light samples.*

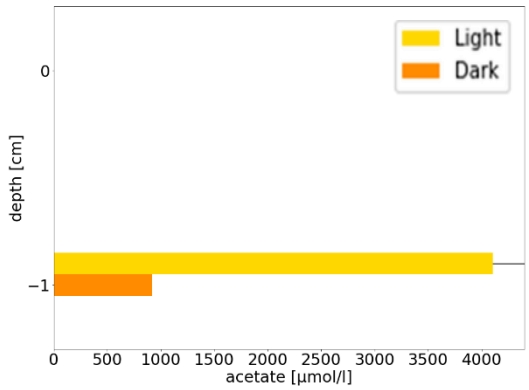

Figure 6.7. Acetate concentrations in a repeated Svalbard sediment incubation, incubated at 0.2 °C for 114 days under light and dark conditions*. Error bars generated from standard deviation of duplicate samples (light). Only one dark sample was measured because of the leak.*

6.4. Detection of other carboxylic acids (propionate, lactate, formate)

Formate and propionate were detected in some samples following incubation, however, they displayed high variability and very few trends could be described. No lactate was found in any of the incubated cryoconite sediment samples. Svalbard samples had the highest concentrations of propionate in the first set of incubations, however after repeating the incubations with new sediment, propionate was much lower and comparable to Antarctic samples after 175 days (Table 6.2). High concentrations of propionate correlated significantly with high concentrations

of acetate after 35 and 175 days in the first set of incubations (Pearson's correlation coefficient 0.82 and 0.99 respectively). Propionate also correlated positively with acetate in Black and Bloom field incubation samples (20 days) (Table 6.3). Antarctic samples had detectable propionate in the first set after 175 days, and none in the second set of incubations (Table 6.2). Concentrations in Greenland margin incubations remained mostly below detection limits throughout.

Formate was on average 3.44 µM ± 4.16 after 35 days and 13.35 ± 45.36 after 175 days in all samples. It did not increase significantly after 175 days when compared to 35 days. It correlated with depth in Greenland margin samples, but not in other samples and it did not correlate with other carboxylic acids.

Table 6.2. Mean propionate concentrations in the cryoconite sediments incubated at 0.2 °C under light and dark conditions. *Standard deviation of triplicate (I incubation set) or duplicate samples (II incubation set) is marked with ± under the mean value (in bold). Samples with concentrations of propionate below the detection limit are indicated with "-". Grey colour indicates no subsamples taken.*

		I incubation set				II incubation set	
		35 days		175 days		114 days	
	depth	light	dark	light	dark	light	dark
Svalbard	0	**65.2** ± 58.8	**23.7** ± 6.9	**839.6**	**691.0**		
	0.5	**50.9** ±64.2	**24.5** ±7.0	**411.6** ±578.4	**736.7**		
	1	**58.9** ±60.9	**26.2** ±4.0	-	**322.4** ±451.5	**58.2** ±1.0	**39.9**
Antarctica	0	-	-	**27.9** ±15.0	**29.3**		
	0.5	-	-	**32.9** ±18.0	**30.7**		
	1	-	-	**38.3** ±16.0	**30.1**	-	-
Greenland	0	-	-	-	-		
	0.5	-	-	**2.6**	-		
	1	-	-	**3.1** ±0.5	-		

Table 6.3. ***Mean propionate concentrations in the cryoconite sediments incubated in situ (Field incubations) and in the laboratory at 0.2 °C under light and dark conditions.*** *Standard deviation of triplicate (Field incubations, I incubation set) or duplicate samples (II incubation set) is marked with ± under the mean value (in bold). Samples with concentrations of propionate below the detection limit are indicated with "-". Grey colour indicates no subsamples taken.*

		Field incubation		I incubation set		II incubation set	
		20 days		92 days		114 days	
Greenland	**depth**	**light**	**dark**	**light**	**dark**	**light**	**dark**
interior (Black and Bloom)	**0**	-	**3.9** ±1.9				
	0.5	-	**3.0** ±0.1				
	1	**3.9** ±0.4	**5.1**	**587.9** ±610.2	**419.8** ±351.4	**46.3** ±9.9	**169.3** ±140.7

6.5. Inorganic ions

Defrosted cryoconite samples (considered t0) had higher concentrations of inorganic anions than cryoconite material incubated for 35 and 175 days (Table 6.4). There were no Greenland margin samples for t0. In the incubated samples, nitrate concentrations did not vary by location or time (Table 6.4). Phosphate was only detectable in Greenland samples. Sulphate decreased in all the samples after 175 days of incubations when compared to the 35 days. There were no correlations of carboxylic acids with sulphate, nitrate or phosphate. The concentrations of inorganic cations (Table 6.5) were also not correlated with carboxylic acids. Inorganic cations were not measured in the defrosted samples (t0).

Table 6.4. ***Mean anions concentrations in the defrosted cryoconite samples and incubated cryoconite sediments.*** *Samples with concentrations below the detection limit are indicated with "-". Grey colour indicates no samples taken.*

	Defrosted samples (t0)			35 days (t1)			175 days (t2)		
	SO_4^{2-}	PO_4^{3-}	NO_3^-	SO_4^{2-}	PO_4^{3-}	NO_3^-	SO_4^{2-}	PO_4^{3-}	NO_3^-
Svalbard	73.8	22.7	73.0	53.2	-	16.0	8.1	-	16.0
Antarctica	123.3	-	33.2	9.2	-	14.8	1.6	-	16.2
Greenland margin				22.6	10.5	15.7	0.0	11.4	14.4
				20 days					
Greenland interior	51.2	-	30.0	6.2	4.0	16.0			

Table 6.5. ***Mean cations concentrations in the incubated cryoconite sediments.*** *Samples with concentrations below the detection limit are indicated with "-".*

	35 days (t1)				175 days (t2)			
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Na^+	K^+	Mg^{2+}	Ca^{2+}
Svalbard	2.8	0.2	15.7	-	2.8	0.9	5.7	6.7
Antarctica	3.8	1.8	13.6	26.7	9.7	3.2	40.2	56.9
Greenland margin	5.1	1.8	5.2	13.2	9.2	5.1	21.6	91.0
	20 days							
Greenland interior	2.1	3.0	-	0.4				

6.6. Fermentation products in anoxic incubations of microbial cultures

Pure microbial cultures of bacteria and yeasts isolated from cryoconite holes (in either oxic or anoxic conditions) were incubated in liquid medium in anaerobic conditions for 70 days and tested for the presence of selected fermentation products. Acetate was found in most samples (42 out of 43, with concentrations ranging from 8.1 to 1931.4 µM, Fig. 6.9). Other carboxylic acids were not uniformly present, with lactate observed in 18 samples (19.0 to 4166.5 µM), formate observed in 22 samples (53.8 to 3129.4 µM) and propionate in 15 samples (2.4 to 3412.8 µM) (Fig. 6.9). Formate was present in all anoxic isolates and lactate was present in most of them, whereas formate and lactate were mostly absent from oxic samples.

Anoxic, oxic and yeast isolates varied significantly in the amount of carboxylic acids produced. For acetate (ANOVA p=0.00), anoxic isolates produced almost five times as much acetate as their oxic counterparts and 20 times more than yeasts isolates (Fig. 6.10). Anoxic isolates also produced much more formate (ANOVA followed by Tukey, p=0.00) and lactate (ANOVA, p=0.00) than their oxic counterparts and yeasts. Oxic bacteria and yeasts were not significantly different.

There was no significant difference in the carboxylic acids accumulated between the field sites from which cryoconite samples were collected and microbes cultured (Fig. 6.9, ANOVA acetate: p=0.46, lactate p=0.31, formate p=0.87). The only notable differences were high concentrations of propionate accumulated in Antarctic isolates (mean = 652.3 µM) when compared to the Svalbard and Greenland interior (mean 1.8 and 0.5 µM respectively, ANOVA p=0.03, Fig. 6.9). The control medium had acetate concentration of 9.1 µM and no other carboxylic acids detected.

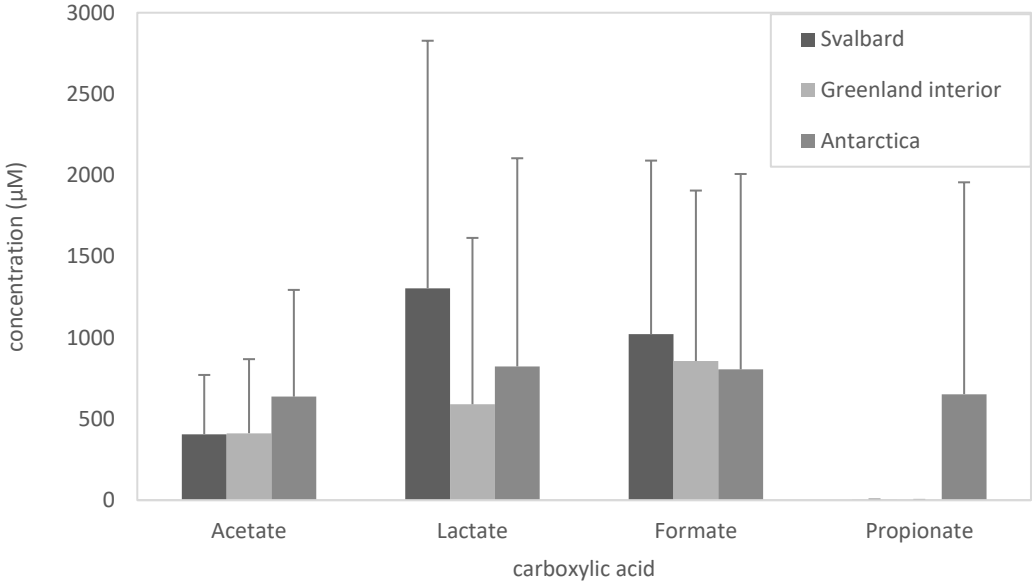

Figure 6.8. Carboxylic acids concentrations produced by microbial isolates from cryoconite holes from Antarctica, Svalbard and Greenland. *Error bars depict standard deviations of concentrations for Svalbard (n=16), Greenland (n=17) and Antarctica (n=10).*

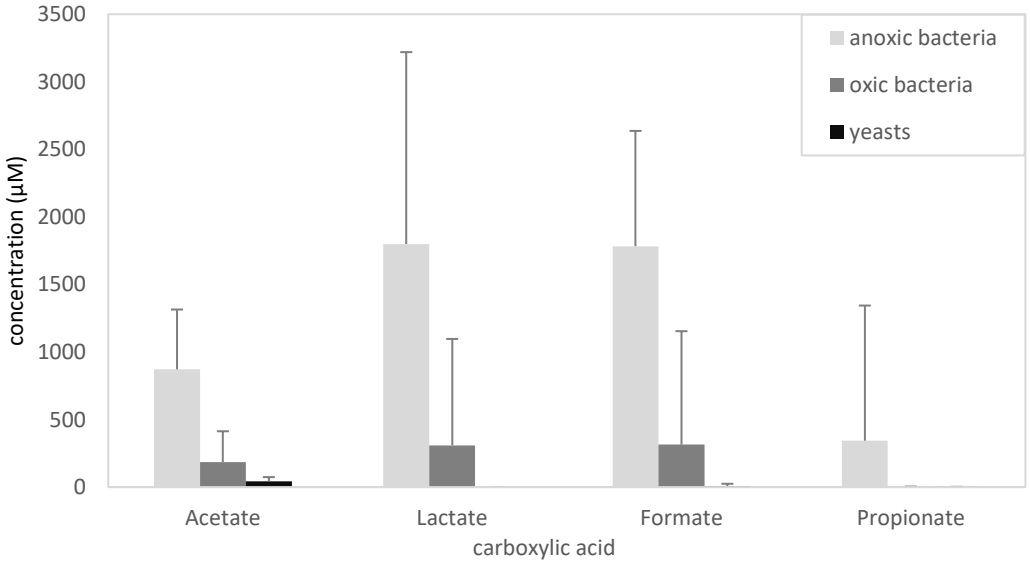

Figure 6.9. Carboxylic acids concentrations produced by microbial isolates from cryoconite holes from Antarctica, Svalbard and Greenland (data from all sites combined) under anaerobic conditions. *Error bars depict standard deviations of concentrations for anoxic isolates (n=19), oxic isolates n=16) and yeasts (n=8).*

6.7. Phototrophic organisms

6.7.1. Fermentation in phototrophic cultures

Microscope observations showed that isolated phototrophic cultures mostly contained one type of phototrophic microorganism with associated heterotrophs (Fig. 6.10). The exception was a culture from Svalbard where alga and cyanobacteria coexisted and could not be separated by at least three generations of subculturing (Fig. 6.10 Svalbard). All the isolates created filamentous mats or flakes (Fig. 6.10) that were subsequently identified by sequencing where possible (Table 6.6). All the cyanobacterial isolates belonged to genus *Phormidesmis* with various associated heterotrophic bacteria. Sequencing analysis revealed an additional phototrophic organism in the Greenland margin sample, represented by a large proportion of OTUs – *Rhodopseudomonas* sp. (purple non-sulphur phototrophic organism). Algae could not be identified due to problems with DNA amplification.

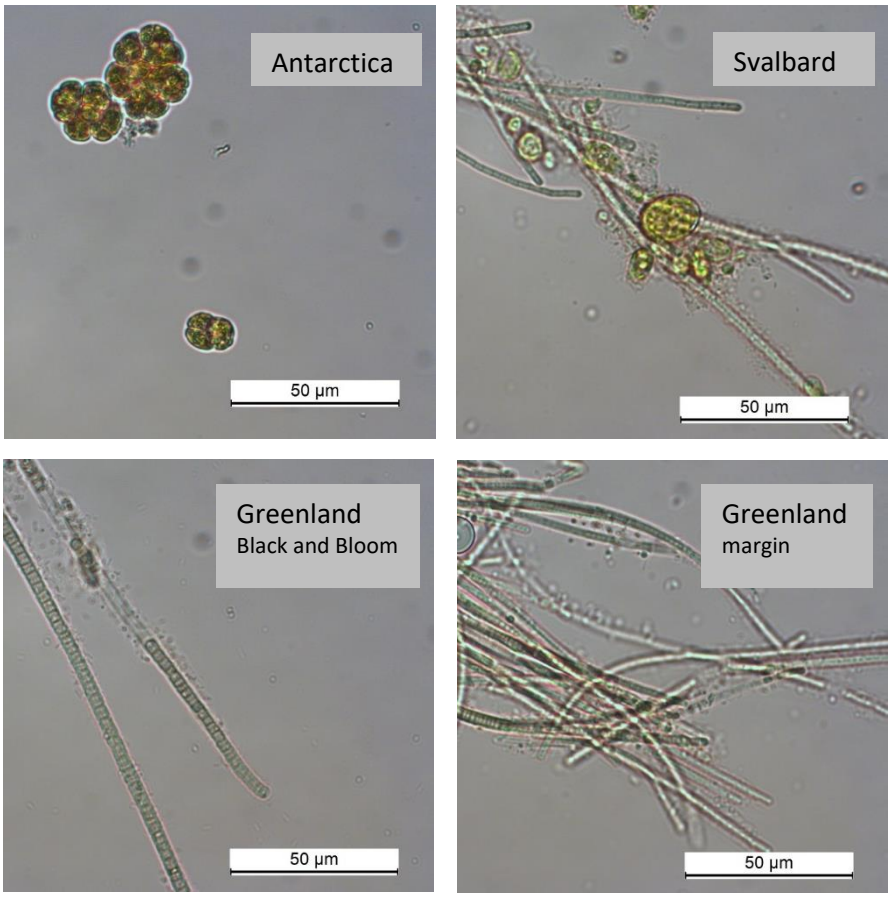

***Figure 6.10. Phototrophic microorganisms cultured from cryoconite holes**. Antarctic alga, Svalbard alga and cyanobacteria, Greenland Black and Bloom cyanobacteria, Greenland margin cyanobacteria*

Table 6.6. Summary of phototrophic microorganisms and dominating associated heterotrophic species isolated from cryoconite holes. *Carboxylic acids concentrations were detected in the incubations of phototrophic microorganisms and associated heterotrophs under anaerobic conditions, supplemented with fermentation medium. No propionate was detected.*

Location	Phototrophs	Associated heterotrophs	Acetate (μM)	Lactate (μM)	Formate (μM)
Antarctica	--	*Flavobacterium araucananum*	694.3	736.0	1951.2
Svalbard	*Phormidesmis sp.*	*Sphingobacteriaceae bacterium*	1174.2	1083.2	2684.3
Greenland margin	*Phormidesmis sp.*	uncultured bacterium	1207.1	1123.5	2796.0
Greenland interior	*Phormidesmis sp.* *Rhodopseudomonas sp.*	*Bacillus endophyticus*	105.4	1940.9	892.1

In order to determine the potential of phototrophic community to produce acetate, cultures were subsequently incubated in 6 different treatments: normal light, high light, high light with low nutrients, dark, anaerobic and anaerobic fermentation (See Methods 3.5.4.4.2). The treatments yielded visible growth (Fig. 6.11 top panel), with green colour where the algae were present and orange colour in cyanobacterial cultures. In the anaerobic fermentation cultures, very few green or orange filaments and aggregates were visible since fermentation prevented growth of phototrophic organisms (Fig. 6.11 bottom panel). Instead, white pellets were discernible, suggesting growth of cohabiting heterotrophic bacteria.

Normal light treatment, which generally served as a positive control with good growth conditions, yielded no carboxylic acids, except for a sample from Antarctica which had 10.7 μM of acetate. Similarly, high light with low nutrients and anaerobic treatments had no detectable carboxylic acids. High light treatment had some very low concentrations of lactate (4.08 μM in Greenland margin sample and 4.66 μM in Greenland Black and Bloom sample), formate (4.29 μM in Greenland Black and Bloom sample) and acetate (12.41 μM in Antarctic sample). Similarly, in the Greenland Black and Bloom sample, the dark treatment had low concentrations of acetate (1.17 μM), lactate (4.14 μM) and formate (3.33 μM). The only treatment with elevated concentrations of carboxylic acids (acetate, lactate, formate) was the anaerobic fermentation treatment (Table 6.6). No propionate was found in any of the samples. Together, the results show very little to no carboxylic acid production by phototrophs and elevated levels were only observed with heterotrophic growth of associated microbes.

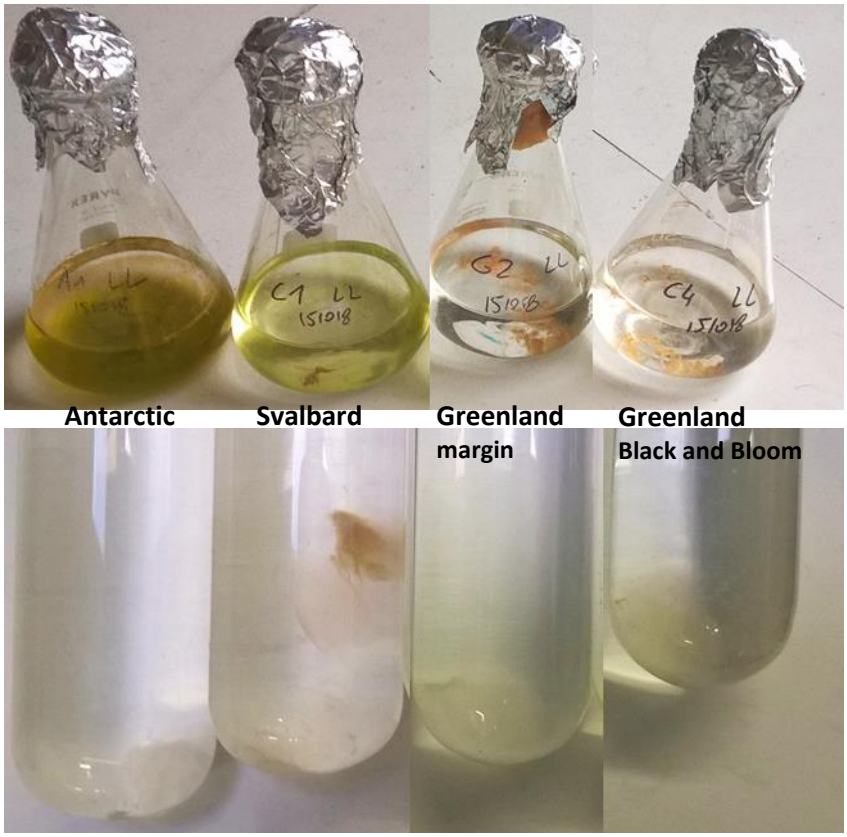

Figure 6.11. Phototrophic growth under normal light condition (top panel). Phototrophic and heterotrophic growth under anaerobic conditions, supplemented with fermentation medium (bottom panel).

6.7.2. Influence of acetate on phototrophs

To assess the influence of acetate alone on the phototrophs, selected cultures were incubated with addition of 1M acetate and their fitness assessed by photophysiology measurements. Following 1h and 24h of incubations with 1M of acetate, the culture from Greenland Black and Bloom sample did not exhibit changes in photochemistry when compared to the control (Fig. 6.12). Similarly, Greenland margin cyanobacterial isolate also did not show any changes after 1h of incubation. The variability of measurements in the Greenland margin isolate was higher than in Black and Bloom, likely because of bigger, denser clumps that were harder to disrupt into a uniform mixture, resulting in self-shading effects.

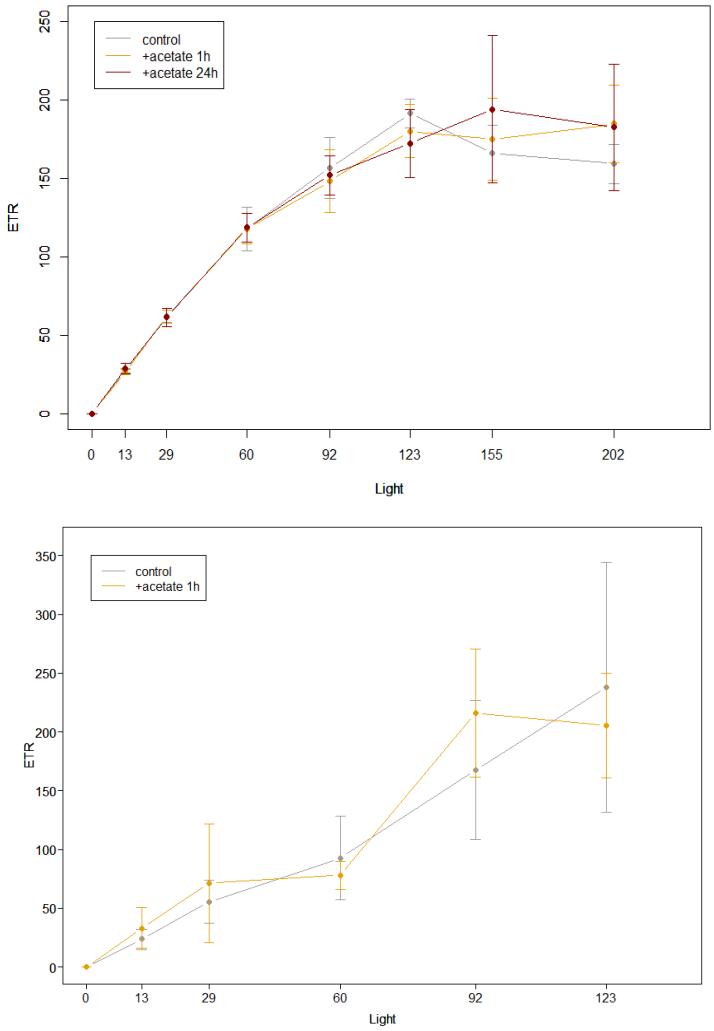

Figure 6.12. Photosynthetic activity measured by saturating light curve method of phototrophic isolates from Greenland Black and Bloom and Greenland margin cryoconite hole incubations. *Electron transport rate (ETR) was measured with and without acetate treatment.*

6.8. Discussion

Carboxylic acids were detected in the water from cryoconite holes and in the pore water from long-term incubations of cryoconite material. Two carboxylic acids were particularly elevated in some (propionate) or most of the samples (acetate).

6.8.1. Acetate in cryoconite holes samples

Acetate concentrations in cryoconite water in the holes on GrIS were very low and most samples had no detectable acetate. Yet, despite potentially unlimited mixing of water in the holes, significant differences were observed between cryoconite water collected from the bottom of the holes (between sediment aggregates) and the upper water column. The acetate (and other organic matter) pool in cryoconite hole therefore seems to be mostly sediment- bound, which agrees with previous research on organic carbon (Stibal et al., 2008; Musilova et al., 2016). The

sediment hosts a bigger, more active microbial community than overlying water column (Anesio et al., 2010). Total cell numbers in the sediments are 10^3-10^5 times higher than in water in Svalbard and Antarctic cryoconite holes (Anesio et al., 2010). Bacterial and primary production rates are 30 times and 1000 times respectively higher in Antarctic cryoconite hole sediments (Anesio et al., 2010; Samui, 2019). Organic carbon and nutrients are bound to the sediment granules (Stibal et al., 2009; Telling et al., 2012; Bagshaw et al., 2013) and likely need freezing and thawing to be released. Lysis of microbes and breaking of soil aggregates releases dissolved organic carbon, which often leads to a subsequent burst of respiration in the surviving microbial population (Larsen et al., 2002; Grogan et al., 2004; Schimel and Mikan, 2005). Such a pulse of nutrients and concomitant increase in microbial activity was observed in cryoconite holes in Antarctica following spring melt (Telling et al., 2014).

Upon thawing of the samples collected in the field and frozen for transport, acetate was released into the water. Most samples released even more acetate following a second freezing and thawing cycle. All the thawed samples from Greenland interior had acetate concentrations several orders of magnitude higher than those measured *in situ*. The limited data on acetate concentrations in the glacial environments are summarized in the Literature review (section 2.6.6), but there are no published data with such high concentrations. Antarctic samples had the lowest concentrations of acetate and Svalbard the highest, and also had the highest variability. Antarctic cryoconite holes have the lowest organic matter content (Edwards et al., 2011; Foreman et al., 2007; Zawierucha et al., in preparation), and so the least labile organic carbon which can be easily recycled and used for fermentation. The amount of acetate likely reflects the dominant type of anoxic metabolism in the cryoconite holes (i.e. fermentation vs sulphate reduction and methanogenesis). Potential mechanisms of acetate accumulation are summarized in Section 6.8.5, and the evidence presented here suggests that fermentation is the most important.

Carboxylic acids were the only organic compounds tested in this study, as limited by laboratory facilities. However, it is likely that acetate and other carboxylic acids constitute a large proportion of DOC in the cryoconite samples following the spring thaw as they are the products of fermentation and no other anoxic metabolism was detected in the samples. Microorganisms remain active in the films of water in the sub-zero temperatures in soils (Rivkina et al., 2000; Price and Sowers, 2004; Schimel and Mikan, 2005), sea ice (Boetius et al., 2015) and glacier ice crystals (Mader et al., 2006; Barletta et al., 2012). The oxygen status of these films is unknown but it is likely that oxygen is scavenged over the winter months and microscale low-oxygen or anoxic conditions develop, in which fermentation can occur. Small, dispersed cryoconite

sediment samples from shallow ice cores (see Methods 3.3.1) also had high acetate concentrations following defrosting, supporting the hypothesis of acetate accumulation during winter months and release upon thawing in the spring. Carboxylic acids released by freeze and thaw are likely important sources of labile C in the beginning of season (Fig. 6.13), to kick start the biogeochemical processes and support the awakening of the ecosystem following the polar night (Telling et al., 2014; Vick-Majors et al., 2014).

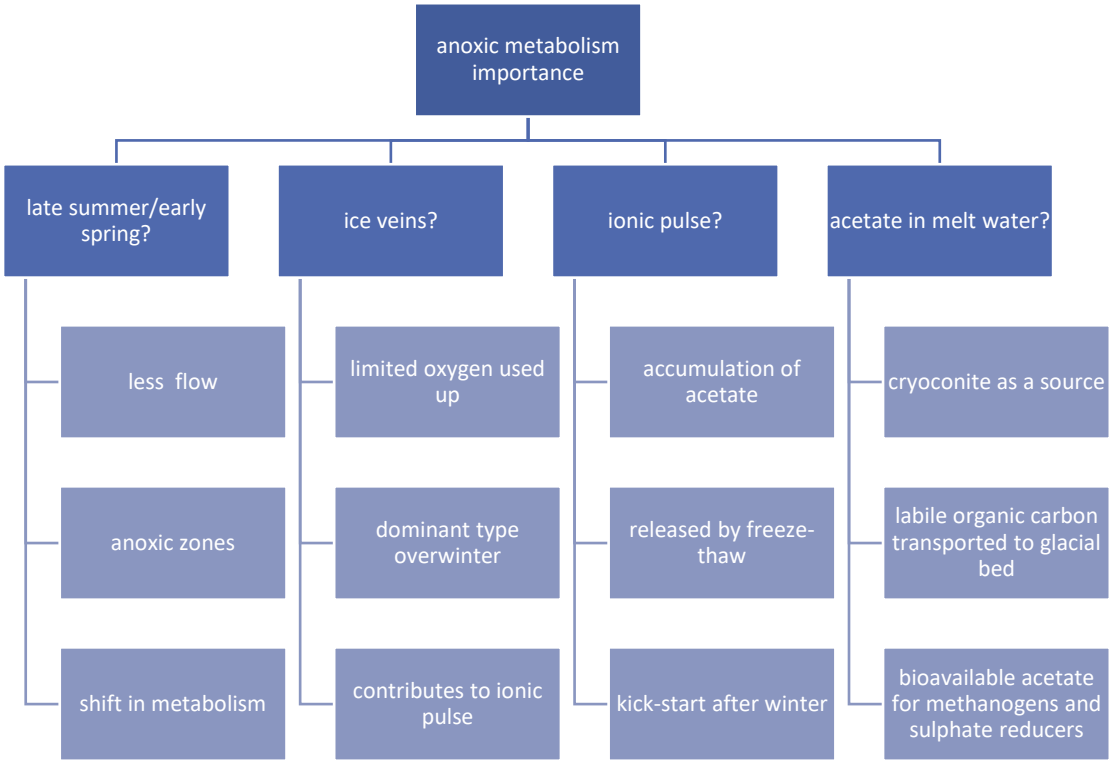

Figure 6.13. Potential mechanisms of importance of anaerobic metabolism on glaciers.

6.8.2. Acetate in cryoconite sediment incubations

In general, other carboxylic acids are less common than acetate in the anaerobic zones of sediments and can be metabolised to acetate (Gottschalk, 1979). Therefore, the prevalence of acetate in the incubations is not surprising. Lactate can be fermented or oxidised to acetate by some of the sulphate reducers (Gottschalk, 1979; Rosenberg et al., 2014b). Incubations with the highest concentrations of propionate (Svalbard, Black and Bloom) correlated with high concentrations of acetate, suggesting potential for product inhibition of acetate generation and accumulation of other, more complex fermentation intermediates, such as propionate (Gottschalk, 1979; Glombitza et al., 2015).

In Black and Bloom field incubations, samples were transferred directly from cryoconite holes to incubation vessels, then incubated at ambient temperatures and light conditions. Any acetate accumulation observed was likely to closely resemble *in situ* processes. The key difference, however, was in the sediment thickness: the incubations had a uniformly thick (1 cm) sediment layer, whereas the sediment in the 'natural' cryoconite holes is irregularly dispersed and frequently redistributed (Irvine-Fynn et al., 2011; Cook et al., 2015). This may explain the discrepancy between the acetate concentrations measured in the field incubation vs. the cryoconite hole waters. An alternative explanation is that the acetate is hydrologically redistributed soon after production in connected cryoconite holes, thus lower concentrations are measured. The results suggest that even short-term thick accumulations of cryoconite sediment may largely affect the composition and release of DOC *in situ*, therefore the sediment thickness is the critical factor. Acetate is not used up in the anoxic layer and not consumed rapidly in the oxic surface layer, causing release and accumulation in the water.

The accumulation of acetate was also observed in the laboratory incubations of cryoconite material from the Arctic (Greenland margin and interior, Svalbard) and Antarctica, but the dynamics of acetate accumulation differ depending on cryoconite source, suggesting a strong influence of local site-specific factors such as microbial community composition or mineral content. After around a month of incubations Greenland margin (Russell glacier) and interior (Black and Bloom) samples had similar concentrations of acetate. However, margin concentrations remained mostly unchanged (with exception of the surface), whereas interior ones were higher in longer incubations. Even finer scale heterogeneity was observed in Svalbard incubations with the highest observed variability of acetate concentrations, as well as in the defrosted cryoconite sediment samples. This suggests the development of different communities within samples with various proportions of groups oxidising acetate and the groups producing it. Local biogeochemical factors affect the structure of microbial community in cryoconite holes even from nearby locations (Weisleitner et al., 2020). The microbial community differed significantly between all sampled locations as discussed in details in Chapter 7. Additionally, cryoconite granules even sourced from one hole differ in the community structure (Uetake et al., 2016). Random distribution of granules in the incubations likely contributed to observed heterogeneity. Discrepancy of acetate accumulation trends in nearby locations such as Greenland margin and interior also demonstrates significance of local conditions and highlights the need for more research on heterogeneity of glacial microbial processes. Greenland margin cryoconite holes are likely influenced by the material from subglacial outflows which could

contain a well-balanced anoxic community (Wadham et al., 2008; Stibal et al., 2012), whereas subglacial material was unlikely to reach the Black and Bloom camp.

Antarctic samples had a steep increase in the acetate concentrations after one month. It seems that initially the production of acetate was slow and/or the consumption was high. Defrosted Antarctic samples had relatively high concentration of sulphate when compared to other locations, which could serve as an inorganic electron acceptor for acetate-consuming sulphate reducers (Gottschalk, 1979). Sulphate was depleted in incubations from all locations over time, suggesting the presence of sulphate reducers in all of the samples. However, not all of the sulphate reducers consume acetate and some produce acetate in the mineralisation of lactate (Gottschalk, 1979). Prolonged incubations also allowed the remobilisation of recalcitrant organic matter and ensured additional carbon source for fermentation (Sanyal et al., 2018). Accumulated cryoconite carbon pool in the Alaskan cryoconite holes was largely composed of the aged carbon, which was not readily bioavailable for microorganisms and mostly mineral-bound (McCrimmon et al., 2018). No significant differences between any of the light and dark incubations suggests that carboxylic acids production does not depend on the ongoing production of organic carbon by primary producers, but rather uses accumulated organic carbon. Stibal et al. (2008) showed that most of the organic matter in cryoconite holes in Svalbard was likely allochthonous and the high organic content exceeded the annual primary production, which makes it likely that some of the organic matter needs longer to be accessed. In Antarctic cryoconite holes from Queen Maude Land, the carbon was also relatively old suggesting that holes were isolated form atmosphere for a long time (even thousands of years) and microorganisms have been recycling the carbon within closed system (Lutz et al., 2019).

Part of differences in the concentrations of carboxylic acids could be induced by technical limitations. Pore water was sampled with a needle through the ports, which were spaced to avoid mixing while aspirating the water. However, a certain degree of mixing was likely to occur and limit the accuracy of the measured differences between depths. The detection limit was not relevant for this study, as most of the measurements were several orders of magnitude above the detection limit. The last source of error could be leaching from the 3D printed vessels or eppendorfs. To exclude such possibility, MilliQ water was incubated as a blank along the laboratory incubations and kept as a blank for the field sampling. The incubated MilliQ water had VFAs below detection limits, and the blank from eppendorfs was significantly lower than the average concentrations found in the field.

Acetate concentrations were comparable between Greenland margin samples incubated in a thin layer (4 mm) in a petri dish and those in a thicker layer (1.5 cm cm) in a vessel. Granules were present in the thin layer (Fig. 3.10 in Methods; Langford et al., 2010), that likely have anoxic centres (Segawa et al., 2014; Poniecka et al., 2018; chapter 4 of this thesis) that support an active anaerobic community capable of fermentation. A thin layer of fluffy aggregates (Fig. 3.10 in Methods) of cryoconite material from Greenland interior (Black and Bloom) produced concentrations of the same order of magnitude as a layer of margin granules, but lower than thick sediment incubations from Black and Bloom site. This suggests that fermentation is ongoing in the natural cryoconite holes over the season to some extent. Even soft aggregates likely promote enough anoxic microniches for the occurrence of fermentation. The potential switch of oxic to anoxic metabolism and consequent change in organic carbon compounds produced requires further investigation.

Finally, it is noteworthy, that although initially, acetate concentrations in the incubations remain within the range of defrosted samples in some of the Black and Bloom, Antarctic and Svalbard samples, all of them exceed the defrosted concentrations with time. Greenland margin samples were not compared to the defrosted samples and they remained stable with time. All the samples were initially supplied with MilliQ water in 2:1 ratio (sediment:water). It cannot be excluded that some of the acetate detected in the t1 were remnants of defrosted samples, but it is highly unlikely that all of it was from this source, particularly since longer incubations show accumulation of acetate.

6.8.3. Acetate in anoxic incubations of pure microbial cultures

Pure microbial cultures of the most abundant cultivable microorganisms were tested for fermentation by measuring the production of carboxylic acids following incubation. All of the isolates but one (Sv4O3: *Mrakia robertii* isolate) produced acetate, and most produced at least one accompanying carboxylic acid. Production of acetate and other carboxylic acids suggests a significant fermentative potential of cryoconite microorganisms and likely ensures fast recycling of labile organic matter, especially low molecular weight DOC, which is present on glaciers (Lawson et al., 2014) and in cryoconite holes (Musilova et al., 2017). Low molecular weight DOC such as glucose and other simple sugars, as well as fatty acids and amino acids, can be directly used in the fermentation process (Gottschalk, 1979; Maier and Pepper, 2015).

Despite differences in the concentrations of acetate in incubations of cryoconite material from different field sites described in the previous subsection, there was no such difference in the pure microbial cultures from all three sites tested. Propionate was the only carboxylic acid

showing location-specific differences. The two comparatively high concentrations were detected in two Antarctic isolates from anoxic incubations and belonged to genus *Tessaracoccus* and *Propionibacteriaceae* family. The members of *Propionibacteriaceae* are able to grow fermentatively and produce acetate and propionate from carbohydrates, such as lactate or glucose (Rosenberg et al., 2014a, 2014b). Consequently, no lactate was found in *Tessaracoccus* cultures and similarly in some other cultures. Any lactate present is therefore fermented, or oxidised by sulphate bacteria.

Anoxic isolates from all sites had better ability to produce acetate effectively than those isolated in oxic conditions and yeasts. Moreover, all of the anoxic isolates produced formate and most of them produced lactate, whereas those two carboxylic acids were only present in few of the oxic and yeast isolates. Perhaps bacteria isolated in the anoxic conditions are better adapted to anaerobic conditions through a larger number of active metabolic pathways for fermentation.

Finally, it is important to note that the microbial cultures do not reflect the environmental conditions nor the metabolic potential of complex community. The cultures were grown on a single, readily available carbon source – glucose, and they had no competing community and no micro-niches with variable conditions which are likely found *in situ*. Laboratory experiments on microbial isolates give us an indication of metabolic potential, such as ability for fermentation of all isolates, which may not be fully expressed *in situ*. Sediment incubations resemble the natural ones more closely and allow long-term experiments and replication, but are not perfect in mimicking glacial environment. Despite these limitations, the culture experiments together with laboratory incubations and limited field observations seem like a feasible and thorough approach to analyse glacial microbiological processes.

6.8.4. Acetate in phototrophic organism metabolism

Filamentous cyanobacteria are the dominating phototrophic microorganisms in cryoconite holes (Wharton et al., 1981; Mueller et al., 2001; Takeuchi et al., 2001; Edwards et al., 2011). Accordingly, Arctic phototrophic microorganisms isolated in this study all belonged to the genus Phormidesmis and created microbial mats made of filaments. Filamentous cyanobacteria in Svalbard samples were accompanied by algae, which could not be identified by DNA sequencing. Isolated Antarctic alga also could not be identified by this method. The algal DNA could not be amplified by PCR despite testing several methods of DNA extraction and several conditions of amplification. Further refining of the method was beyond the scope of this study.

Interestingly, DNA sequencing showed that in the Greenland interior (Black and Bloom) sample, Phormidesmis was accompanied by another phototrophic bacterium - *Rhodopseudomonas sp.* – a purple non-sulphur phototrophic organism belonging to Proteobacteria. Photosynthetic Proteobacteria (along with Stramenopiles and Actinobacteria) were identified before by functional gene analyses as potentially important within cryoconite holes worldwide (Cameron et al., 2012). Among them one of the amplified functional genes (nitrous oxide reductase gene) had an identity of 82-84% to *Rhodopseudomonas palustris,* potentially suggesting a presence of *Rhodopseudomonas* genus in the holes. Proteobacterial genera such as *Rhodobacter* and *Rhodopila* as well as members of Chloroflexi phylum were also present in the clone library of bacteria from Svalbard cryoconite holes (Edwards et al., 2011).

Stress conditions, especially high light with limited nutrients, can induce production of carbon-rich exudates to get rid of excess carbon assimilated by photosynthesis (Pannard et al., 2016). Acetate is one of such exudates (Abed, 2010; Pannard et al., 2016). However, high light with low nutrients (lower nitrogen and phosphorus than in the other treatments) did not promote production of carboxylic acids. Phototrophic growth was crudely assessed visually by observing visible green flakes and orange filaments. In the fermentation treatment, the vials were dominated with white pellets suggesting growth of associated heterotrophic microorganisms, rather than growth of phototrophs themselves, which could explain elevated carboxylic acids concentrations in this treatment.

Anaerobic conditions alone did not yield any detectable carboxylic acids, despite cyanobacteria generally being capable of fermentation (Stal and Moezelaar, 1997). Most fermentation pathways in cyanobacteria have acetate as end product (Stal and Moezelaar, 1997), however it is possible that isolates from cryoconite holes used another fermentation pathway, with glycolate as end product. This is mostly found in combination with formate and/or traces of oxalate (Heyer and Krumbein, 1991); neither glycolate not oxalate concentrations were checked in this study. However, glycolate degradation potential was tested in the chapter 5 and it was utilised as a single carbon source by some of the microbial isolates from cryoconite holes.

Acetate is not only a fermentation product, but it is also known to disrupt photosynthesis and other cellular processes resulting in growth inhibition (Russell and Diez-Gonzalez, 1998; Grime et al., 2008; Pinhal et al., 2019), but disruptive influence of acetate on phototrophic microorganisms was not detected. Overall, the phototroph-dominated community was not responsible for high concentrations of carboxylic acids in any treatments applied when compared to the sediment incubations or heterotrophic isolates' incubations. However, when the anaerobic heterotrophic growth was promoted by addition of glucose in anoxic conditions, all the samples produced

carboxylic acids in excess. An unlimited supply of carbon substrate, together with anaerobic conditions, effectively promoted heterotrophic growth and anaerobic fermentation. It cannot be excluded that phototrophic microorganisms, especially cyanobacteria, also produced carboxylic acids in such conditions as they are capable of fermentation (Stal and Moezelaar, 1997), however, it is unlikely given the lack of fermentation products in anoxic conditions mentioned earlier. Therefore heterotrophic bacteria seem to be responsible for the majority of carboxylic acids presence in cryoconite material.

6.8.5. Decoupling of fermentation and terminal oxidation

During mineralisation of organic matter, microorganisms preferentially use the more energy-yielding electron acceptors first, leading to depletion of oxygen, followed by nitrate, iron and manganese and then sulphate. This 'thermodynamic ladder' of electron acceptors yielding less and less energy creates zones in the sediments inhabited by aerobes, followed by nitrate and manganese reducers, iron reducers, sulphate reducers, and finally methanogens utilising the fermentation products to create methane. However, the zonation based on thermodynamics alone was recently challenged and iron reducers, sulphate reducers and methanogens often coexist in mutualistic relationship in subsurface instead of competing for energy. The distribution of microorganisms depends not only on available energy resources, but also on ecological and physiological factors which are yet to be determined. Microenvironments enable localised higher concentrations of compounds and altered pH when compared to the bulk of water body. An example of such environment could be a biofilm around the fermenting organic matter creating a microniche with acidic pH and increased acetate concentrations when compared to the bulk alkaline water of the aquifer (Bethke et al., 2011; Barletta et al., 2012). Evidence presented in Chapter 4 shows that microenvironments are prevalent in cryoconite holes.

Acetate and other carboxylic acids likely produced in such microniches and anoxic layers of cryoconite sediment seem to not be used up in cryoconite sediment. In the anaerobic mineralisation of organic matter, fermentation is an intermediate step in the oxidation of carbon (Glombitza et al., 2015). The fermentation products are then further oxidised by acetogens, sulphate reducers and methanogens. Methanogenesis is considered the dominant terminal metabolic pathway in freshwater environments (Weston and Joye, 2005), so in a well-balanced ecosystem the concentrations of intermediates such as carboxylic acids are usually low and coupled with terminal steps of organic matter degradation. Usually an accumulation of fermentation products only occurs when the conditions change and there is a transient

imbalance in the environment (Glombitza et al., 2015). The terminal steps of mineralisation in the cryoconite holes therefore seem to be non-efficient or non-existent.

In the glacial biome, methanogens and sulphate-reducers are mostly associated with anoxic subglacial sediments (Wadham et al., 2004, 2008; Anesio and Laybourn-Parry, 2012; Stibal et al., 2012b). However, anoxic niches are present (Chapter 4; Poniecka et al., 2018) and some strict anaerobes were cultured from cryoconite sediment (Zdanowski et al., 2017) or identified in molecular studies. Methanogenic Archaea were only reported in two molecular studies of cryoconite holes to date (Cameron et al., 2012). The majority of detected Archaea were identified as *Thaumarchaeota* and were likely involved in nitrogen cycling and the remaining sequences belonged to methanogenic classes: *Methanobacteriaceae* and *Methanomicrobia*. The same phyla were identified in the Arctic, in Svalbard cryoconite holes, with prevalence of *Thaumarchaeota* (Lutz et al., 2017). Contrary, another molecular study of Svalbard cryoconite holes failed to detect Archaea despite of the use of primers specific for methanogenic taxa (Edwards et al., 2011). The culture-dependent study also failed to isolate methanogens or identify them by molecular methods in Antarctic cryoconite holes and anaerobic enrichments (Zdanowski et al., 2017). This suggests, that although some methanogenic taxa can be found within cryoconite holes, perhaps they are limited and if the conditions are not right, their numbers fall below detection limits.

In Alpine cryoconite, archaeal sequences represented only a small minority of metagenome (0.1%) (Edwards et al., 2013), although the detection of sequences in molecular studies does not equal functionality. Rates of methane production decrease with decreasing temperatures and acetate accumulation was observed in the freshwater environments at low temperatures even where methanogens were present (Weston and Joye, 2005).

Sulphate reducers are usually the most important terminal oxidisers in marine sediments, with acetate being the most important substrate (Finke et al., 2007). They can be divided into two distinct functional groups (Gottschalk, 1979). The incomplete oxidizers utilise various organic acids and alcohols in production of acetate. *Desulfovibrio* genus and most *Desulfotomaculum* species are examples of genera for which acetate is the end product of their metabolism. The complete oxidisers are able to oxidize substrates, such as acetate, completely to carbon dioxide with sulphate as the electron acceptor. *Desulfotomaculum acetoxidans* was the first sulphate-reducing bacteria shown to grow on acetate as a sole energy and carbon source (Spring et al., 2009). Sulphate reducers were isolated from cryoconite holes in a culture-dependent experiment (Zdanowski et al., 2017). Among the most abundant anaerobic genera, only one sulphate-reducing genus was identified – *Desulfosporosinus*. Members of the genus grow in anaerobic

conditions on lactate as the electron donor and produce acetate (Ramamoorthy et al., 2006). They are not able to grow on acetate (or propionate or glucose). In the absence of sulphate, they are also able to grow as homoacetogens with H_2 and CO_2, and to grow fermentatively in the absence of sulphate, which would further contribute to the accumulation of acetate in cryoconite holes.

Functional genes likely responsible for sulphate reduction and denitrification were detected in cryoconite holes in Austrian Alps (Edwards et al., 2013). However, the functional genes presence does not necessarily imply activity, which depends on gene regulation (Cocolin and Rantsiou, 2014). It is more an indication of genetic potential, which might or might not be fully used. Franzetti et al. (2016) on the other hand found the abundance of marker genes for sulphate reduction to be negligible (dsrAB) in Italian Alps and Pakistan Himalayas. Metagenomic studies of Antarctic, Greenland or Svalbard cryoconite holes were not published to date, so additional molecular studies of the presence, abundance and activity of genes present in the cryoconite holes are needed to confirm the relative importance of fermentation compared to other anaerobic metabolism.

The data presented here show that fermentation and acetate accumulation are prevalent in cryoconite holes, but the relative this process for the glacial environment remains enigmatic. The potential importance of observed phenomena is summarised in figure 6.13.

6.8.6. Can we expect more accumulations in the future?

Although no modelling of future of the cryoconite holes has been done yet, it is easy to imagine that in the warmer scenario we will expect larger accumulation of cryoconite sediment. It can be visualised by a melt season progression on Greenland Ice Sheet. In the beginning of the season, the one might observe dispersed cryoconite material and relatively small cryoconite holes scattered in the landscape, with a uniform layer of tiny cryoconite accumulation in the shallow ice cores (Fig. 3.5 in Methods). As the season progresses, the holes often connect and create larger holes with thicker sediment (Irvine-Fynn et al., 2011). Cryoconite material also accumulates on the bottom of the streams and large accumulations develop after stream drainage (Fig. 3.5 in Methods). Alternatively, Antarctica can serve as an example of a cold climate scenario, with Svalbard and Greenland representing a warmer scenario. Antarctic melt season is shorter and the holes do not defrost fully, retaining a perennial ice lid and staying mostly in the same spots for years. Arctic cryoconite holes are more transient, with holes and accumulations appearing and disappearing over the period of days to weeks (Cook et al., 2016). Even in the Arctic, cryoconite holes acts as long term sites of sediment storage, despite all the mixing and

flushing by meltwater (Cook et al., 2016). This ensures enhanced microbial activity and a potential for microbial growth and organic matter build-up over the years, while allowing scope for disaggregation, redistribution, accumulation and sudden changes by dynamic hydrology (Hodson et al., 2007; Irvine-Fynn et al., 2011; Cook et al., 2016).

Cryoconite material generally has a long residence time in the supraglacial local ecosystem and its displacement happens on a very small spatial scale (Irvine-Fynn et al., 2011). Typically, material is redistributed to a single layer of granules maintaining the linear relationship between the mass of cryoconite sediment and area of the hole (Cook et al., 2010, 2015). Long residence time of granules, can result in the growth of bigger granules with heterotrophic inner layers (Cook et al., 2010) and eventually anoxic centres. However, there is no direct evidence so far that under future melt scenarios there will more accumulations of cryoconite material or bigger granules. Higher melt can also lead to accumulation of dispersed cryoconite in the streams where it creates thick deposits (Hodson et al., 2007), or to creation of deep, isolated holes with thicker sediment (Irvine-Fynn et al., 2011). Although far more surveys are required to resolve the dynamics of cryoconite debris on the glaciers, it seems that anoxic zones might play a significant role in the future melt scenarios.

6.9. Summary

Acetate is an important intermediate in the anaerobic decomposition of organic matter, which is usually consumed by methanogens, sulphate and iron reducers. If there is no balance in the ecosystem between fermenters and acetate consumers, it accumulates. Despite low concentrations of acetate detected in various glacial environments and in cryoconite water, large quantities of acetate can be released upon freezing and thawing of the cryoconite sediment, suggesting there is decoupling between fermenters and subsequent consumers. Moreover, when incubated, cryoconite sediment produces high concentrations acetate, sometimes accompanied by other carboxylic acids. Accumulated fermentation products are likely entrapped within the granules and cryoconite matrix and later released by freeze and thaw. Even Antarctic cryoconite material, with typically the lowest content of organic matter and shortest growth season when compared to the Arctic ones, produces large amounts of VFAs and releases some following freeze-thaw. Production of acetate is not limited to thick sediment accumulations, but rather depends on localised anoxic niches, even if they are very small. Acetate is present even in the very small samples of dispersed cryoconite from the ice cores and it accumulates in the water over time in the long-term incubations of a thin layer of granules. Microorganisms isolated from cryoconite sediment produce acetate under anaerobic conditions and the anoxic part of the

community seems better suited to fermentation. The abundance of methanogens and sulphate reducers will be determined in Chapter 7, but the evidence in this chapter shows that they are not efficient enough to process all the products. Anaerobic bacteria in cryoconite sediment seem likely to have an important role in creating a pool of labile organic carbon in the end of the season, when potential accumulations of cryoconite and smaller meltwater flow would promote development of anoxic niches and accumulation of fermentation products.

Chapter 7

Microbial community structure

7.1. Introduction

Microbial isolates from cryoconite holes were able to grow both in aerobic and anaerobic conditions (chapter 5). Products of anaerobic metabolisms were detected in the anoxic zones of cryoconite incubations (chapter 6). Significant fermentative potential of cryoconite microorganisms was shown (chapter 6). In this chapter, the changes of microbial community structure in the incubations will be explored following long-term anoxia (chapter 4). Particularly, the abundance of fermentative groups and terminal oxidisers such as methanogens and sulphate reducers will be determined.

7.2. Differences in community structure

At all time points in all incubations, Proteobacteria were the dominant phyla (36% in Antarctica, 27% in Greenland interior (Black and Bloom), 59% in Greenland margin, 38% in Svalbard), followed by Bacteroidetes (15%, 21%, 11%, 23% in Antarctic, Greenland interior, Greenland margin and Svalbard samples respectively; Fig.7.1). Other ubiquitous phyla in Antarctic samples were Cyanobacteria and Actinobacteria (18 and 17% respectively). In Greenland interior (Black and Bloom) samples, Cyanobacteria and Chloroflexi as well as Armatimonadetes were numerous (11%, 19% and 10% respectively), whereas Actinobacteria were present in low abundance (2%). In Greenland margin samples Caldiserica bacteria were plentiful (13%), whereas Cyanobacteria were present in very low abundance (1%). In Svalbard samples, Firmicutes, along with Cyanobacteria and Actinobacteria were numerous (11%, 11% and 11% respectively). Acidobacteria were present in low abundance in all sampled regions (4%, 2%, 1%, 2% in Antarctic, Greenland interior, Greenland margin and Svalbard samples, respectively).

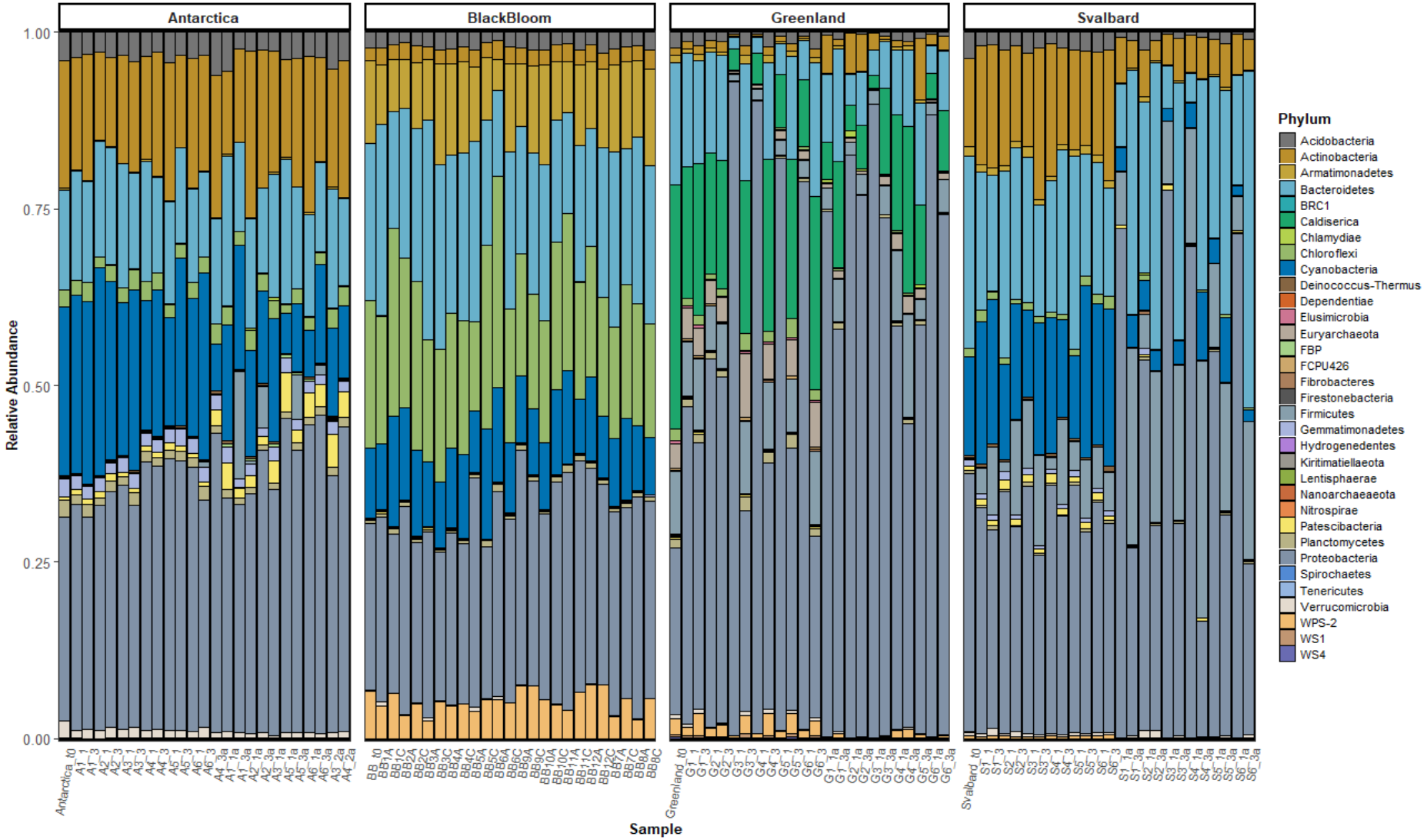

Figure 7.1. Relative abundance of microbial phyla in incubations of cryoconite material from Antarctica, Greenland interior, Greenland margin and Svalbard.

There was a strong spatial structuring in the incubations from the four different locations. NMDS analysis showed the primary clustering was by location (Fig. 7.2), regardless of incubation time or depth. PCoA analysis of Bray-Curtis distance showed the same trends (Appendix E). The differences in diversity between locations were confirmed by other diversity measures (Observed richness, Chao1, ACE, Shannon, Simpson, Inverse Simpson, Fisher; Appendix E). Greenland margin samples clustered closer to Svalbard than to Greenland interior samples (Black and Bloom) in NMDS and PCoA analysis, but not in the most of other diversity matrices (including richness Chao1, ACE, Shannon, Simpson, Inverse Simpson, Fisher; see Appendix E).

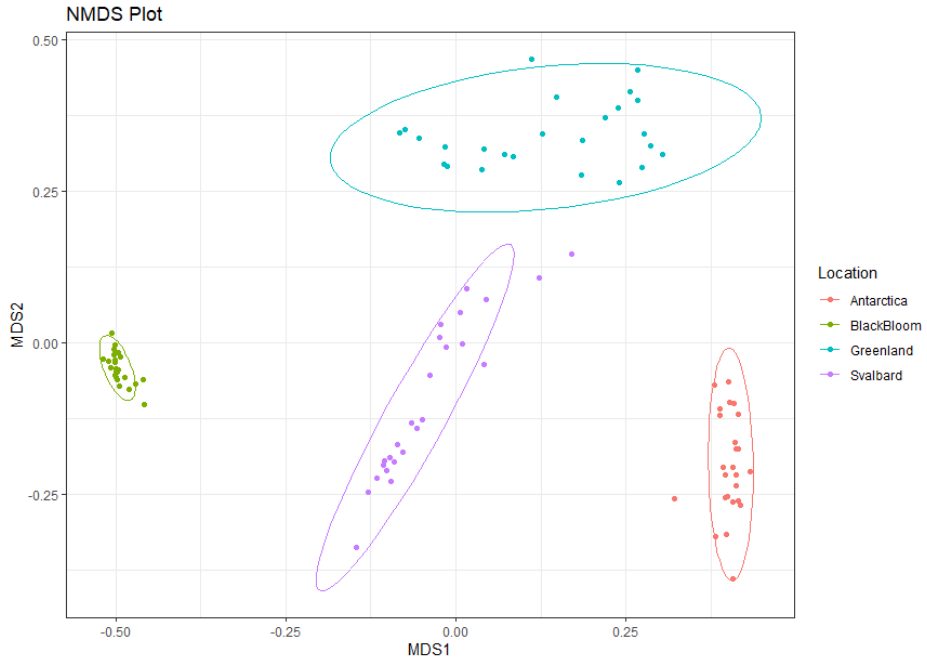

Figure 7.2. NMDS analysis of diversity of microbial community in cryoconite material incubations.

The differences between microbial communities in the incubations were compared by: polar region (Arctic vs Antarctic samples); location (Antarctica, Greenland margin, Greenland interior, Svalbard); time of incubations (0, 35, 175 days except of Greenland interior which was only incubated for 20 days); acetate concentrations (the concentrations of acetate in the sediment pore water sampled at the same depth and time); light levels (light vs dark incubations); and depth (samples taken at 0, 0.5 and 1cm). The major differences in bacterial community structure between locations were confirmed by ANOSIM analysis (Table 7.1). Significant differences were observed when the samples were divided by polar region, showing that the Antarctic community is distinct from Arctic communities, despite their diversity. The changes in community structure of incubations were also observed with time (0, 35 and 175 days), and there were small but

significant differences between samples related to acetate concentrations. Light treatment and depth did not significantly affect the community.

Table 7.1. ANOSIM analysis of differences of community structure depending on selected variables. *Significance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Variable	ANOSIM statistics R	Significance	
polar region	0.46	1×10^{-4}	***
location	0.85	1×10^{-4}	***
time	0.21	1×10^{-4}	***
acetate	0.07	0.02	*
light	-0.005	0.56	
depth	-0.008	0.62	

SIMPER analysis revealed the phyla responsible for the observed differences between locations (Table 7.2). The majority of these differences were accounted to Proteobacteria phylum. Chloroflexi contributed the most to the discrepancies between Greenland interior and other samples. Caldiserica were important in Greenland margin samples, as also seen in Fig 7.3. Firmicutes contributed to difference between Svalbard and Antarctica or Greenland interior, but not between Svalbard and Greenland margin. These differences are visually discernible in the heatmap of phyla (Figure 7.3).

Table 7.2. SIMPER analysis of phyla contributing to observed differences between locations. *Compared groups – pairs of samples sets from different locations being compared; Average dissimilarity – SIMPER measure of dissimilarity based on Bray-Curtis distances; St. dev. – Standard deviation of average dissimilarity measure; Cumulative contribution – Sum of contribution of each phyla to dissimilarity between sample sets; Significance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Compared groups	Average dissimilarity	St. dev.	Cumulative contribution	Significance	
Greenland_Antarctica					
Proteobacteria	1.27×10^{-1}	9.25×10^{-2}	0.27	0.01	**
Cyanobacteria	8.78×10^{-2}	3.59×10^{-2}	0.46	0.01	**
Actinobacteria	6.68×10^{-2}	2.51×10^{-2}	0.60	0.01	**
Caldiserica	6.29×10^{-2}	4.52×10^{-2}	0.74	0.01	**
Bacteroidetes	2.80×10^{-2}	2.19×10^{-2}	0.79	1.00	
Greenland_BlackBloom					
Proteobacteria	1.59×10^{-1}	1.05×10^{-1}	0.28	0.01	**
Chloroflexi	9.28×10^{-2}	1.92×10^{-2}	0.45	0.01	**
Caldiserica	6.29×10^{-2}	4.52×10^{-2}	0.56	0.01	**
Bacteroidetes	5.33×10^{-2}	3.09×10^{-2}	0.66	0.02	*
Cyanobacteria	5.04×10^{-2}	1.54×10^{-2}	0.75	0.08	.

Greenland_Svalbard					
Proteobacteria	1.38×10^{-1}	9.70×10^{-2}	0.31	0.01	**
Bacteroidetes	6.66×10^{-2}	4.87×10^{-2}	0.45	0.01	**
Caldiserica	6.29×10^{-2}	4.52×10^{-2}	0.59	0.01	**
Cyanobacteria	5.27×10^{-2}	3.60×10^{-2}	0.71	0.01	**
Actinobacteria	4.30×10^{-2}	2.76×10^{-2}	0.80	0.02	*
Antarctica_BlackBloom					
Chloroflexi	8.57×10^{-2}	1.88×10^{-2}	0.22	0.01	**
Actinobacteria	7.13×10^{-2}	1.54×10^{-2}	0.39	0.01	**
Armatimonadetes	5.13×10^{-2}	1.25×10^{-2}	0.52	0.01	**
Cyanobacteria	4.47×10^{-2}	2.67×10^{-2}	0.64	0.79	
Proteobacteria	4.40×10^{-2}	2.76×10^{-2}	0.75	1.00	
Antarctica_Svalbard					
Proteobacteria	6.16×10^{-2}	5.75×10^{-2}	0.20	1.00	
Firmicutes	5.37×10^{-2}	4.25×10^{-2}	0.38	0.01	**
Cyanobacteria	5.16×10^{-2}	3.49×10^{-2}	0.55	0.04	*
Bacteroidetes	5.00×10^{-2}	4.05×10^{-2}	0.72	0.03	*
Actinobacteria	3.59×10^{-2}	2.47×10^{-2}	0.83	0.86	
BlackBloom_Svalbard					
Chloroflexi	9.34×10^{-2}	1.89×10^{-2}	0.22	0.01	**
Proteobacteria	6.41×10^{-2}	7.47×10^{-2}	0.37	1.00	
Firmicutes	5.68×10^{-2}	4.33×10^{-2}	0.50	0.01	**
Armatimonadetes	4.88×10^{-2}	1.27×10^{-2}	0.62	0.01	**
Bacteroidetes	4.27×10^{-2}	3.49×10^{-2}	0.72	0.57	

7.3. Location-specific trends

All the incubations took place in the same incubation vessels and developed anoxia over time (Chapter 4). They were kept in the same conditions, except for the Greenland interior samples that were incubated under *in situ* light and temperature on the ice. Despite the same incubation conditions and prolonged anoxia observed in the incubation vessels, community structure did not converge over time, but rather the location-specific differences were preserved. Because of this strong spatial patterns, further analyses were undertaken on individual subsets based on location. The heatmap of dominant phyla in all the treatments grouped by location and time demonstrated clear differences as incubation time increased (Fig. 7.3). Enrichments of various phyla over time were location-specific, but there were some universal trends: Proteobacteria were enriched in all incubations and Cyanobacterial abundance generally decreased with time; they were almost undetectable in Greenland margin samples (Fig 7.3). Svalbard and Antarctic incubations were enriched in Firmicutes. Relative abundance of Caldiserica and Chloroflexi, as well as Euryarcheota, decreased gradually in Greenland margin samples. Actinobacteria, by

contrast, increased in Greenland margin samples, decreased in Svalbard and remained mostly unchanged in Antarctica and Greenland interior over time. Patescibacteria increased notably in Antarctic samples. An exception to these trends was the Greenland interior (Black and Bloom) samples, which remained mostly unchanged with time.

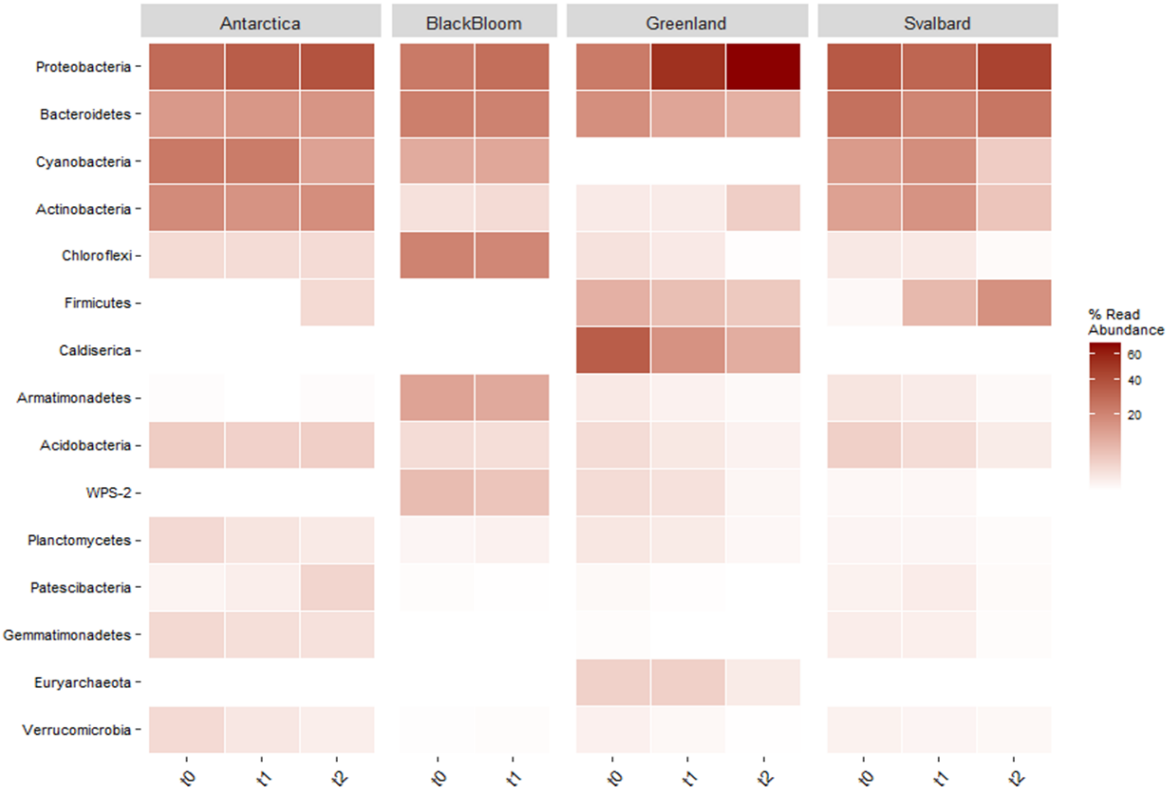

Figure 7.3. Heatmap of most abundant phyla in the cryoconite material incubations, divided by location and by time*.t1 = 35 days (excepting Black and Bloom where t1 = 20 days), t2 = 175 days.*

The genera which increased the most in relative abundance over time among all samples except Greenland interior (Black and Bloom) were assigned to the Proteobacteria phylum (Fig. 7.4) with *Rhodoferax* (Comamonadaceae) being followed by *Pseudomonas* (Pseudomonadaceae). *Clostridium* genus also visibly increased. The trends of other abundant genera were location-specific. For example, *Caldiserisum*, belonging to Caldiserica phylum, diminished remarkably with time in Greenland margin samples (from 34.3% to 9.4%). *Paludibacter* (Bacteroidetes phylum) increased notably in Greenland margin and Svalbard samples with time. There was also a considerable increase in *Cairneyella* (Proteobacteria) in Svalbard samples at the final time point of incubation. Most abundant genera in Greenland interior (Black and Bloom) samples, similarly to phyla, remained mostly unchanged.

Figure 7.4. Heatmap of most abundant genera in the cryoconite material incubations, divided by location and by time. *t1 = 35 days (excepting Black and Bloom where t1 = 20 days), t2 = 175 days.*

7.3.1. Community structure in Antarctic incubations

In Antarctic samples, significant differences in community structure were observed over time (Table 7.3). Differences depending on depth were significant, although small (Table 7.3) and microbial communities of different depths did not overlap in PCoA analysis (Fig. 7.5). Community structure also varied with acetate concentration detected in the pore water. Less notable differences in ANOSIM analysis were detected between light regimes, and the microbial communities from different light regimes largely overlapped in PCoA analysis (Fig. 7.5).

Table 7.3. ANOSIM analysis of differences of community structure depending on environmental variables in Antarctic samples. *Acetate – acetate concentrations in the pore water; significance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Location	***Variable***	***ANOSIM statistics R***	***Significance***	
Antarctica	time	0.66	1×10^{-4}	***
	acetate	0.52	1×10^{-4}	***
	light	0.19	0.01	*
	depth	0.124	0.04	*

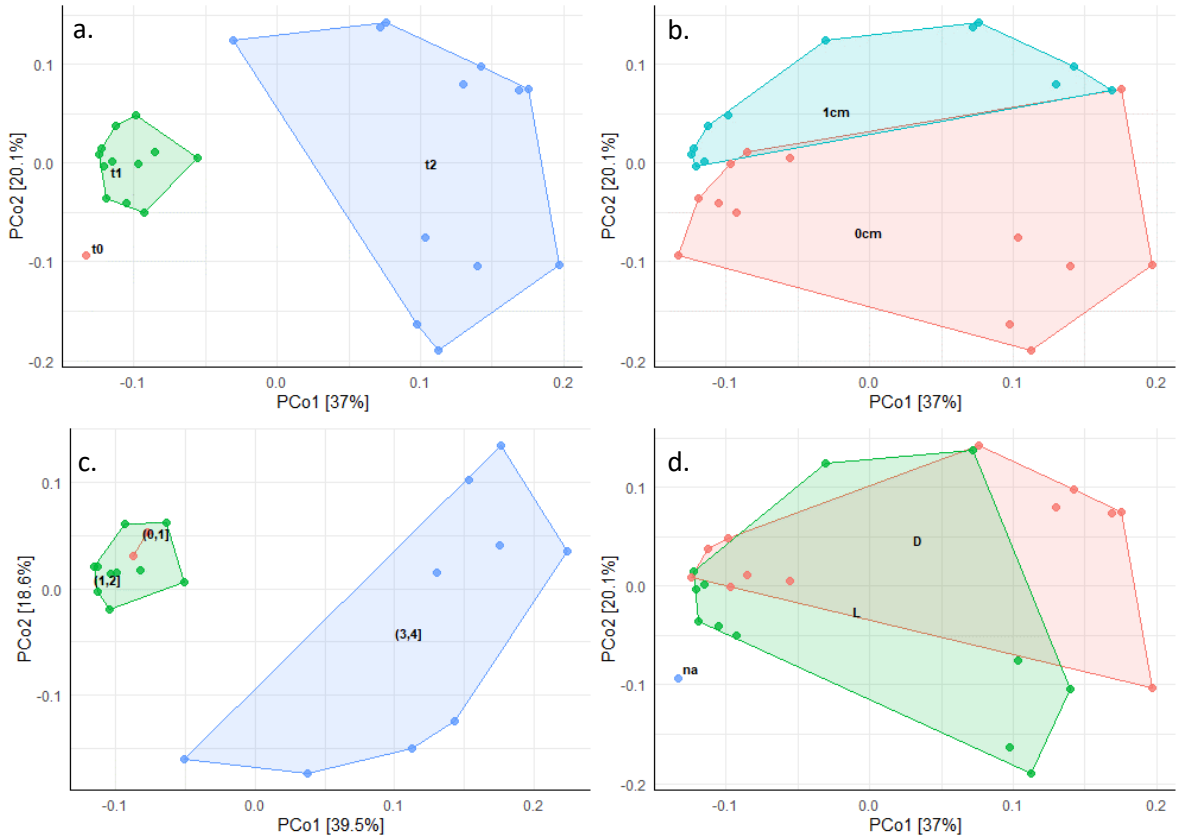

Figure 7.5. PCoA analysis of diversity of Antarctic communities *depending on: a. Time (t0, t1=35 days, t2=175 days); b. Depth (0cm, 1 cm); c. Acetate ((0,1] = acetate concentration 1-10 µM, (1,2] = 10-100 µM, (3,4] = 1000-10000 µM); d. Light (na = t0, D = dark, L = light).*

7.3.2. Community structure in Svalbard incubations

In Svalbard samples, similarly to the Antarctic samples, significant differences in community structure were observed over time (Table 7.4). Differences depending on depth were significant, although small (Table 7.4) and microbial communities of different depths did not overlap in PCoA analysis (Fig. 7.6). The community structure did not differ depending on acetate concentration detected in the pore water, nor between the light regimes, in contrast with the Antarctic samples.

Table 7.4. ANOSIM analysis of differences of community structure depending on environmental variables in Svalbard samples. *acetate – acetate concentrations in the pore water;* ***s****ignificance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Location	*Variable*	*ANOSIM statistics R*	*Significance*	
Svalbard	time	0.68	1×10^{-4}	***
	acetate	0.03	0.30	
	light	-0.04	0.72	
	depth	0.11	0.04	*

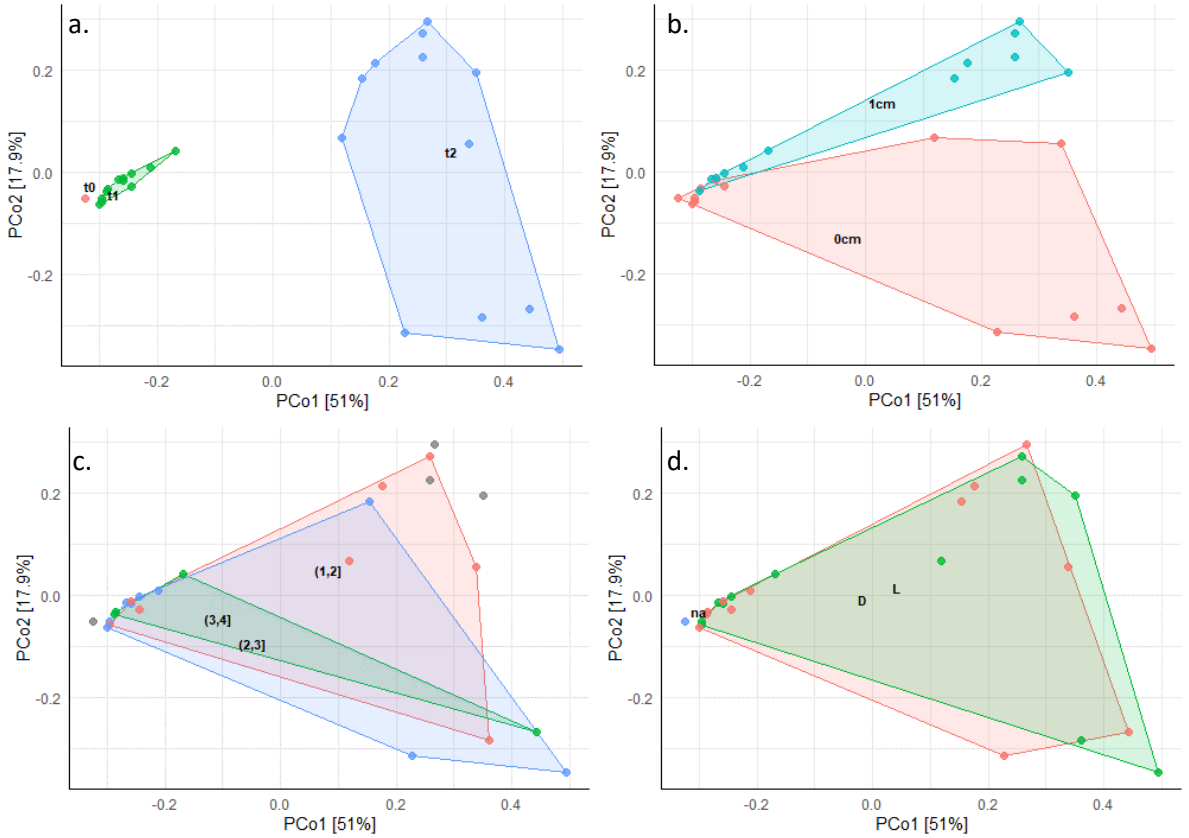

Figure 7.6. PCoA analysis of diversity of Svalbard communities *depending on: a. Time (t0, t1=35 days, t2=175 days); b. Depth (0cm, 1 cm); c. Acetate ((1,2] = acetate concentration 10-100 µM, (2,3] = 100-1000 µM), (3,4] = 1000-10000 µM); d. Light (na = t0, D = dark, L = light).*

7.3.3. Community structure in Greenland margin incubations

In Greenland margin samples, similarly to Antarctic and Svalbard samples, significant differences in community structure were observed over time (Table 7.5). Differences depending on depth were significant, although small (Table 7.5), but microbial communities of different depths did overlap in PCoA analysis (Fig. 7.7). Unlike Antarctic samples, community structure did not differ depending on acetate concentration detected in the pore water, nor in the different light treatments, which was similar to Svalbard samples.

Table 7.5. ANOSIM analysis of differences of community structure depending on environmental variables in Greenland margin samples. *acetate – acetate concentrations in the pore water; significance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Location	*Variable*	*ANOSIM statistics R*	*Significance*	
Greenland	time	0.38	9×10^{-4}	***
margin	acetate	0.12	0.14	
	light	0.04	0.21	
	depth	0.16	0.03	*

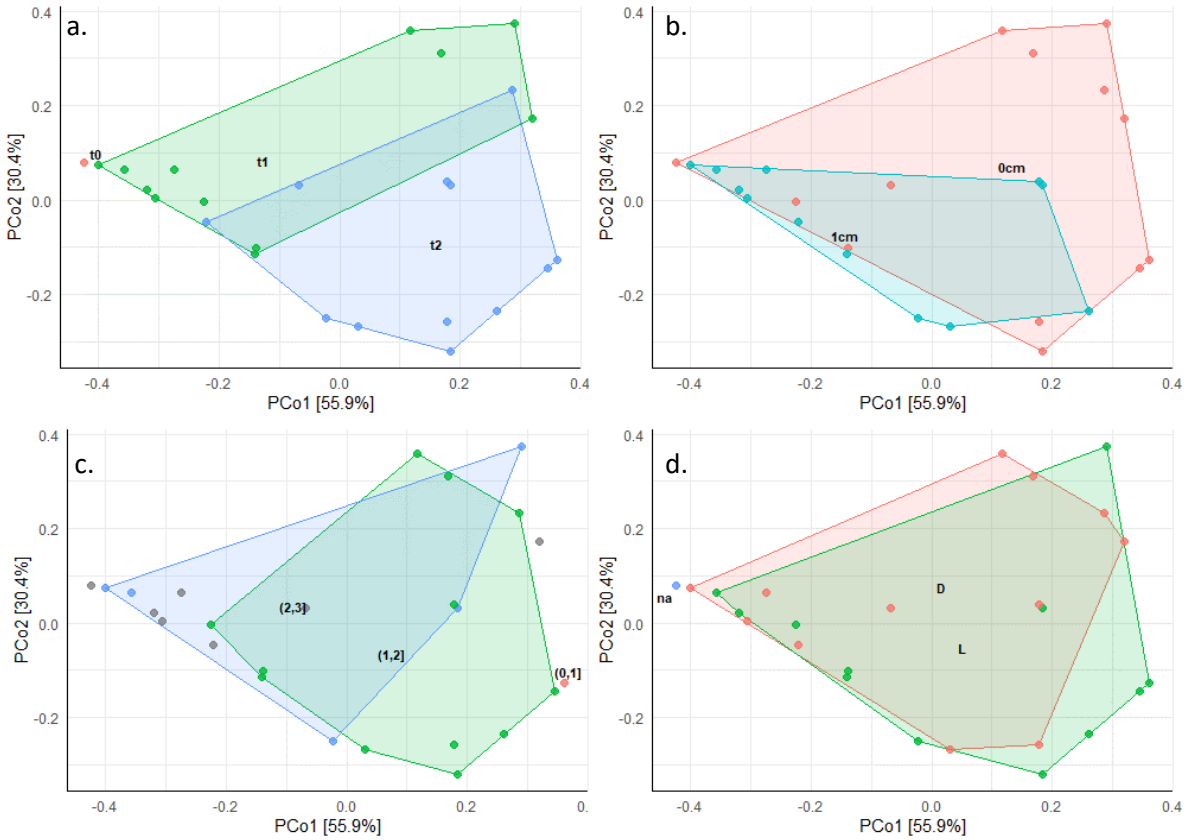

Figure 7.7. PCoA analysis of diversity of Greenland margin communities *depending on: a. Time (t0, t1=35 days, t2=175 days); b. Depth (0cm, 1 cm); Acetate ((0,1] = acetate concentration 1-10 µM, (1,2] = 10-100 µM, (2,3] = 100-1000 µM); d. Light (na = t0, D = dark, L = light).*

7.3.4. Community structure in Greenland interior incubations

Black and Bloom incubations had shorter incubation times (t1=20) than other incubations (t1=35) and only two time points were sampled. Moreover, samples were incubated under in situ conditions, with consequently different temperature, light and nutrients. Contrary to the lab incubations, no differences in microbial community structure were detected over time (Table 7.6). The only significant difference was between light regimes, (ANOSIM statistics=0.27, p=0.00). Notably, there were no differences between lidded and unlidded samples (Fig. 7.8).

Table 7.6. ANOSIM analysis of differences of community structure depending on environmental variables in Greenland interior (Black and Bloom) samples. *acetate – acetate concentrations in the pore water; significance – *** = p<0.001, ** = p<0.01, *= p<0.05, . = p<0.1*

Location	*Variable*	*ANOSIM statistics R*	*Significance*	
Greenland	time	-0.06	0.51	
Black and Bloom	acetate	0.13	0.08	.
	light	0.27	0.001	***
	depth	0.032	0.26	
	lid	0.006	0.37	

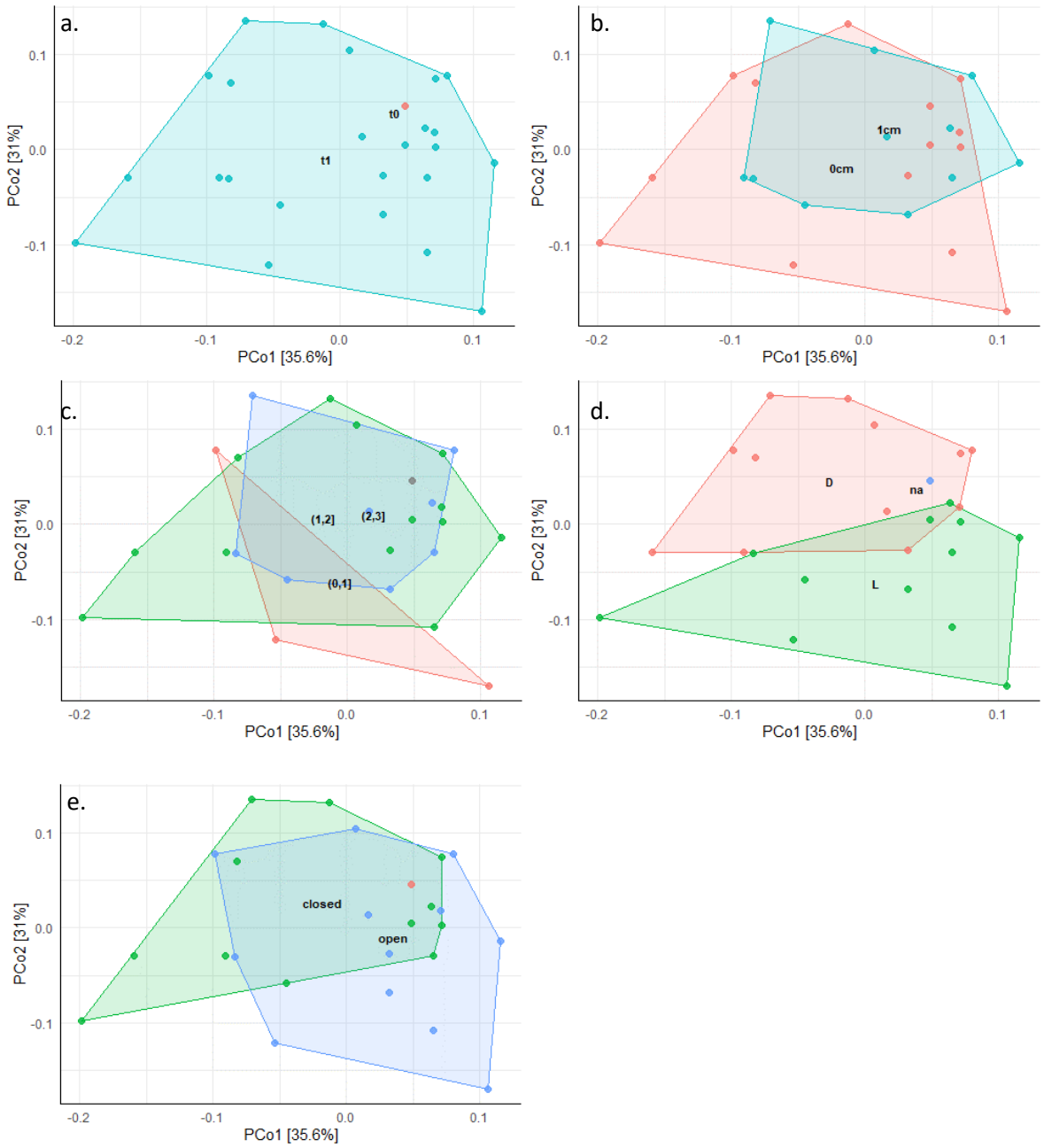

Figure 7.8. PCoA analysis of diversity of Greenland interior communities depending *on: a. Time (t0, t1=20 days, t2=175 days); b. Depth (0cm, 1 cm); Acetate ((0,1] = acetate concentration 1-10 µM, (1,2] = 10-100 µM, (2,3] = 100-1000 µM); d. Light (na = t0, D = dark, L = light); e. Lid (closed = lid present, open = lid absent)*

7.3.5. Acetate consumers

The microbial communities from cryoconite incubations were screened for potential acetate consumers, namely methanogens and sulphate reducers. Methanogens were identified to family level, whereas sulphate reducers could be identified down to genus level. Methanogens, dominated by *Methanoregulaceae* family, were relatively abundant in the Greenland margin samples and virtually not detected in other samples (Fig. 7.9). Sulphate reducers, mostly belonging to *Geobacter* and *Desulfosporosinus* genera, were present in most of the Greenland margin and Svalbard samples as well as in a few Antarctic samples (Fig. 7.10).

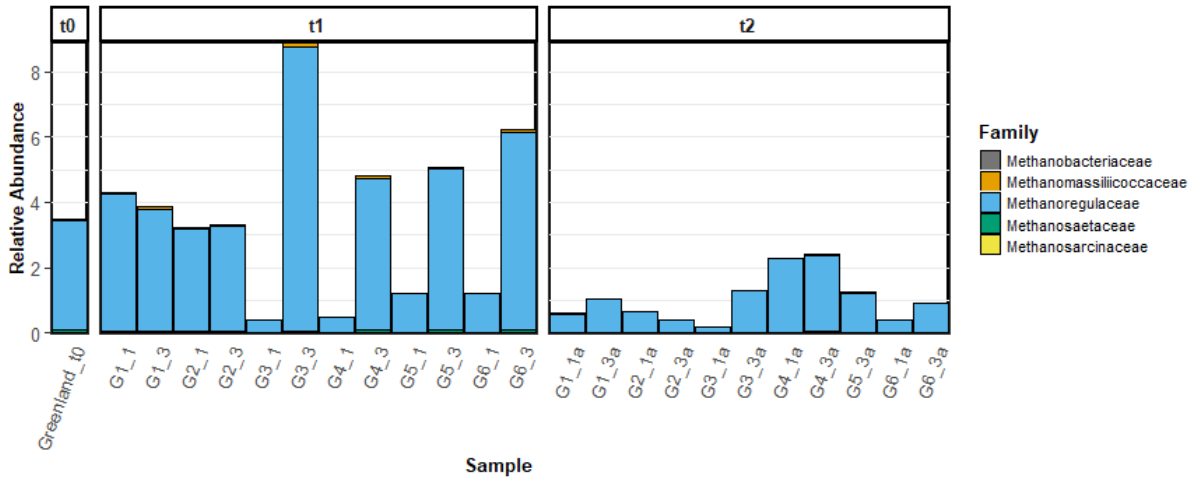

Figure 7.9. Abundance of methanogens in Greenland margin cryoconite incubations over time (t0, t1, t2). *t1 = 35 days, t2 = 175 days. Greenland_t0….G6_3a indicate individual samples at three different time points.*

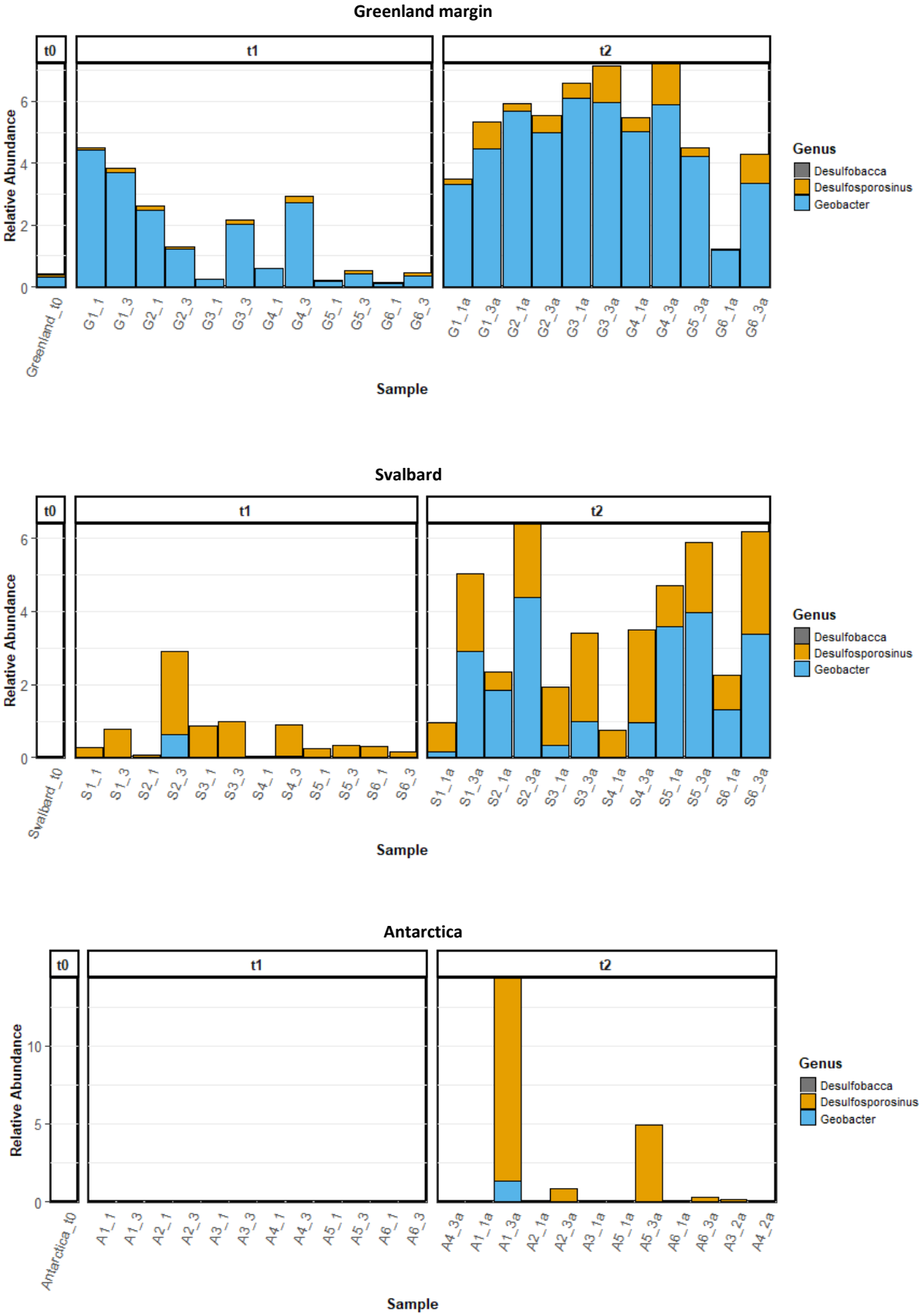

Figure 7.10. Abundance of methanogens in Greenland margin, Svalbard and Antarctic incubations over time (t0, t1, t2). *t1 = 35 days, t2 = 175 days. Greenland_t0…A4_2a indicate individual samples at three different time points.*

7.4. Discussion

7.4.1. Microbial community structure

Sequencing of rRNA gene amplicons is a wide-spread technique allowing to survey the composition of bacterial communities (Nemergut et al., 2011; Hu et al., 2016; Malard et al., 2019). Identifying taxa via 16S rRNA gene amplification has some inherent technical limitations. For example, in the analysis of freshwater microbial community, several errors were generated during the preparation steps and some differences were observed between replicates (Poretsky et al., 2014). The 16S rDNA sequencing provided lower sensitivity and resolution when compared to the metagenomic analysis of the same data. However, 16S rDNA is much more affordable when compared to metagenomic sequencing (Earl et al., 2018). Lower sensitivity and resolution is partially due to sequencing of only a relatively short part of 16S rRNA gene region, which in turn provides the reliable classification at the family or genus level, rather than species level (Earl et al., 2018). The heterogeneity between copies of the 16S rDNA in many species further adds to difficulty of reliably identifying microorganisms at the species level (Dahllöf et al., 2000). Relatively low resolution means that some rare taxa may be omitted in the analysis. However, this study targeted groups which were abundant enough to influence the detectable concentrations of carboxylic acids. Rare taxa at very low abundance would be unlikely to have any effect and the importance of capturing information regarding such taxa was not a goal of the study.

Nevertheless, 16S rDNA analysis allows to detect broad shifts in the community over time. It is commonly used for assessing community diversity and dynamics also in glacial settings (Lutz et al., 2015; Anesio et al., 2017). It was used to successfully detect shifts in community in cryoconite material on Greenland Ice Sheet (Musilova et al., 2015) and to describe the biogeography of cryoconite microorganisms across the globe (Darcy et al., 2018). Having in mind the mentioned limitations, 16S rDNA seems to offer a good compromise between accuracy and reliability of the data, costs and data analysis difficulty.

The microbial communities from all locations were dominated by Proteobacteria and Bacteroidetes, with varying proportion of Cyanobacteria and Actinobacteria, which is consistent with other studies (Edwards et al., 2011, 2014; Cameron et al., 2012; Musilova et al., 2015; Stibal et al., 2015; Lutz et al., 2017; Weisleitner et al., 2020). The greatest differences between locations are associated with Proteobacteria phylum. Proteobacteria are the most abundant in all the locations and comprise a diverse group of bacteria, therefore it is not surprising that their contribution to the differences has the greatest impact. The results point to the strong

dependence of community development on the source of cryoconite material (Musilova et al., 2015; Darcy et al., 2018; Weisleitner et al., 2020). Cryoconite microbial communities in Antarctica show significant spatial structuring and worldwide demonstrate decreasing similarity to each other with increasing distance (Darcy et al., 2018). The total phylogenetic and functional potential of the community is a result of the selection by the environmental conditions and dispersal, as well as stochastic factors (Schmidt et al., 2012; Darcy et al., 2018; Zawierucha et al., 2019). An example is the correlation of abundance of cyanobacteria in cryoconite holes with inorganic nitrogen (Stibal et al., 2006). The *Polaromonas* genus is globally distributed, despite differences in environmental conditions, thanks to dormancy genes facilitating survival of long-distance transport (Darcy et al., 2011).

Manipulative microcosm experiments were used to investigate the effect of environmental conditions on the microbial community structure from cryospheric habitats. These experiments showed that the organisms that were better adapted were enriched in response to artificial environmental pressure (Schmidt et al., 2012; Darcy and Schmidt, 2016). In this study, despite the same incubation conditions, the community structure did not converge towards the same pattern, but rather remained different. Most of the differences could be explained by cryoconite origin, as confirmed by PCoA, NMDS and ANOSIM analyses. The community structure of cryoconite holes is therefore strongly related to abiotic factors including depth and diameter of the holes, organic matter content or mineral diversity (Weisleitner et al., 2020). The results presented in this thesis did not show clustering of Greenland samples when compared to more distant samples from Svalbard and Antarctica, contrary to the findings of Darcy et al. (2018). This suggests not only significant diversity of cryoconite holes on a global scale, but also between relatively proximal Arctic samples. This strengthens the rationale for analysing each location separately.

The differences between Greenland margin and interior samples are likely an interplay of location determining the dust and sediment input, as well as melt water flow (Hodson et al., 2010; Telling et al., 2012; Stibal et al., 2015). Moreover, Greenland margin samples were specifically sampled for thicker sediment layers and big drained holes sometimes referred to as a ‘cryoconite mantle’ (Hodson et al., 2008). Such thick accumulations on the margin of the glacier are hypothesised to have incorporated subglacial debris from nearby outflows and dynamic geomorphological processes on the margin (Knight et al., 2002); and they are often anoxic (chapter 4). Subglacial samples were collected before on the Russell and Leverett glaciers on the margin of GrIS from pressure ridges and sediment bands thrust to the glacier surface (Stibal et al., 2012; Nixon et al., 2017). The debris from melting old ice together with wind-blown particles

in the ablation zone was shown to aggregate and melt into cryoconite hole (Bøggild et al., 2010). The potential inclusion of a distinctive subglacial microbial community in these samples, as well as prolonged anaerobic environment, is likely a contributing factor to observed differences between the Greenland margin and interior samples in this study. Seeding from subglacial sediment admixing is also a likely reason for observed higher abundance of methanogenic and sulphate reducing groups discussed below in subsection 7.4.2.

In all three long-term incubations (excepting the shorter Black and Bloom incubation), the community changed significantly over time. Temporal changes of cryoconite holes community on a short time-scale (one summer) were previously observed on an Alpine glacier, although this is traditionally regarded as a more transient environment than polar glaciers (Pittino et al., 2018). By contrast, the Greenland margin community composition remained stable over the ablation season (Musilova et al., 2015) after an initial rapid community change following snow melt. This suggests that bacterial community of cryoconite holes is prone to shift following fluctuations in abiotic conditions. Seasonal variations of nutrients, freeze-thaw events and hydrological connections influence the otherwise stable cryoconite hole environment (Fountain et al., 2008; Telling et al., 2014). Periods of unstable conditions, such as initial snow melt or the thaw associated with the beginning of the season (Telling et al., 2014; Musilova et al., 2015), likely lead to a shift in a community structure. Musilova et al. (2015) found a rapid increase in Bacteroidetes and a decrease in Firmicutes and Acidobacteria in the first weeks of ablation season. Contrarily in this study, Firmicutes were one of the main groups enriched over time in the incubations.

7.4.2. Changes within community with prolonged anoxia and characteristic taxa

Chapter 4 explored the development of anoxic conditions over time; this also is likely to affect the community composition with time. Chloroflexi bacteria were particularly abundant in Greenland interior samples when compared to the other samples. Chloroflexi comprise a physiologically diverse group which occurs in various anaerobic environments, ranging from hot springs to sludge communities (Speirs et al., 2019). They are known for fermentation and granulation capabilities. However, they are not limited to anaerobic conditions, and were found in aerobic conditions as well, both in mesophilic and thermophilic conditions and they accommodate heterotrophic and phototrophic metabolisms (Speirs et al., 2019). Together with Cyanobacteria, they probably contribute to production of organic carbon in cryoconite holes (Cameron et al., 2012). Low abundances of Chloroflexi were found in alpine glacier cryoconite

holes (Weiland-Bräuer et al., 2017) and at relatively high abundance on Tibetan glaciers (Liu et al., 2017) and a Svalbard glacier (Gokul et al., 2016). Sequences related to Chloroflexi were also identified within Antarctic, Svalbard and Greenland margin cryoconite holes (Cameron et al., 2012). In this study, they are particularly abundant in Black and Bloom samples, potentially being one of the key players in formation of granules and the fermentation and accumulation of acetate.

Svalbard and Antarctic samples were enriched in Firmicutes over time. This phylum contains many anaerobic microorganisms such as lactic acid bacteria or sulphate-reducers (e.g. *Desulfotomaculum* spp.). A number of *Clostridium* species are fermenters and produce acetate among many other fermentation products (such as butyrate, CO_2, and H_2). In the soil, many Firmicutes bacteria (especially clostridia) inhabit anoxic micro-niches devoid of oxygen by intensely-respirating aerobes (Madigan et al., 2012). The development of micro-niches was speculated in cryoconite holes (Zdanowski et al., 2017) and would explain enrichment in Firmicutes in prolonged anoxic conditions. Moreover, members of Firmicutes are enveloped in a rigid cell wall (De Vos et al., 2009) and create spores which are highly resistant to environmental stresses (Parkes and Sass, 2009), making them suitable inhabitants of demanding icy environment.

Caldiserica were abundant in Greenland margin. Caldiserica is still an enigmatic phylum with only one cultured representative so far, isolated from the hot springs (Mori et al., 2009). However, genomic studies revealed that this phylum can be found in a wide range of environment including sediments, permafrost and ice. Its wide metabolic potential was revealed by metagenomic studies, including sulphate reduction, amino acid degradation and fermentation with formate and acetate as the end products (Martinez et al., 2019). Despite their seemingly good potential for survival in extreme anaerobic conditions, their abundance decreased with time in Greenland margin samples with the concurrent increase in Actinobacteria. The reasons for this are unknown, but could be because of decreasing sulphate concentrations (chapter 6) or lack of other specific terminal acceptors.

Actinobacteria are ubiquitous in a wide diversity of niches, including soil, marine and freshwater sediments (Ul-Hassan and Wellington, 2009). The majority of Actinobacteria are capable of decomposing complex organic matter, including recalcitrant substrates, by producing a variety of extracellular hydrolytic enzymes. They are often regarded as key organisms in carbon recycling (Puttaswamygowda et al., 2019). A lack of input of labile carbon sources in the prolonged incubations could explain the increase of the more versatile Actinobacteria in Greenland margin samples. Their remarkable capacity for degradation of organic substances could make them a

keystone phylum in cryoconite community (Hodson et al., 2007; Cameron et al., 2012; Sanyal et al., 2018).

Patescibacteria were present in small numbers in all cryoconite samples and showed an increase in Antarctic samples with time. Patescibacteria are mostly found in the cold and most oligotrophic environments on Earth, such as groundwater, deep sea sediments, permafrost, and the continental deep subsurface (Herrmann et al., 2019). Members of this phylum seem to belong mainly to ultra-small class size, which ensures the optimal surface-to-volume ratio in the extremely nutrient-poor environments. As the nutrients likely were depleted over time in the long-term incubations of cryoconite material, Patescibacteria seem to be well suited to outcompete other phyla in such extremely challenging conditions. Moreover, they are mostly found in anaerobic conditions and are thought to possess fermentative metabolism, but are able to survive in some oxic habitats such as groundwater (Herrmann et al., 2019). Hence, they are able to survive the long-term anaerobic conditions of cryoconite incubations and they likely contribute to the acetate production. However, a detailed inventory of their fermentative capabilities and physiological limits is yet to be determined.

More detailed analysis of the lower taxonomic rank – dominant genera - demonstrated that *Pseudomonas* and *Rhodoferax,* as well as *Paludibacter,* became enriched over time. Among these, *Paludibacter* is a relatively unrecognized genus with only a few cultivated strains. *Paludibacter* is a strictly anaerobic, chemoorganotrophic genus, capable of fermentation with acetate and propionate as major products (Ueki et al., 2006). It was previously found in the anaerobic enrichments from Antarctic cryoconite (Zdanowski et al., 2017), so could be particularly well-adapted to anaerobic conditions in cryoconite holes. Members of *Rhodoferax* are purple non-sulphur bacteria and were found to have a range of anaerobic metabolisms including the anoxygenic photoorganotrophy and fermentation. They were capable of utilising various organic substrates including acetate (Finneran et al., 2003; Jung et al., 2004). *Rhodoferax* spp. was found before in polar environments and cryoconite holes in Himalaya (Sanyal et al., 2018) and in Antarctica (Sommers et al., 2018). Its growth in long-term incubations was likely elevated thanks to favourable conditions including anoxia, darkness and high concentrations of acetate as a carbon source. Pseudomonas, on the other hand, is believed to be strictly aerobic (Carrión et al., 2011; Shin et al., 2012). However, it was found in the anaerobic enrichment of cryoconite material both in this study and at Zdanowski et al. (2017). In the incubations, Pseudomonas was enriched with time despite the development of anaerobic conditions, suggesting that at least some members of *Pseudomonas* genus are well adapted to anoxia. Some pathogenic species were shown to be able to create anaerobic biofilms by excreting

exopolysaccharides (e.g. *Pseudomonas aeruginosa*) (Yoon et al., 2002), which would be advantageous in cryoconite holes. However, pathogenic species are best suited to warm temperatures, which suggests that there is another subgroup of *Pseudomonas* genus, able to survive both cold conditions and lack of oxygen. *Pseudomonas* is relatively common in cold environments and was found in cryoconite holes before (Carrión et al., 2011; Shin et al., 2012; Singh et al., 2014).

7.4.3. Acetate producers and consumers

Fermentative microorganisms form a heterogeneous group across different phyla. The group includes strict anaerobes and facultative anaerobes. As anoxia can develop and change rapidly in the cryoconite sediments, it is likely that many of the microorganisms are facultative anaerobes. In fact, all of the abundant cultivable microorganisms analysed in chapter 2 were facultative anaerobes. In the absence of oxygen, some of the facultative anaerobes switch to fermentation, while others switch to anaerobic respiration using inorganic electron acceptors (Sandle, 2019)*.* No comprehensive review of fermentation capabilities across different phyla is available in the literature. Some of the abundant phyla found in cryoconite sediments are known for fermentative capabilities, such as members of Firmicutes (especially *Clostridium* genus) Caldiserica, Chloroflexi and Actinobacteria. But the precise analysis of enrichment of fermentative phyla is impossible with simple 16S rDNA profiling of the community. A metagenomic approach or even transcriptomic approach would be needed to fully understand the fermentative potential and its changes over time or depending on the oxygen status of the sediment. The metagenomic approach was used to screen for various genes in cryoconite holes, including genes linked to inorganic nutrients cycling (Edwards et al., 2013), anoxygenic phototrophic metabolism (Franzetti et al., 2016), degradation of anthropogenic contaminants (Hauptmann et al., 2017) and antibiotic resistance (Makowska et al., 2020). However, no metagenomic analysis has yet revealed the genes responsible for fermentation.

Given the challenges in identifying the community changes of acetate producers, it is hard to estimate how much the observed changes in acetate concentrations (chapter 6) are related to increase in the relative abundance of fermenters. Nevertheless, accumulation of acetate is the result of the imbalance in the utilisation of acetate which does not match its production. The main groups responsible for consumption of acetate in the environment are methanogenic archaea and sulphate reducers (Finke and Jørgensen, 2008). Until recently, strict anaerobic microorganisms, especially methanogens, acetogens, sulphate reducers or nitrifiers were believed to be absent from oxygen-rich cryoconite holes (Christner et al., 2003; Stibal and

Tranter, 2007) and methanogenesis and sulphate reduction was believed to be restricted to subglacial environments (Wadham et al., 2008; Anesio and Laybourn-Parry, 2012). More recently, methanogens belonging to *Methanobacteriaceae* and *Methanomicrobia* were identified in Antarctic, but not Arctic cryoconite holes (Cameron et al., 2012). In a metagenomic snapshot of an Alpine cryoconite hole, a small proportion of the metagenome was aligned to *Methanomicrobiales* and some reads were assigned to functional category of sulphate reducers (Edwards et al., 2013). *Methanobacteria* and *Methanomicrobia* classes were also present at low abundance in Svalbard cryoconite holes (Lutz et al., 2017). *The Desulfosporosinus* genus known for sulphate reduction capabilities has been identified in cryoconite material from maritime Antarctica (Zdanowski et al., 2017), but there are no published reports of sulphate reducers in Arctic cryoconite holes. Relative abundance of methanogens and sulphate reducers in incubations of cryoconite material in this study was very variable, but they were present. Greenland margin samples were particularly rich in methanogenic archaea, whereas most known sulphate reducers were detected in both Greenland margin and Svalbard samples and in a very few Antarctic samples. The high abundance of acetate consumers in the Greenland margin samples may explain their relatively low concentrations of acetate when compared to other incubations. Varying abundance of sulphate reducers in Svalbard samples may in turn be responsible for the great variability of acetate concentrations in Svalbard incubations.

Among the sulphate reducers detected in the incubations, *Geobacter* spp. uses acetate as an electron donor in reduction of iron and other metal ions, elemental sulphur, and fumarate (Caccavo et al., 1994). Acetate consumption is a primary source of energy for some members of the genus, as they lack the metabolic pathways responsible for sugar uptake (Methé et al., 2003). In addition to the aforementioned methanogens, relatively high abundance of *Geobacter* in Greenland margin samples is likely another reason for lower acetate concentrations when compared to incubations from other regions. *Geobacter* was a dominant sulphate-reducer in the subglacial environments of Leverett and Russell glaciers (Nixon et al., 2017). The second sulphate reducer detected at notable abundance belongs to a group of incomplete oxidisers. Known *Desulfosporosinus* species oxidise lactate to acetate and do not further oxidise acetate (Ramamoorthy et al., 2006; Lee et al., 2009), in consequence contributing to the accumulation by not only producing acetate, but also likely competing for electron acceptors with *Geobacter*. The final detected sulphate-reducing and acetate-oxidising genus (Göker et al., 2011) – *Desulfobacca* - was only present in one Greenland margin sample at low abundance. Sulphate-reducers and methanogens in Greenland margin samples are likely seeded from nearby subglacial debris and thrive when the anoxic conditions occur in cryoconite material. However,

they do not dominate the anaerobic community as evidence by presence of fermentation products in the incubations.

The diversity and abundance of microbial groups with fermentative potential in cryoconite material from all locations is therefore higher than of those with acetate-degrading capabilities. Perhaps the dynamic conditions of cryoconite holes favour the facultative anaerobes with greater nutrient-scavenging abilities over the strict anaerobes, like sulphate reducers and methanogens. The former can grow in both oxygen-rich and oxygen-poor conditions with various sources of organic carbon utilised by fermentation or oxic respiration. The latter, restricted to anaerobic microniches, demonstrate slower growth and lesser abilities to use organic carbon, which probably limits their expansion.

7.5. Summary

The microbial community of cryoconite holes varies by location, which is likely a result of local environmental factors such as sediment sources, nutrients provided with melt water and physical conditions. The initial composition of samples changes significantly over time under selected environmental conditions, but the differences depending on location remain visible. Anoxia, which develops in the incubations over time, promotes enrichment of certain microbial groups such as Firmicutes. Other enriched phyla, for example Chloroflexi, Patescibacteria or Actinobacteria, also seem well suited to anaerobic, nutrient-poor environments and members of these groups were shown to have fermentative potential. Terminal oxidisers of fermentation products such as methanogens and sulphate reducers were present in the incubations in variable abundances. Their presence was likely a reason for variable acetate concentrations in the Svalbard samples, and for low concentrations in Greenland margin samples. Microorganisms with potential fermentative capabilities are more abundant than terminal oxidisers, likely because the former are better adapted to fluctuating environment conditions such as oxygen concentrations. Persistent and transient anaerobic conditions in cryoconite sediment promote the enrichment of facultatively anaerobic taxa with fermentative potential over strict anaerobes.

Chapter 8

Conclusions

The primary aim of this research project was to get a better understanding of microbial processes in the supraglacial habitat of cryoconite holes, assessing their influence on biogeochemistry of the holes and impact on surrounding ecosystems. The availability of nutrients and other abiotic factors controls microbial activity, which in turn determines the biogeochemical properties of the cryoconite holes examined. Four research hypotheses were formulated and investigated. This section will revisit those hypotheses and draw subsequent conclusions. It concludes by addressing the limitations of this thesis, persisting knowledge gaps and directions for future research.

8.1. Hypotheses revisited

8.1.1. H1: Cryoconite holes as a heterogenous habitat

The first hypothesis stated that cryoconite holes are a heterogenous habitat with diverse niches for microorganisms. Oxygen profiles through cryoconite sediment were able to demonstrate biological activity with a greater resolution than bulk measurements of the water above the sediment, and to reveal processes in cryoconite which is not water-covered, for example, on supraglacial stream banks and in larger holes (Fountain et al., 2004; Hodson et al., 2008; Cook et al., 2016). The results show that biological activity within cryoconite is extremely sensitive to morphological controls and perturbation, and hence is spatially heterogeneous.

H1a: Thick layers of sediment and granule formation restricts oxygen diffusion and allows development of anoxic niches.

Oxygen is quickly used up for microbial activity, creating anoxic zones within otherwise well-oxygenated supraglacial habitats. These results contradict the belief that cryoconite holes should generally be regarded as aerobic environments (Zdanowski et al., 2017), but support the view that the primary control on biological activity is the structure of the sediment ('biocryomorphology', (Cook et al., 2015)). The degree of hydrological connectivity and consequent residence time of the cryoconite is also a strong control, by first allowing the build-up of a stable community structure (Bagshaw et al., 2016a, 2016b), and second by supplying

nutrients via meltwater (Hodson et al., 2005; Stibal and Tranter, 2007). Closed laboratory incubations had much lower productivity levels than field incubations that were open, but these are likely not wholly representative of the real system since inputs from ice melt below could not be included. When the holes remain hydrologically isolated, the melt of underlying ice is a likely source of nutrients. This was somewhat simulated when subsampling of the sediment introduced a small amount of fresh water and caused a peak in activity, but requires confirmation by future laboratory simulations. The constant downward melt of the cryoconite holes during the summer ablation season also seems to be an important mechanism for driving circulation within the sediment layer.

H1b: The development and extent of anoxia depends on the structure of the sediment, which varies by location.

Anoxia could be detected in the incubations of cryoconite material from all studied locations, but the individual patterns of oxygen consumption and production differed. The difference between Arctic and Antarctic samples could be partly explained by higher initial heterotrophic activity of Arctic communities, shown by faster oxygen consumption in the shallow layers of the sediment following perturbations. Arctic communities are likely better adapted to a changeable environment, whereas Antarctic cryoconite holes are considered stable, as they often remain in the same spot isolated from the atmosphere for years at a time (Tranter et al., 2004). Heterotrophic activity and consequent oxygen consumption was lower in Antarctic samples, which could be a consequence of more nutrient-deprived environment (Bagshaw et al., 2013). The oxygen profile was influenced not only by microbial activity, but also by the sediment structure (granule size, thickness, overlying water depth). Although the most common morphology of cryoconite deposit observed in the studied sector of the GrIS was the 'classic' circular, water-filled structure, where anoxia was not observed, the large deposits ('drained and accumulated') were home to comparatively large microbial habitats, much of which were anoxic. Such accumulations were also found in Svalbard. Although no surface accumulations were reported from Antarctica, cryoconite sediments are often thick, which creates a habitat potentially suitable for anaerobes.

8.1.2. H2: Extreme limits of cryoconite microorganisms

The supraglacial environment is characterized by extreme conditions, namely: fluctuating oxygen concentrations; freeze-thaw cycles; fluctuating salinity and pH; fluctuating temperatures; variable availability of carbon sources. It was therefore hypothesised that the ecophysiological

capabilities of microorganisms inhabiting cryoconite holes would reflect the environmental conditions.

The generally oxygen-rich cryoconite holes do harbour an active, culturable anaerobic community. Anaerobic cultivability was better in the coldest conditions tested, which suggests their adaptation to winter conditions and dominance in the beginning or end of the melt season, when anoxic conditions are likely to occur.

H2a: The most abundant members of microbial community are the most successful and demonstrate wide plasticity in a broad range of environmental conditions.

Cultured representatives of the most abundant species could withstand a wide range of physical stresses, including extreme pH and salinity. pH tolerance broadly reflected the values found in cryoconite holes from different locations. Although no high salinities are commonly found on glaciers, microorganisms were shown to tolerate extreme values. These may occur transiently in cryoconite holes, for example during freeze-thaw cycles. Alternatively, the microbial community is seeded from nearby saline habitats, which include arid soils or persistent melt ponds. Microorganisms inhabiting cryoconite holes do not appear to be endemic to cryoconite hole environment, but rather demonstrate similarities to nearby polar locations. Cryoconite microorganisms used a wide range of substrates and as a community were effective in scavenging limited carbon sources. Their metabolic capabilities seem to depend not only on the genetic affiliation, but also fitness of the culture and phenotypic differences between closely related species. Such phenotypic differences are especially likely, as the bacteria with a high 16S rRNA gene similarity showed differences in their physiology.

H2b: Isolates of the most abundant cultivable Antarctic microorganisms are characterized by a higher resistance to harsh environments when compared to the abundant microorganisms from Arctic locations.

The most abundant Antarctic isolates were different phylogenetically and physiologically from Arctic cultures. Antarctic isolates on average showed greater resistance to freezing and thawing cycles and greater use of variable carbon sources when compared to Arctic isolates, suggesting that such adaptations are more important in the harsher physical and biogeochemical conditions of Antarctic cryoconite holes. Overall, there were similar total cell numbers and the same range of salinity tolerance in both samples sets, and a few genera could be found in both Arctic and Antarctic cryoconite holes. This demonstrates that cryoconite hole microorganisms from both polar regions share some physiological traits.

8.1.3. H3: Anaerobic metabolism in cryoconite holes

Cryoconite holes are a heterogenous habitat which harbour anoxic niches, inhabited by a viable anaerobic and facultatively anaerobic community. This anaerobic community will remain active despite the fluctuating oxygen conditions and switch to anaerobic metabolisms when the oxygen becomes depleted.

H3a Fermentation is the most common anaerobic metabolism and its products such as acetate and other carboxylic acids will be detected in the cryoconite pore water.

The most common intermediate of anaerobic remineralisation of organic matter is acetate. High concentrations of acetate were measured in the pore water from long-term incubations of cryoconite material and defrosted sediment samples. Additionally, most of the cultured microorganisms were able to ferment under an anaerobic atmosphere and produced acetate and other carboxylic acids as anaerobic metabolism products. Acetate was also found, in much smaller concentrations, *in situ* in the cryoconite holes on the Greenland Ice Sheet. The results suggest that fermentation is a common process in cryoconite holes under anoxic conditions. Released metabolites are most likely bound to the sediment and could be released through freeze and thaw processes. This may contribute to the previously observed spring 'ionic pulse' of nutrients (Telling et al., 2014).

H3b Fermentation products released to oxic layers in the upper parts of the sediment will likely become oxidised, which will lower their concentration in the overlying oxygenated water in comparison to deeper sediment layers.

The difference in concentrations between different sediment layers was only observed in the Greenland cryoconite incubations. Moreover, concentrations of carboxylic acids released from incubations of sediment from different locations varied over time. These differences are likely an interplay of individual sediment structure, nutrient supply and microbial community composition at the different sites in Antarctica, Svalbard and Greenland.

H3c Dormant/inactive strict anaerobes such as sulphate reducers or methanogens would become active in the anaerobic niches and utilise fermentation products.

Strict anaerobes are often the terminal oxidisers and will utilise intermediate anaerobic products (e.g. acetate, lactate) as well as terminal electron acceptors (e.g. sulphate) in the process of complete mineralisation of organic matter. However, in cryoconite holes their activity seems to be limited. The accumulation of acetate and other carboxylic acids in the pore water suggests that the ecosystem is imbalanced and the oxidation rates of intermediate metabolites are

insufficient. Such imbalance is often found in dynamic environments, such as coastal sediments (Finke et al., 2007; Glombitza et al., 2015). Fermentation seems to be the dominant type of anaerobic metabolism in the cryoconite holes.

8.1.4. H4: Anaerobic communities in cryoconite holes

Prolonged anoxia changes the community structure and promotes the growth of microorganisms adapted to anaerobic conditions. Previous anaerobic enrichments found an increase in strict anaerobic species with time (Zdanowski et al., 2017). Similarly, abundant facultative anaerobic microorganisms were also isolated in this study. Analysis of the microbial community structure revealed that the communities change significantly with time, with the exception of the shorter *in situ* incubations.

H4a Prolonged incubations will have a major influence on the microbial assemblages and a significant change of microbial community structure will be observed.

Bacterial communities of cryoconite holes are prone to change following fluctuations of abiotic factors (Musilova et al., 2015; Pittino et al., 2018). The prolonged incubations were characterised by development and persistence of anoxic conditions. Limited oxygen availability likely put a strong selective pressure on communities from different locations and they were expected to start resembling each other. However, even though a significant shift in bacterial populations was observed, the location of the sampled cryoconite holes remained the strongest factor differentiating the communities. The initial differences between microbial diversity of the incubations remained visible throughout long-term incubations. Some common trends were observed between samples, such as enrichment of anaerobic Firmicutes in Svalbard and Antarctic samples, but mostly each location developed in a unique way.

H4b Prolonged anaerobic conditions were hypothesised to promote the growth of microbial groups present in cryoconite holes in low abundances and capable of anaerobic metabolisms, such as fermenters, sulphate reducers or methanogens.

The growth of Firmicutes, including fermenting genus *Clostridium* and sulphate-reducing genus *Desulfosporosinus* was previously stimulated in Antarctic cryoconite (Zdanowski et al., 2017). In most incubations of this study, *Clostridium* also increased in abundance, as did *Desulfosporosinus* in Greenland margin, Svalbard and a few of Antarctic samples. Other fermenting bacteria were harder to identify as they form a heterogeneous group across different phyla. However, many of the abundant phyla found in the incubations contain genera known for fermentative capabilities, such as members of Caldiserica, Chloroflexi and Actinobacteria. On the other hand, the groups

commonly known for terminal oxidation of fermentation products, especially acetate, seem to be less abundant and very variable. Greenland margin samples were particularly rich in methanogenic archaea, whereas most known sulphate reducers were detected in both Greenland margin and Svalbard samples and in a very few Antarctic samples. High abundance of acetate consumers in Greenland margin samples may explain their relatively low concentrations of acetate when compared to other incubations and might be explained by the influence of exposed subglacial sediments at the margin of ice sheet. In the incubations from all the other locations, low abundance of acetate consumers is one of the factors responsible for the observed accumulation of acetate.

8.2. Synthesis

Cryoconite holes are diverse environments characterised by spatially heterogenous geochemical properties. Such heterogeneity promotes development of microbial communities well adapted to rapidly changing extreme conditions. The results provide evidence for rapid development of anoxia in cryoconite holes and the existence of niches with limited oxygen. Anaerobic zones can be found in the sediment layers, which were previously assumed to be well oxygenated. The heterotrophic microorganisms isolated from the cryoconite hole samples worldwide are well adapted to fluctuating oxygen concentrations. All the most abundant cultivable species are facultatively anaerobic and demonstrate fermentative capabilities. They can also withstand the extreme conditions of the supraglacial environment, including fluctuating temperatures and associated freezing and thawing, extreme and varying geochemical conditions such as wide range of pH and high salinity, and limited nutrients. Antarctic isolates show greater resistance to freezing and thawing cycles and greater use of variable carbon sources when compared to Arctic ones, which likely reflects harsher conditions of Antarctic cryoconite holes. Anoxic conditions are likely to occur in the beginning and end of the melt season, promoting adaptation of anaerobic community to winter conditions, which is reflected in their better cultivability in the coldest conditions tested. Their fermentation products are likely bound to the sediment and released to the cryoconite water in the beginning of the melt season after thawing. Consequently, facultative anaerobic heterotrophs are likely to be important players in the reactivation of the community after the polar night.

Production and accumulation of acetate and other carboxylic acids in cryoconite holes could serve as a source of labile organic carbon for subglacial environments, which are rich in terminal oxidisers such as methanogens and sulphate reducers. Those groups are scarce in the sampled cryoconite holes and incubations, resulting in decoupling of the intermediate and terminal stages

of anaerobic degradation of organic matter and consequent accumulation of intermediate products. The exception are Greenland margin samples, which may contain some subglacial material. In the incubations with prolonged anoxic conditions, other enriched phyla are known for fermentative capabilities, but a full assessment of fermentation potential based on simple taxonomic analysis is impossible. Despite this limitation, fermentation seems to be a dominant anaerobic metabolism in the cryoconite hole ecosystem.

These advancements in the current knowledge of biogeochemical processes and microbial activity are critical as cryoconite holes are a key component of supraglacial environments, contributing to the storage and release of meltwater to the rest of the glacial system. Complete understanding of the extent of anaerobic processes on glaciers and the consequent release of labile carbon to the downstream environments will contribute to future models of biological activity on glaciers and its contribution to global carbon cycle.

8.3. Recommendations for future work

While several significant findings have been made throughout this thesis, there remains much to understand regarding microbial activity, anaerobic zones and processes in cryoconite holes.

8.3.1. Field incubations and measurements in other locations

The first limitation of this research was limiting the field measurements and incubations to one small sampling area of Greenland Ice Sheet. This could not be overcome because of logistic constraints. Cryoconite holes worldwide are very variable in terms of sediment structure, year-to-year persistence, geochemical properties and microbial communities. Therefore, more expansive studies of spatiotemporal heterogeneity of cryoconite holes from different regions with particular focus on dynamics of anaerobic niches need to be conducted for comparison and upscaling purposes.

8.3.2. Polar night transition

Another important aspect not investigated in this research and mostly overlooked in the literature is transition between polar night and polar day and associated dynamic changes in microbial activity. A major input of nutrients released from the ice thaw was observed in cryoconite studies in Antarctica. Significant changes of community structure in the beginning of the season in Greenland were also associated with the initial melt. Low melt rates associated with the transition period would likely create favourable conditions for creation of anaerobic

zones and resulting anaerobic metabolism. A research methodology tackling logistic constraints on sampling during late winter conditions is needed to answer questions about the polar night microbial processes on glaciers.

8.3.3. Metagenomic and transcriptomic studies

Finally, molecular analysis of microbial communities with the use of 16S rDNA sequencing did not allow for targeted investigation of the fermentative potential of cryoconite microorganisms. Some information could be inferred from the previous knowledge about the taxa occurring within the holes, especially in the anaerobic conditions. However, metagenomic and metatranscriptomic studies would allow for the quantification of the fermentation genes and their activity, revealing a potential and extent of anaerobic metabolisms in cryoconite holes.

Bibliography

Abed, R. M. M. (2010). Interaction between cyanobacteria and aerobic heterotrophic bacteria in the degradation of hydrocarbons. *International Biodeterioration and Biodegradation*, *64*(1). https://doi.org/10.1016/j.ibiod.2009.10.008

Amoroso, A., Domine, F., Esposito, G., Morin, S., Savarino, J., Nardino, M., Montagnoli, M., Bonneville, J.-M., Clement, J.-C., & Ianniello, A. (2009). Microorganisms in dry polar snow are involved in the exchanges of reactive nitrogen species with the atmosphere. *Environmental Science & Technology*, *44*(2), 714–719.

Andersen, K., Kirkegaard, R., Karst, S., & Albertsen, M. (2018). ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. *BioRxiv*. https://doi.org/10.1101/299537

Anesio, A. M., Hodson, A. J., Fritz, A., Psenner, R., & Sattler, B. (2009). High microbial activity on glaciers: importance to the global carbon cycle. *Global Change Biology*, *15*(4), 955–960.

Anesio, A. M., & Laybourn-Parry, J. (2012). Glaciers and ice sheets as a biome. *Trends in Ecology & Evolution*, *27*(4), 219–225. https://doi.org/10.1016/j.tree.2011.09.012

Anesio, A. M., Lutz, S., Nathan, A., Chrismas, M., & Benning, L. G. (2017). The microbiome of glaciers and ice sheets. *Npj Biofilms and Microbiomes*, *3*, 1.

Anesio, A. M., Mindl, B., Laybourn-Parry, J., Hodson, A. J., & Sattler, B. (2007). Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). *Journal of Geophysical Research: Biogeosciences (2005–2012)*, *112*(G4).

Anesio, A. M., Sattler, B., Foreman, C., Telling, J., Hodson, A., Tranter, M., & Psenner, R. (2010a). Carbon fluxes through bacterial communities on glacier surfaces. *Annals of Glaciology*. https://doi.org/10.3189/172756411795932092

Anesio, A. M., Sattler, B., Foreman, C., Telling, J., Hodson, A., Tranter, M., & Psenner, R. (2010b). Carbon fluxes through bacterial communities on glacier surfaces. *Annals of Glaciology*, *51*(56), 32–40.

Angle, J. C., Morin, T. H., Solden, L. M., Narrowe, A. B., Smith, G. J., Borton, M. A., Rey-Sanchez, C., Daly, R. A., Mirfenderesgi, G., Hoyt, D. W., Riley, W. J., Miller, C. S., Bohrer, G., & Wrighton, K. C. (2017). Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. *Nature Communications*, *8*(1).

https://doi.org/10.1038/s41467-017-01753-4

Arrigo, K. R. (2014). Sea Ice Ecosystems. *Annual Review of Marine Science*, *6*(1), 439–467. https://doi.org/10.1146/annurev-marine-010213-135103

Azam, F., Smith, D. C., Steward, G. F., & Hagström, Å. (1994). Bacteria-organic matter coupling and its significance for oceanic carbon cycling. *Microbial Ecology*, *28*(2). https://doi.org/10.1007/BF00166806

Bagshaw, E. A., Tranter, M., Wadham, J. L., Fountain, A. G., Dubnick, A., & Fitzsimons, S. (2016). Processes controlling carbon cycling in Antarctic glacier surface ecosystems. *Geochemical Perspectives Letters*, *2*(1), 44–54. https://doi.org/10.7185/geochemlet.1605

Bagshaw, E A, Tranter, M., Wadham, J. L., Fountain, A. G., Dubnick, A., & Fitzsimons, S. (2016). Processes controlling carbon cycling in Antarctic glacier surface ecosystems. *Geochemical Perspectives Letters*, *2*(0), 44–54. https://doi.org/http://dx.doi.org/10.7185/geochemlet.1605

Bagshaw, E A, Tranter, M., Wadham, J. L., Fountain, A. G., & Mowlem, M. (2011). High-resolution monitoring reveals dissolved oxygen dynamics in an Antarctic cryoconite hole. *Hydrological Processes*, *25*(18), 2868–2877. https://doi.org/10.1002/hyp.8049

Bagshaw, E. A., Wadham, J. L., Mowlem, M., Tranter, M., Eveness, J., Fountain, A. G., & Telling, J. (2011). Determination of dissolved oxygen in the cryosphere: A comprehensive laboratory and field evaluation of fiber optic sensors. *Environmental Science and Technology*, *45*(2), 700–705. https://doi.org/10.1021/es102571j

Bagshaw, Elizabeth A. (2008). *The Biogeochemistry of Cryoconite Holes on Glaciers in Taylor Valley, Antarctica*. University of Bristol.

Bagshaw, Elizabeth A., Tranter, M., Fountain, A. G., Welch, K. A., Basagic, H., & Lyons, W. B. (2007). Biogeochemical evolution of cryoconite holes on Canada Glacier, Taylor Valley, Antarctica. *Journal of Geophysical Research: Biogeosciences*, *112*(4). https://doi.org/10.1029/2007JG000442

Bagshaw, Elizabeth A., Tranter, M., Fountain, A. G., Welch, K., Basagic, H. J., & Lyons, W. B. (2013). Do cryoconite holes have the potential to be significant sources of C, N, and P to downstream depauperate ecosystems of Taylor Valley, Antarctica? *Arctic, Antarctic, and Alpine Research*, *45*(4), 440–454. https://doi.org/10.1657/1938-4246-45.4.440

Bagshaw, Elizabeth A, Wadham, J. L., Tranter, M., Perkins, R., Morgan, A., Williamson, C. J.,

Fountain, A. G., Fitzsimons, S., & Dubnick, A. (2016). Response of Antarctic cryoconite microbial communities to light. *FEMS Microbiology Ecology*, *92*(6), fiw076. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4864406/pdf/fiw076.pdf

Bakermans, C. (2008). Limits for microbial life at subzero temperatures. In *Psychrophiles: From Biodiversity to Biotechnology* (pp. 17–28). Springer.

Bakermans, C., & Skidmore, M. (2011a). Microbial respiration in ice at subzero temperatures (− 4 C to− 33 C). *Environmental Microbiology Reports*, *3*(6), 774–782. http://onlinelibrary.wiley.com/store/10.1111/j.1758-2229.2011.00298.x/asset/j.1758-2229.2011.00298.x.pdf?v=1&t=ixswli4i&s=9e95ed31c39df06b8a9b3b18a12cc9b959429f8b

Bakermans, C., & Skidmore, M. L. (2011b). Microbial metabolism in ice and brine at− 5 C. *Environmental Microbiology*, *13*(8), 2269–2278. http://onlinelibrary.wiley.com/store/10.1111/j.1462-2920.2011.02485.x/asset/j.1462-2920.2011.02485.x.pdf?v=1&t=ixswkyea&s=967e2d452808ffa0d1fc585e2b07671124986414

Banwell, A., Hewitt, I., Willis, I., & Arnold, N. (2016). Moulin density controls drainage development beneath the Greenland ice sheet. *Journal of Geophysical Research: Earth Surface*, *121*(12), 2248–2269. https://doi.org/10.1002/2015JF003801

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016). Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*. https://doi.org/10.1128/mmbr.00019-15

Barletta, R. E., Priscu, J. C., Mader, H. M., Jones, W. L., & Roe, C. H. (2012). Chemical analysis of ice vein microenvironments: II. Analysis of glacial samples from Greenland and Antarctica. *Journal of Glaciology*, *58*(212), 1109–1118. https://doi.org/10.3189/2012JoG12J112

Bartelme, R. P., Custer, J. M., Dupont, C. L., Espinoza, J. L., Torralba, M., Khalili, B., & Carini, P. (2020). Influence of Substrate Concentration on the Culturability of Heterotrophic Soil Microbes Isolated by High-Throughput Dilution-to-Extinction Cultivation. *MSphere*, *5*(1), e00024-20. https://doi.org/10.1128/mSphere.00024-20

Bellas, C. M., Anesio, A. M., Telling, J., Stibal, M., Tranter, M., & Davis, S. (2013). Viral impacts on bacterial communities in Arctic cryoconite. *Environmental Research Letters*, *8*(4). https://doi.org/10.1088/1748-9326/8/4/045021

Bethke, C. M., Sanford, R. A., Kirk, M. F., Jin, Q., & Flynn, T. M. (2011). The thermodynamic ladder in geomicrobiology. *American Journal of Science*, *311*(3). https://doi.org/10.2475/03.2011.01

Bhatia, M. P., Das, S. B., Xu, L., Charette, M. A., Wadham, J. L., & Kujawinski, E. B. (2013). Organic carbon export from the Greenland ice sheet. *Geochimica et Cosmochimica Acta*, *109*, 329–344. https://doi.org/10.1016/j.gca.2013.02.006

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., & Rapp, J. Z. (2015). Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677–690.

Bøggild, C. E., Brandt, R. E., Brown, K. J., & Warren, S. G. (2010). The ablation zone in northeast greenland: Ice types, albedos and impurities. *Journal of Glaciology*, *56*(195). https://doi.org/10.3189/002214310791190776

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Boyd, E. S., Hamilton, T. L., Havig, J. R., Skidmore, M. L., & Shock, E. L. (2014). Chemolithotrophic Primary Production in a Subglacial Ecosystem. *Applied and Environmental Microbiology*, *80*(19), 6146 LP – 6153. https://doi.org/10.1128/AEM.01956-14

Boyd, E. S., Skidmore, M., Mitchell, A. C., Bakermans, C., & Peters, J. W. (2010). Methanogenesis in subglacial sediments. *Environmental Microbiology Reports*, *2*(5), 685–692. https://doi.org/10.1111/j.1758-2229.2010.00162.x

Bradley, J. A., Singarayer, J. S., & Anesio, A. M. (2014). Microbial community dynamics in the forefield of glaciers. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1795), 20140882. http://rspb.royalsocietypublishing.org/content/royprsb/281/1795/20140882.full.pdf

Bulat, S. A. (2016). Microbiology of the subglacial Lake Vostok: first results of borehole-frozen lake water analysis and prospects for searching for lake inhabitants. *Phil. Trans. R. Soc. A*, *374*(2059), 20140292.

http://rsta.royalsocietypublishing.org/content/roypta/374/2059/20140292.full.pdf

Caccavo, F., Lonergan, D. J., Lovley, D. R., Davis, M., Stolz, J. F., & McInerney, M. J. (1994). Geobacter sulfurreducens sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Applied and Environmental Microbiology*, *60*(10). https://doi.org/10.1128/aem.60.10.3752-3759.1994

Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic. *FEMS Microbiology Ecology*, *82*(2), 254–267. http://femsec.oxfordjournals.org/content/femsec/82/2/254.full.pdf

Cameron, K. A., Stibal, M., Chrismas, N., Box, J., & Jacobsen, C. S. (2016). Nitrate addition has minimal short-term impacts on Greenland ice sheet supraglacial prokaryotes. *Environmental Microbiology Reports*.

Carpenter, E. J., Lin, S., & Capone, D. G. (2000). Bacterial activity in South Pole snow. *Applied and Environmental Microbiology*, *66*(10), 4514–4517. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC92333/pdf/am004514.pdf

Carrión, O., Miñana-Galbis, D., Montes, M. J., & Mercadé, E. (2011). Pseudomonas deceptionensis sp. nov., a psychrotolerant bacterium from the antarctic. *International Journal of Systematic and Evolutionary Microbiology*, *61*(10). https://doi.org/10.1099/ijs.0.024919-0

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. In *International Dairy Journal*. https://doi.org/10.1016/j.idairyj.2004.02.001

Cavicchioli, R, Charlton, T., Ertan, H., Mohd Omar, S., Siddiqui, K. S., & Williams, T. J. (2011). Biotechnological uses of enzymes from psychrophiles. *Microbial Biotechnology*, *4*(4), 449–460. https://doi.org/10.1111/j.1751-7915.2011.00258.x

Cavicchioli, Ricardo. (2015). Microbial ecology of Antarctic aquatic systems. *Nature Reviews Microbiology*, *13*(11), 691–706. https://doi.org/10.1038/nrmicro3549

Cavicchioli, Ricardo. (2016). On the concept of a psychrophile. In *ISME Journal* (Vol. 10, Issue 4, pp. 793–795). Nature Publishing Group. https://doi.org/10.1038/ismej.2015.160

Chandler, D. M., Wadham, J. L., Lis, G. P., Cowton, T., Sole, A., Bartholomew, I., Telling, J., Nienow, P., Bagshaw, E. B., & Mair, D. (2013). Evolution of the subglacial drainage system

beneath the Greenland Ice Sheet revealed by tracers. *Nature Geoscience*, *6*(3), 195–198.

Chaudhary, D. K., Khulan, A., & Kim, J. (2019). Development of a novel cultivation technique for uncultured soil bacteria. *Scientific Reports*, *9*(1), 6666. https://doi.org/10.1038/s41598-019-43182-x

Christner, B. C., Kvitko, B. H., & Reeve, J. N. (2003). Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles*, *7*(3), 177–183. https://doi.org/10.1007/s00792-002-0309-0

Christner, B. C., Mosley-Thompson, E., Thompson, L. G., & Reeve, J. N. (2003). Bacterial recovery from ancient glacial ice. *Environmental Microbiology*, *5*(5), 433–436. http://onlinelibrary.wiley.com/store/10.1046/j.1462-2920.2003.00422.x/asset/j.1462-2920.2003.00422.x.pdf?v=1&t=ixswla1l&s=10d42fbe84a27399f0d381c66819cd9ed398d36a

Christner, B. C., Skidmore, M. L., Priscu, J. C., Tranter, M., & Foreman, C. M. (2008). Bacteria in subglacial environments. In *Psychrophiles: From Biodiversity to Biotechnology* (pp. 51–71). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-74335-4_4

Cocolin, L., & Rantsiou, K. (2014). Molecular Biology: Transcriptomics. In *Encyclopedia of Food Microbiology: Second Edition* (pp. 803–807). Elsevier Inc. https://doi.org/10.1016/B978-0-12-384730-0.00436-5

Collins, T., & Margesin, R. (2019). Psychrophilic lifestyles: mechanisms of adaptation and biotechnological tools. In *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-019-09659-5

Cook, J., Hodson, A., Telling, J., Anesio, A., Irvine-Fynn, T., & Bellas, C. (2010). The mass-area relationship within cryoconite holes and its implications for primary production. *Annals of Glaciology*, *51*(56), 106–110. https://doi.org/10.3189/172756411795932038

Cook, J. M., Hodson, A. J., Anesio, A. M., Hanna, E., Yallop, M., Stibal, M., Telling, J., & Huybrechts, P. (2012). An improved estimate of microbially mediated carbon fluxes from the Greenland ice sheet. *Journal of Glaciology*, *58*(212), 1098–1108. https://doi.org/10.3189/2012JoG12J001

Cook, Joseph, Edwards, A., & Hubbard, A. (2015). Biocryomorphology: Integrating Microbial Processes with Ice Surface Hydrology, Topography, and Roughness. *Frontiers in Earth Science*, *3*, 78.

Cook, Joseph, Edwards, A., Takeuchi, N., & Irvine-Fynn, T. (2016). Cryoconite: The dark biological secret of the cryosphere. *Progress in Physical Geography*, *40*(1), 66–111. https://doi.org/10.1177/0309133315616574

Cook, Joseph M, Edwards, A., Bulling, M., Mur, L. A. J., Cook, S., Gokul, J. K., Cameron, K. A., Sweet, M., & Irvine-Fynn, T. D. L. (2016). Metabolome-mediated biocryomorphic evolution promotes carbon fixation in Greenlandic cryoconite holes. *Environmental Microbiology*, *18*(12), 4674–4686.

Cook, Joseph Mitchell, Hodson, A. J., & Irvine-Fynn, T. D. L. (2016). Supraglacial weathering crust dynamics inferred from cryoconite hole hydrology. *Hydrological Processes*, *30*(3). https://doi.org/10.1002/hyp.10602

Courtright, E. M., Wall, D. H., & Virginia, R. A. (2001). Determining habitat suitability for soil invertebrates in an extreme environment: The McMurdo Dry Valleys, Antarctica. *Antarctic Science*. https://doi.org/10.1017/S0954102001000037

Cragg, B. A., & Kemp, A. E. S. (1995). Bacterial Profiles in Deep Sediment Layers from the Eastern Equatorial Pacific Ocean, Site 851. In *Proceedings of the Ocean Drilling Program, 138 Scientific Results*. https://doi.org/10.2973/odp.proc.sr.138.130.1995

Dahl, T. A., Midden, W. R., & Hartman, P. E. (1989). Comparison of killing of gram-negative and gram-positive bacteria by pure singlet oxygen. *Journal of Bacteriology*. https://doi.org/10.1128/jb.171.4.2188-2194.1989

Dahllöf, I., Baillie, H., & Kjelleberg, S. (2000). rpoB-Based Microbial Community Analysis Avoids Limitations Inherent in 16S rRNA Gene Intraspecies Heterogeneity. *Applied and Environmental Microbiology*, *66*(8), 3376 LP – 3380. https://doi.org/10.1128/AEM.66.8.3376-3380.2000

Darcy, J. L., & Schmidt, S. K. (2016). Nutrient limitation of microbial phototrophs on a debris-covered glacier. *Soil Biology and Biochemistry*, *95*. https://doi.org/10.1016/j.soilbio.2015.12.019

Darcy, John L, Gendron, E. M. S., Sommers, P., Porazinska, D. L., & Schmidt, S. K. (2018). Island Biogeography of Cryoconite Hole Bacteria in Antarctica's Taylor Valley and Around the World . In *Frontiers in Ecology and Evolution* (Vol. 6, p. 180). https://www.frontiersin.org/article/10.3389/fevo.2018.00180

Darcy, John L, Lynch, R. C., King, A. J., Robeson, M. S., & Schmidt, S. K. (2011). Global

distribution of Polaromonas phylotypes-evidence for a highly successful dispersal capacity. *PLoS One*, *6*(8), e23742. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3163589/pdf/pone.0023742.pdf

de Man, J. C. (1983). MPN tables, corrected. *European Journal of Applied Microbiology and Biotechnology*. https://doi.org/10.1007/BF00508025

De Smet, W. H., & Van Rompu, E. A. (1994). Rotifera and Tardigrada from some cryoconite holes on a Spitsbergen (Svalbard) glacier. *BELG.J.ZOOL.*, *124*(1).

De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K.-H., & Whitman, W. B. (2009). Bergey's manual of systematic bacteriology, 2nd Ed. Vol.3. The Firmicutes. In *Bergey's Manual of Systematic Bacteriology* (Vol. 3, Issue 9). https://doi.org/10.1007/978-0-387-68489-5

Decho, A. W., & Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSs) in ocean systems. *Frontiers in Microbiology*, *8*(MAY). https://doi.org/10.3389/fmicb.2017.00922

Dieser, M., Greenwood, M., & Foreman, C. M. (2010). Carotenoid pigmentation in Antarctic heterotrophic bacteria as a strategy to withstand environmental stresses. *Arctic, Antarctic, and Alpine Research*. https://doi.org/10.1657/1938-4246-42.4.396

Doran, P. T., McKay, C. P., Clow, G. D., Dana, G. L., Fountain, A. G., Nylen, T., & Lyons, W. B. (2002). Valley floor climate observations from the McMurdo Dry Valleys, Antarctica, 1986–2000. *Journal of Geophysical Research: Atmospheres (1984–2012)*, *107*(D24), ACL 13-1-ACL 13-12.

Earl, J. P., Adappa, N. D., Krol, J., Bhat, A. S., Balashov, S., Ehrlich, R. L., Palmer, J. N., Workman, A. D., Blasetti, M., Sen, B., Hammond, J., Cohen, N. A., Ehrlich, G. D., & Mell, J. C. (2018). Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *Microbiome*, *6*(1), 190. https://doi.org/10.1186/s40168-018-0569-2

Edwards, A., Anesio, A. M., Rassner, S. M., Sattler, B., Hubbard, B., Perkins, W. T., Young, M., & Griffith, G. W. (2011a). Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *The ISME Journal*, *5*(1), 150–160. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3105670/pdf/ismej2010100a.pdf

Edwards, A., Anesio, A. M., Rassner, S. M., Sattler, B., Hubbard, B., Perkins, W. T., Young, M., &

Griffith, G. W. (2011b). Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *ISME Journal*, *5*(1), 150–160. https://doi.org/10.1038/ismej.2010.100

Edwards, A., Mur, L. A. J., Girdwood, S. E., Anesio, A. M., Stibal, M., Rassner, S. M. E., Hell, K., Pachebat, J. A., Post, B., Bussell, J. S., Cameron, S. J. S., Griffith, G. W., Hodson, A. J., & Sattler, B. (2014). Coupled cryoconite ecosystem structure-function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiology Ecology*, *89*(2), 222–237. https://doi.org/10.1111/1574-6941.12283

Edwards, A., Pachebat, J. A., Swain, M., Hegarty, M., Hodson, A. J., Irvine-Fynn, T. D. L., Rassner, S. M. E., & Sattler, B. (2013). A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environmental Research Letters*, *8*(3). https://doi.org/10.1088/1748-9326/8/3/035003

Edwards, A., Rassner, S. M. E., Anesio, A. M., Worgan, H. J., Irvine-Fynn, T. D. L., Williams, H. W., Sattler, B., & Griffith, G. W. (2013). Contrasts between the cryoconite and ice-marginal bacterial communities of Svalbard glaciers. *Polar Research*, *32*. https://doi.org/10.3402/polar.v32i0.19468

Eiler, A., Langenheder, S., Bertilsson, S., & Tranvik, L. J. (2003). Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology*, *69*(7), 3701–3709.

Fallowfield, H. J., & Daft, M. J. (1988). The extracellular release of dissolved organic carbon by freshwater cyanobacteria and algae and the interaction with lysobacter cp-1. *British Phycological Journal*, *23*(4). https://doi.org/10.1080/00071618800650351

Fawley, M. W., & Fawley, K. P. (2004). A simple and rapid technique for the isolation of DNA from microalgae. *Journal of Phycology*, *40*(1). https://doi.org/10.1111/j.0022-3646.2004.03-081.x

Fernandez-Buces, N., Siebe, C., Cram, S., & Palacio, J. L. (2006). Mapping soil salinity using a combined spectral response index for bare soil and vegetation: A case study in the former lake Texcoco, Mexico. *Journal of Arid Environments*, *65*(4), 644–667.

Ferrario, C., Pittino, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Azzoni, R. S., Diolaiuti, G., Franzetti, A., Ambrosini, R., & Villa, S. (2017). Bacteria contribute to pesticide degradation in cryoconite holes in an Alpine glacier. *Environmental Pollution*, *230*, 919–926. https://doi.org/https://doi.org/10.1016/j.envpol.2017.07.039

Ferry, J. G. (1992). Methane from acetate. In *Journal of Bacteriology* (Vol. 174, Issue 17). https://doi.org/10.1128/jb.174.17.5489-5495.1992

Finke, N., & Jørgensen, B. B. (2008). Response of fermentation and sulfate reduction to experimental temperature changes in temperate and Arctic marine sediments. *ISME Journal*, *2*(8). https://doi.org/10.1038/ismej.2008.20

Finke, N., Vandieken, V., & Jørgensen, B. B. (2007). Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. *FEMS Microbiology Ecology*, *59*(1), 10–22. https://doi.org/10.1111/j.1574-6941.2006.00214.x

Finneran, K. T., Johnsen, C. V., & Lovley, D. R. (2003). Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *International Journal of Systematic and Evolutionary Microbiology*, *53*(3). https://doi.org/10.1099/ijs.0.02298-0

Fonseca, F., Béal, C., & Corrieu, G. (2001). Operating conditions that affect the resistance of lactic acid bacteria to freezing and frozen storage. *Cryobiology*. https://doi.org/10.1006/cryo.2001.2343

Foreman, C. M., Sattler, B., Mikucki, J. A., Porazinska, D. L., & Priscu, J. C. (2007). Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica. *Journal of Geophysical Research: Biogeosciences*, *112*(4). https://doi.org/10.1029/2006JG000358

Fountain, A. G., Basagic, H. J., & Niebuhr, S. (2016). Glaciers in equilibrium, McMurdo Dry Valleys, Antarctica. *Journal of Glaciology*, *62*(235), 976–989. https://doi.org/10.1017/jog.2016.86

Fountain, A. G., Lyons, W. B., Burkins, M. B., Dana, G. L., Doran, P. T., Lewis, K. J., McKnight, D. M., Moorhead, D. L., Parsons, A. N., Priscu, J. C., Wall, D. H., Wharton Jr., R. A., & Virginia, R. A. (1999). Physical Controls on the Taylor Valley Ecosystem, Antarctica. *BioScience*, *49*(12), 961–971. https://doi.org/10.1525/bisi.1999.49.12.961

Fountain, A. G., Nylen, T. H., Monaghan, A., Basagic, H. J., & Bromwich, D. (2010). Snow in the McMurdo Dry Valleys, Antarctica. *International Journal of Climatology*, *30*(5), 633–642. https://doi.org/10.1002/joc.1933

Fountain, A. G., Nylen, T. H., Tranter, M., & Bagshaw, E. (2008). Temporal variations in physical and chemical features of cryoconite holes on Canada Glacier, McMurdo Dry Valleys,

Antarctica. *Journal of Geophysical Research: Biogeosciences*, *113*(G1).

Fountain, A. G., Tranter, M., Nylen, T. H., Lewis, K. J., & Mueller, D. R. (2004). Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. *Journal of Glaciology*, *50*(168), 35–45. https://doi.org/10.3189/172756504781830312

Franzetti, A, Pittino, F., Gandolfi, I., Azzoni, R. S., Diolaiuti, G., Smiraglia, C., Pelfini, M., Compostella, C., Turchetti, B., Buzzini, P., & Ambrosini, R. (2020). Early ecological succession patterns of bacterial, fungal and plant communities along a chronosequence in a recently deglaciated area of Italian Alps. *FEMS Microbiology Ecology*. https://doi.org/10.1093/femsec/fiaa165

Franzetti, Andrea, Navarra, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Minora, U., Azzoni, R. S., Diolaiuti, G., Smiraglia, C., & Ambrosini, R. (2017). Temporal variability of bacterial communities in cryoconite on an alpine glacier. *Environmental Microbiology Reports*, *9*(2), 71–78. https://doi.org/10.1111/1758-2229.12499

Franzetti, Andrea, Tagliaferri, I., Gandolfi, I., Bestetti, G., Minora, U., Mayer, C., Azzoni, R. S., Diolaiuti, G., Smiraglia, C., & Ambrosini, R. (2016). Light-dependent microbial metabolisms drive carbon fluxes on glacier surfaces. *ISME Journal*. https://doi.org/10.1038/ismej.2016.72

Friedmann, E. I., & Weed, R. (1987). Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic cold desert. *Science*, *236*(4802), 703–705. http://science.sciencemag.org/content/sci/236/4802/703.full.pdf

Gajda, R. T. (1958). CRYOCONITE PHENOMENA ON THE GREENLAND ICE CAP IN THE THULE AREA. *Canadian Geographer / Le Géographe Canadien*, *3*(12). https://doi.org/10.1111/j.1541-0064.1958.tb01802.x

Garneau, M., Roy, S., Lovejoy, C., Gratton, Y., & Vincent, W. F. (2008). Seasonal dynamics of bacterial biomass and production in a coastal arctic ecosystem: Franklin Bay, western Canadian Arctic. *Journal of Geophysical Research: Oceans (1978–2012)*, *113*(C7).

Gawor, J., Grzesiak, J., Sasin-Kurowska, J., Borsuk, P., Gromadka, R., Górniak, D., Świątecki, A., Aleksandrzak-Piekarczyk, T., & Zdanowski, M. K. (2016). Evidence of adaptation, niche separation and microevolution within the genus Polaromonas. *Extremophiles*, *20*(4), 403–413.

Glombitza, C., Jaussi, M., Røy, H., Seidenkrantz, M.-S., Lomstein, B. A., & Jørgensen, B. B. (2015). Formate, acetate, and propionate as substrates for sulfate reduction in sub-arctic sediments of Southwest Greenland. *Frontiers in Microbiology*, *6*, 846. https://doi.org/10.3389/fmicb.2015.00846

Göker, M., Teshima, H., Lapidus, A., Nolan, M., Lucas, S., Hammon, N., Deshpande, S., Cheng, J. F., Tapia, R., Han, C., Goodwin, L., Pitluck, S., Huntemann, M., Liolios, K., Ivanova, N., Pagani, I., Mavromatis, K., Ovchinikova, G., Pati, A., … Klenk, H. P. (2011). Complete genome sequence of the acetate-degrading sulfate reducer Desulfobacca acetoxidans type strain (ASRB2 T). *Standards in Genomic Sciences*, *4*(3). https://doi.org/10.4056/sigs.2064705

Gokul, J. K., Hodson, A. J., Saetnan, E. R., Irvine-Fynn, T. D. L., Westall, P. J., Detheridge, A. P., Takeuchi, N., Bussell, J., Mur, L. A. J., & Edwards, A. (2016). Taxon interactions control the distributions of cryoconite bacteria colonizing a High Arctic ice cap. *Molecular Ecology*, *25*(15). https://doi.org/10.1111/mec.13715

Gottschalk, G. (1979). *Bacterial Metabolism*. Springer US. https://doi.org/10.1007/978-1-4684-0465-4

Gradinger, R. R. (2001). Adaptation of Arctic and Antarctic ice metazoa to their habitat1 1Presented at the 94th Annual Meeting of the Deutsche Zoologische Gesellschaft in Osnabrück, June 4–8, 2001. *Zoology*, *104*(3), 339–345. https://doi.org/https://doi.org/10.1078/0944-2006-00039

Grannas, A. M., Bogdal, C., Hageman, K. J., Halsall, C., Harner, T., Hung, H., Kallenborn, R., Klán, P., Klánová, J., & Macdonald, R. W. (2013). The role of the global cryosphere in the fate of organic contaminants. *Atmospheric Chemistry and Physics*, *13*(6), 3271–3305.

Grant, W. D., & Jones, B. E. (2000). Alkaline environments. *Encyclopaedia of Microbiology*, *1*, 126–133.

Grime, J. M. A., Edwards, M. A., Rudd, N. C., & Unwin, P. R. (2008). Quantitative visualization of passive transport across bilayer lipid membranes. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(38). https://doi.org/10.1073/pnas.0803720105

Grogan, P., Michelsen, A., Ambus, P., & Jonasson, S. (2004). Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology and Biochemistry*, *36*(4), 641–654.

https://doi.org/https://doi.org/10.1016/j.soilbio.2003.12.007

Grossart, H. P., & Simon, M. (2007). Interactions of planktonic algae and bacteria: Effects on algal growth and organic matter dynamics. *Aquatic Microbial Ecology*, *47*(2). https://doi.org/10.3354/ame047163

Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J. C., Diderichsen, B., & Plemenitaš, A. (2003). Extremophilic fungi in arctic ice: A relationship between adaptation to low temperature and water activity. *Physics and Chemistry of the Earth*. https://doi.org/10.1016/j.pce.2003.08.056

Hall, K., Thorn, C. E., Matsuoka, N., & Prick, A. (2002). Weathering in cold regions: some thoughts and perspectives. *Progress in Physical Geography*, *26*(4), 577–603.

Hansen, A. A., Herbert, R. A., Mikkelsen, K., Jensen, L. L., Kristoffersen, T., Tiedje, J. M., Lomstein, B. A., & Finster, K. W. (2007). Viability, diversity and composition of the bacterial community in a high Arctic permafrost soil from Spitsbergen, Northern Norway. *Environmental Microbiology*. https://doi.org/10.1111/j.1462-2920.2007.01403.x

Hatayama, K., Esaki, K., & Ide, T. (2013). Cellulomonas soli sp. nov. and Cellulomonas oligotrophica sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*. https://doi.org/10.1099/ijs.0.038364-0

Hauptmann, A. L., Sicheritz-Pontén, T., Cameron, K. A., Bælum, J., Plichta, D. R., Dalgaard, M., & Stibal, M. (2017). Contamination of the Arctic reflected in microbial metagenomes from the Greenland ice sheet. *Environmental Research Letters*. https://doi.org/10.1088/1748-9326/aa7445

Hauptmann, A. Z. E. L., Sicheritz-Pontén, T., Cameron, K. A., Bælum, J., Plichta, D. R., Dalgaard, M. D., & Stibal, M. (2017). Contamination of the Arctic reflected in microbial metagenomes from the Greenland ice sheet. *Environmental Research Letters*, *12*(7).

Hawkings, J., Wadham, J., Tranter, M., Telling, J., Bagshaw, E., Beaton, A., Simmons, S., Chandler, D., Tedstone, A., & Nienow, P. (2016). The Greenland Ice Sheet as a hot spot of phosphorus weathering and export in the Arctic. *Global Biogeochemical Cycles*, *30*(2), 191–210.

Hawkings, J.R., Wadham, J. L., Tranter, M., Lawson, E., Sole, A., Cowton, T., Tedstone, A. J., Bartholomew, I., Nienow, P., Chandler, D., & Telling, J. (2015). The effect of warming climate on nutrient and solute export from the Greenland Ice Sheet. *Geochemical*

Perspectives Letters, *1*(1), 94–104. https://doi.org/10.7185/geochemlet.1510

Hawkings, Jon R., Hatton, J. E., Hendry, K. R., de Souza, G. F., Wadham, J. L., Ivanovic, R., Kohler, T. J., Stibal, M., Beaton, A., Lamarche-Gagnon, G., Tedstone, A., Hain, M. P., Bagshaw, E., Pike, J., & Tranter, M. (2018). The silicon cycle impacted by past ice sheets. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-05689-1

Herrmann, M., Wegner, C.-E., Taubert, M., Geesink, P., Lehmann, K., Yan, L., Lehmann, R., Totsche, K. U., & Küsel, K. (2019). Predominance of Cand. Patescibacteria in Groundwater Is Caused by Their Preferential Mobilization From Soils and Flourishing Under Oligotrophic Conditions . In *Frontiers in Microbiology* (Vol. 10, p. 1407). https://www.frontiersin.org/article/10.3389/fmicb.2019.01407

Heyer, H., & Krumbein, W. E. (1991). Excretion of fermentation products in dark and anaerobically incubated cyanobacteria. *Archives of Microbiology*, *155*(3). https://doi.org/10.1007/BF00252213

Hodson, A., Anesio, A. M., Ng, F., Watson, R., Quirk, J., Irvine-Fynn, T., Dye, A., Clark, C., Mccloy, P., Kohler, J., & Sattler, B. (2007). A glacier respires: Quantifying the distribution and respiration CO2 flux of cryoconite across an entire arctic supraglacial ecosystem. *Journal of Geophysical Research: Biogeosciences*, *112*(4). https://doi.org/10.1029/2007JG000452

Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J., & Sattler, B. (2008). Glacial ecosystems. In *Ecological Monographs* (Vol. 78, Issue 1, pp. 41–67). https://doi.org/10.1890/07-0187.1

Hodson, A., Bøggild, C., Hanna, E., Huybrechts, P., Langford, H., Cameron, K., & Houldsworth, A. (2010). The cryoconite ecosystem on the Greenland ice sheet. *Annals of Glaciology*, *51*(56). https://doi.org/10.3189/172756411795931985

Hodson, A., Brock, B., Pearce, D., Laybourn-Parry, J., & Tranter, M. (2015). Cryospheric ecosystems: a synthesis of snowpack and glacial research. *Environmental Research Letters*, *10*(11), 110201. https://doi.org/http://dx.doi.org/10.1088/1748-9326/10/11/110201

Hodson, A., Cameron, K., Bøggild, C., Irvine-Fynn, T., Langford, H., Pearce, D., & Banwart, S. (2010). The structure, biological activity and biogeochemistry of cryoconite aggregates upon an arctic valley glacier: Longyearbreen, Svalbard. *Journal of Glaciology*, *56*(196), 349–362. https://doi.org/10.3189/002214310791968403

Hodson, A. J., Mumford, P. N., Kohler, J., & Wynn, P. M. (2005). The High Arctic glacial ecosystem: new insights from nutrient budgets. *Biogeochemistry*, *72*(2), 233–256.

Hoffman, P. F. (2016). Cryoconite pans on Snowball Earth: supraglacial oases for Cryogenian eukaryotes? *Geobiology*, *14*(6). https://doi.org/10.1111/gbi.12191

Holland, A.T., Williamson, C. J., Sgouridis, F., Tedstone, A. J., McCutcheon, J., Cook, J. M., Poniecka, E., Yallop, M. L., Tranter, M., & Anesio, A. M. (2019). Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet). *Biogeosciences*, *16*(16). https://doi.org/10.5194/bg-16-3283-2019

Holland, Alexandra T, Bergk Pinto, B., Layton, R., Williamson, C. J., Anesio, A. M., Vogel, T. M., Larose, C., & Tranter, M. (2020). Over Winter Microbial Processes in a Svalbard Snow Pack: An Experimental Approach. In *Frontiers in Microbiology* (Vol. 11, p. 1029). https://www.frontiersin.org/article/10.3389/fmicb.2020.01029

Hood, E., Battin, T. J., Fellman, J., O'Neel, S., & Spencer, R. G. M. (2015). Storage and release of organic carbon from glaciers and ice sheets. *Nature Geoscience*, *8*(2), 91–96. https://doi.org/10.1038/ngeo2331

Hood, E., Fellman, J., Spencer, R. G. M., Hernes, P. J., Edwards, R., D'Amore, D., & Scott, D. (2009). Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature*, *462*(7276), 1044–1047. https://doi.org/10.1038/nature08580

Hu, X., & Cai, W. (2011). An assessment of ocean margin anaerobic processes on oceanic alkalinity budget. *Global Biogeochemical Cycles*, *25*(3).

Hu, Y. O. O., Karlson, B., Charvet, S., & Andersson, A. F. (2016). Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the baltic sea. *Frontiers in Microbiology*, *7*(MAY). https://doi.org/10.3389/fmicb.2016.00679

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Irvine-Fynn, T. D. L., Bridge, J. W., & Hodson, A. J. (2011). In situ quantification of supraglacial cryoconite morphodynamics using time-lapse imaging: an example from Svalbard. *Journal of Glaciology*, *57*(204), 651–657.

Jordan, S., Hutchings, M. I., & Mascher, T. (2008). Cell envelope stress response in Gram-positive bacteria. In *FEMS Microbiology Reviews*. https://doi.org/10.1111/j.1574-6976.2007.00091.x

Jørgensen, A. S., & Andreasen, F. (2007). Mapping of permafrost surface using ground-penetrating radar at Kangerlussuaq Airport, western Greenland. *Cold Regions Science and Technology*, *48*(1). https://doi.org/10.1016/j.coldregions.2006.10.007

Jung, D. O., Achenbach, L. A., Karr, E. A., Takaichi, S., & Madigan, M. T. (2004). A gas vesiculate planktonic strain of the purple non-sulfur bacterium Rhodoferax antarcticus isolated from lake Fryxell, Dry Valleys, Antarctica. *Archives of Microbiology*, *182*(2–3). https://doi.org/10.1007/s00203-004-0719-8

Jungblut, A. D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D. R., Burns, B. P., & Neilan, B. A. (2005). Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environmental Microbiology*. https://doi.org/10.1111/j.1462-2920.2005.00717.x

Junge, K., Eicken, H., & Deming, J. W. (2004). Bacterial Activity at −2 to −20°C in Arctic Wintertime Sea Ice. *Applied and Environmental Microbiology*, *70*(1), 550 LP – 557. https://doi.org/10.1128/AEM.70.1.550-557.2004

Kaczmarek, Ł., Jakubowska, N., Celewicz-Gołdyn, S., & Zawierucha, K. (2016). The microorganisms of cryoconite holes (algae, Archaea, bacteria, cyanobacteria, fungi, and Protista): A review. In *Polar Record* (Vol. 52, Issue 2, pp. 176–203). Cambridge University Press. https://doi.org/10.1017/S0032247415000637

Kaksonen, A. H., Plumb, J. J., Robertson, W. J., Spring, S., Schumann, P., Franzmann, P. D., & Puhakka, J. A. (2006). Novel thermophilic sulfate-reducing bacteria from a geothermally active underground mine in Japan. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.72.5.3759-3762.2006

Kastovská, K., Elster, J., Stibal, M., & Santrůcková, H. (2005). Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high arctic). *Microbial Ecology*. https://doi.org/10.1007/s00248-005-0246-4

Keskitalo, J., Lepparanta, M., & Arvola, L. (2013). First records of primary producers of epiglacial and supraglacial lakes in western Dronning Maud Land, Antarctica. *Polar Biology*, *36*(10), 1441–1450. https://doi.org/10.1007/s00300-013-1362-0

Kleinteich, J., Hildebrand, F., Bahram, M., Voigt, A. Y., Wood, S. A., Jungblut, A. D., Küpper, F. C., Quesada, A., Camacho, A., Pearce, D. A., Convey, P., Vincent, W. F., Zarfl, C., Bork, P., & Dietrich, D. R. (2017). Pole-to-Pole Connections: Similarities between Arctic and Antarctic Microbiomes and Their Vulnerability to Environmental Change. *Frontiers in Ecology and Evolution*, *5*(NOV), 137. https://doi.org/10.3389/fevo.2017.00137

Knight, P., Waller, R., Patterson, C., Jones, A., & Robinson, Z. (2002). Discharge of debris from ice at the margin of the Greenland ice sheet. *Journal of Glaciology*, *48* (161), 192-198. doi:10.3189/172756502781831359

Kolber, Z. S., Prášil, O., & Falkowski, P. G. (1998). Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1367*(1), 88–106. https://doi.org/https://doi.org/10.1016/S0005-2728(98)00135-2

Koziol, K. A., Moggridge, H. L., Cook, J. M., & Hodson, A. J. (2019). Organic carbon fluxes of a glacier surface: A case study of Foxfonna, a small Arctic glacier. *Earth Surface Processes and Landforms*, *44*(2), 405–416. https://doi.org/https://doi.org/10.1002/esp.4501

Köpke, B., Wilms, R., Engelen, B., Cypionka, H., & Sass, H. (2005). Microbial diversity in coastal subsurface sediments: A cultivation approach using various electron acceptors and substrate gradients. *Applied and Environmental Microbiology*, *71*(12), 7819–7830. https://doi.org/10.1128/AEM.71.12.7819-7830.2005

Lamarche-Gagnon, G., Wadham, J. L., Sherwood Lollar, B., Arndt, S., Fietzek, P., Beaton, A. D., Tedstone, A. J., Telling, J., Bagshaw, E. A., Hawkings, J. R., Kohler, T. J., Zarsky, J. D., Mowlem, M. C., Anesio, A. M., & Stibal, M. (2019). Greenland melt drives continuous export of methane from the ice-sheet bed. *Nature*, *565*(7737), 73–77. https://doi.org/10.1038/s41586-018-0800-0

Langford, H. (2012). *The microstructure, biogeochemistry and aggregation of Arctic cryoconite granules.* University of Sheffield.

Langford, H., Hodson, A., Banwart, S., & Bøggild, C. (2010). The microstructure and biogeochemistry of Arctic cryoconite granules. *Annals of Glaciology*, *51*(56), 87–94. https://doi.org/10.3189/172756411795932083

Langford, H. J., Irvine-Fynn, T. D. L., Edwards, A., Banwart, S. A., & Hodson, A. J. (2014). A spatial investigation of the environmental controls over cryoconite aggregation on Longyearbreen glacier, Svalbard. *Biogeosciences*, *11*(19), 5365–5380.

Larsen, K. S., Jonasson, S., & Michelsen, A. (2002). Repeated freeze–thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology*, *21*(3), 187–195. https://doi.org/https://doi.org/10.1016/S0929-1393(02)00093-8

Lawson, E. C., Wadham, J. L., Tranter, M., Stibal, M., Lis, G. P., Butler, C. E. H., Laybourn-Parry, J., Nienow, P., Chandler, D., & Dewsbury, P. (2014). Greenland Ice Sheet exports labile organic carbon to the Arctic oceans. *Biogeosciences*, *11*(14), 4015–4028. https://doi.org/10.5194/bg-11-4015-2014

Lawson, Emily C, Bhatia, M. P., Wadham, J. L., & Kujawinski, E. B. (2014). Continuous summer export of nitrogen-rich organic matter from the Greenland Ice Sheet inferred by ultrahigh resolution mass spectrometry. *Environmental Science & Technology*, *48*(24), 14248–14257.

Lawson, Emily Charlotte. (2012). *Investigating Carbon Sourcing and Cycling in Subglacial Environments*. University of Bristol.

Lee, Y. J., Romanek, C. S., & Wiegel, J. (2009). Desulfosporosinus youngiae sp. nov., a sporeforming, sulfate-reducing bacterium isolated from a constructed wetland treating acid mine drainage. *International Journal of Systematic and Evolutionary Microbiology*, *59*(11). https://doi.org/10.1099/ijs.0.007336-0

Lee, Y. M., Kim, S.-Y., Jung, J., Kim, E. H., Cho, K. H., Schinner, F., Margesin, R., Hong, S. G., & Lee, H. K. (2011). Cultured bacterial diversity and human impact on alpine glacier cryoconite. *The Journal of Microbiology*, *49*(3), 355–362.

Legrand, M., Preunkert, S., Wagenbach, D., Cachier, H., & Puxbaum, H. (2003). A historical record of formate and acetate from a high-elevation Alpine glacier: Implications for their natural versus anthropogenic budgets at the European scale. *Journal of Geophysical Research: Atmospheres*, *108*(24). https://doi.org/10.1029/2003jd003594

Legrand, M, Preunkert, S., Jourdain, B., Guilhermet, J., Faïn, X., Alekhina, I., & Petit, J. R. (2013). Water-soluble organic carbon in snow and ice deposited at Alpine, Greenland, and Antarctic sites: a critical review of available data and their atmospheric relevance. *Past*, *9*, 2195–2211.

Legrand, Michel, & Mayewski, P. (1997). Glaciochemistry of polar ice cores: A review. In *Reviews of Geophysics* (Vol. 35, Issue 3). https://doi.org/10.1029/96RG03527

Leslie, A. (1879). *The Arctic Voyages of Adolf Erik Nordenskiöld: 1858-1879*. Macmillan and

Company.

Liu, Y., Vick-Majors, T. J., Priscu, J. C., Yao, T., Kang, S., Liu, K., Cong, Z., Xiong, J., & Li, Y. (2017). Biogeography of cryoconite bacterial communities on glaciers of the Tibetan Plateau. *FEMS Microbiology Ecology*, *93*(6). https://doi.org/10.1093/femsec/fix072

Luimstra, V. M., Schuurmans, J. M., Verschoor, A. M., Hellingwerf, K. J., Huisman, J., & Matthijs, H. C. P. (2018). Blue light reduces photosynthetic efficiency of cyanobacteria through an imbalance between photosystems I and II. *Photosynthesis Research*, *138*(2), 177–189. https://doi.org/10.1007/s11120-018-0561-5

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2015). Microbial diversity on Icelandic glaciers and ice caps. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00307

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2017). Linking microbial diversity and functionality of arctic glacial surface habitats. *Environmental Microbiology*, *19*(2), 551–565.

Lutz, S., Ziolkowski, L. A., & Benning, L. G. (2019). The biodiversity and geochemistry of cryoconite holes in queen maud land, East Antarctica. *Microorganisms*. https://doi.org/10.3390/microorganisms7060160

Lyons, W. B., Welch, K. A., Fountain, A. G., Dana, G. L., Vaughn, B. H., & McKnight, D. M. (2003). Surface glaciochemistry of Taylor Valley, southern Victoria Land, Antarctica and its relationship to stream chemistry. *Hydrological Processes*, *17*(1), 115–130.

Maccario, L., Sanguino, L., Vogel, T. M., & Larose, C. (2015). Snow and ice ecosystems: not so extreme. *Research in Microbiology*, *166*(10), 782–795. https://doi.org/https://doi.org/10.1016/j.resmic.2015.09.002

Macdonald, M. L., Wadham, J. L., Telling, J., & Skidmore, M. L. (2018). Glacial Erosion Liberates Lithologic Energy Sources for Microbes and Acidity for Chemical Weathering Beneath Glaciers and Ice Sheets . In *Frontiers in Earth Science* (Vol. 6, p. 212). https://www.frontiersin.org/article/10.3389/feart.2018.00212

Mader, H. M., Pettitt, M. E., Wadham, J. L., Wolff, E. W., & Parkes, R. J. (2006). Subsurface ice as a microbial habitat. *Geology*, *34*(3), 169–172. https://doi.org/10.1130/G22096.1

Madigan, M., Martinko, J., Bender, K., Buckley, D., & Stahl, D. (2012). *Brock Biology of Microorganisms 13th Edition*.

Mai-Prochnow, A., Clauson, M., Hong, J., & Murphy, A. B. (2016). Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. *Scientific Reports*. https://doi.org/10.1038/srep38610

Maier, R. M., & Pepper, I. L. (2015a). Bacterial Growth. In *Environmental Microbiology: Third Edition*. https://doi.org/10.1016/B978-0-12-394626-3.00003-X

Maier, R. M., & Pepper, I. L. (2015b). Chapter 3 - Bacterial Growth. In *Environmental Microbiology (Third edition)* (pp. 37–56). Academic Press. https://doi.org/http://dx.doi.org/10.1016/B978-0-12-394626-3.00003-X

Malard, L. A., Anwar, M. Z., Jacobsen, C. S., & Pearce, D. A. (2019). Biogeographical patterns in soil bacterial communities across the Arctic region. *FEMS Microbiology Ecology*, *95*(9). https://doi.org/10.1093/femsec/fiz128

Makhalanyane, T. P., Van Goethem, M. W., & Cowan, D. A. (2016). Microbial diversity and functional capacity in polar soils. In *Current Opinion in Biotechnology* (Vol. 38, pp. 159–166). Elsevier Ltd. https://doi.org/10.1016/j.copbio.2016.01.011

Makowska, N., Zawierucha, K., Nadobna, P., Piątek-Bajan, K., Krajewska, A., Szwedyk, J., Iwasieczko, P., Mokracka, J., & Koczura, R. (2020). Occurrence of integrons and antibiotic resistance genes in cryoconite and ice of Svalbard, Greenland, and the Caucasus glaciers. *Science of the Total Environment*, *716*. https://doi.org/10.1016/j.scitotenv.2020.137022

Margesin, R., & Fell, J. W. (2008). Mrakiella cryoconiti gen. nov., sp. nov., a psychrophilic, anamorphic, basidiomycetous yeast from alpine and arctic habitats. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *58*(12), 2977–2982. https://doi.org/10.1099/ijs.0.2008/000836-0

Margesin, Rosa, Fonteyne, P.-A., Schinner, F., & Sampaio, J. P. (2007). Rhodotorula psychrophila sp. nov., Rhodotorula psychrophenolica sp. nov. and Rhodotorula glacialis sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments. *International Journal of Systematic and Evolutionary Microbiology*, *57*(9), 2179–2184. https://doi.org/10.1099/ijs.0.65111-0

Margesin, Rosa, & Miteva, V. (2011). Diversity and ecology of psychrophilic microorganisms. *Research in Microbiology*, *162*(3), 346–361. http://ac.els-cdn.com/S0923250810002810/1-s2.0-S0923250810002810-main.pdf?_tid=40b217e2-9e92-11e5-8af3-00000aacb35d&acdnat=1449678849_133e423ec90dd30081cb07a838b8857d

Margesin, Rosa, Schumann, P., Zhang, D.-C., Redzic, M., Zhou, Y.-G., Liu, H.-C., & Schinner, F. (2012). Arthrobacter cryoconiti sp. nov., a psychrophilic bacterium isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *62*(2), 397–402. https://doi.org/doi:10.1099/ijs.0.031138-0

Margesin, Rosa, Spröer, C., Schumann, P., & Schinner, F. (2003). Pedobacter cryoconitis sp. nov., a facultative psychrophile from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *53*(5), 1291–1296. https://doi.org/doi:10.1099/ijs.0.02436-0

Margesin, Rosa, Spröer, C., Zhang, D.-C., & Busse, H.-J. (2012). Polaromonasglacialis sp. nov. and Polaromonas cryoconiti sp. nov., isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *62*(11), 2662–2668. https://doi.org/doi:10.1099/ijs.0.037556-0

Margesin, Rosa, Zacke, G., & Schinner, F. (2002). Characterization of heterotrophic microorganisms in alpine glacier cryoconite. *Arctic, Antarctic, and Alpine Research*, *34*(1), 88–93. https://doi.org/10.2307/1552512

Martens-Habbena, W., & Sass, H. (2006). Sensitive determination of microbial growth by nucleic acid staining in aqueous suspension. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.72.1.87-95.2006

Martinez, M. A., Woodcroft, B. J., Ignacio Espinoza, J. C., Zayed, A. A., Singleton, C. M., Boyd, J. A., Li, Y.-F., Purvine, S., Maughan, H., Hodgkins, S. B., Anderson, D., Sederholm, M., Temperton, B., Bolduc, B., Saleska, S. R., Tyson, G. W., Rich, V. I., Saleska, S. R., Tyson, G. W., & Rich, V. I. (2019). Discovery and ecogenomic context of a global Caldiserica-related phylum active in thawing permafrost, Candidatus Cryosericota phylum nov., Ca. Cryosericia class nov., Ca. Cryosericales ord. nov., Ca. Cryosericaceae fam. nov., comprising the four species C. *Systematic and Applied Microbiology*, *42*(1), 54–66. https://doi.org/https://doi.org/10.1016/j.syapm.2018.12.003

Masclet, P., Hoyau, V., Jaffrezo, J. L., & Cachier, H. (2000). Polycyclic aromatic hydrocarbon deposition on the ice sheet of Greenland. Part I: superficial snow. *Atmospheric Environment*, *34*(19), 3195–3207.

McCrimmon, D. O., Bizimis, M., Holland, A., & Ziolkowski, L. A. (2018). Supraglacial microbes use young carbon and not aged cryoconite carbon. *Organic Geochemistry*, *118*, 63–72. https://doi.org/10.1016/j.orggeochem.2017.12.002

McIntyre, N. F. (1984). Cryoconite hole thermodynamics. *Canadian Journal of Earth Sciences*, *21*(2), 152–156. https://doi.org/10.1139/e84-016

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Meneghel, J., Passot, S., Dupont, S., & Fonseca, F. (2017). Biophysical characterization of the Lactobacillus delbrueckii subsp. bulgaricus membrane during cold and osmotic stress and its relevance for cryopreservation. *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-016-7935-4

Methé, B. A., Nelson, K. E., Eisen, J. A., Paulsen, I. T., Nelson, W., Heidelberg, J. F., Wu, D., Wu, M., Ward, N., Beanan, M. J., Dodson, R. J., Madupu, R., Brinkac, L. M., Daugherty, S. C., DeBoy, R. T., Durkin, A. S., Gwinn, M., Kolonay, J. F., Sullivan, S. A., … Fraser, C. M. (2003). Genome of Geobacter sulfurreducens: Metal Reduction in Subsurface Environments. *Science*, *302*(5652). https://doi.org/10.1126/science.1088727

Michaud, L., Caruso, C., Mangano, S., Interdonato, F., Bruni, V., & Lo Giudice, A. (2012). Predominance of Flavobacterium, Pseudomonas, and Polaromonas within the prokaryotic community of freshwater shallow lakes in the northern Victoria Land, East Antarctica. *FEMS Microbiology Ecology*. https://doi.org/10.1111/j.1574-6941.2012.01394.x

Mikucki, J. A., Pearson, A., Johnston, D. T., Turchyn, A. V, Farquhar, J., Schrag, D. P., Anbar, A. D., Priscu, J. C., & Lee, P. A. (2009). A Contemporary Microbially Maintained Subglacial Ferrous "Ocean". *Science*, *324*(5925), 397–400. http://www.sciencemag.org/content/324/5925/397.full.pdf

Mori, K., Yamaguchi, K., Sakiyama, Y., Urabe, T., & Suzuki, K. -i. (2009). Caldisericum exile gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel bacterial phylum, Caldiserica phyl. nov., originally called the candidate phylum OP5, and description of Caldisericaceae fam. nov., Caldisericales ord. nov. and Caldisericia classis nov. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *59*(11), 2894–2898. https://doi.org/10.1099/ijs.0.010033-0

Moyer, C. L., & Morita, R. Y. (2007). Psychrophiles and psychrotrophs. *ELS*.

Mueller, D. R., Vincent, W. F., Pollard, W. H., & Fritsen, C. H. (2001). Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats. *Nova Hedwigia Beiheft*, *123*, 173–198.

Muñoz, P. A., Márquez, S. L., González-Nilo, F. D., Márquez-Miranda, V., & Blamey, J. M. (2017). Structure and application of antifreeze proteins from Antarctic bacteria. *Microbial Cell Factories*. https://doi.org/10.1186/s12934-017-0737-2

Musilova, M., Tranter, M., Bamber, J. L., Takeuchi, N., & Anesio, A. (2016). Experimental evidence that microbial activity lowers the albedo of glaciers. *Geochemical Perspectives Letters*, *2*(2), 106–116. https://doi.org/10.7185/geochemlet.1611

Musilova, M, Tranter, M., Bennett, S. A., Wadham, J., & Anesio, A. M. (2015). Stable microbial community composition on the Greenland Ice Sheet. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/fmicb.2015.00193

Musilova, Michaela, Tranter, M., Wadham, J., Telling, J., Tedstone, A., & Anesio, A. M. (2017). Microbially driven export of labile organic carbon from the Greenland ice sheet. *Nature Geoscience*, *10*(5), 360–365. https://doi.org/10.1038/ngeo2920

Nemergut, D. R., Costello, E. K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S. K., Fierer, N., Townsend, A. R., Cleveland, C. C., Stanish, L., & Knight, R. (2011). Global patterns in the biogeography of bacterial taxa. *Environmental Microbiology*, *13*(1). https://doi.org/10.1111/j.1462-2920.2010.02315.x

Nichols, C. M., Lardière, S. G., Bowman, J. P., Nichols, P. D., Gibson, J. A. E., & Guézennec, J. (2005). Chemical characterization of exopolysaccharides from Antarctic marine bacteria. *Microbial Ecology*. https://doi.org/10.1007/s00248-004-0093-8

Nixon, S. L., Telling, J. P., Wadham, J. L., & Cockell, C. S. (2017). Viable cold-tolerant iron-reducing microorganisms in geographically diverse subglacial environments. *Biogeosciences*, *14*(6). https://doi.org/10.5194/bg-14-1445-2017

O'Donnell, E. C., Wadham, J. L., Lis, G. P., Tranter, M., Pickard, A. E., Stibal, M., Dewsbury, P., & Fitzsimons, S. (2016). Identification and analysis of low-molecular-weight dissolved organic carbon in subglacial basal ice ecosystems by ion chromatography. *Biogeosciences*, *13*(12). https://doi.org/10.5194/bg-13-3833-2016

Odland, J. Ø., Donaldson, S., Dudarev, A., & Carlsen, A. (2016). AMAP assessment 2015: human health in the Arctic. *International Journal of Circumpolar Health*, *75*.

Pannard, A., Pédrono, J., Bormans, M., Briand, E., Claquin, P., & Lagadeuc, Y. (2016). Production of exopolymers (EPS) by cyanobacteria: impact on the carbon-to-nutrient ratio of the particulate organic matter. *Aquatic Ecology*, *50*(1). https://doi.org/10.1007/s10452-015-

9550-3

Park, J. I., Grant, C. M., Attfield, P. V., & Dawes, I. W. (1997). The freeze-thaw stress response of the yeast Saccharomyces cerevisiae is growth phase specific and is controlled by nutritional state via the RAS- Cyclic AMP signal transduction pathway. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.63.10.3818-3824.1997

Parkes, R. J., & Sass, H. (2009). *Deep Sub-Surface* (M. B. T.-E. of M. (Third E. Schaechter (ed.); pp. 64–79). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012373944-5.00275-3

Parkes, R. J., Taylor, J., & Jørck-Ramberg, D. (1984). Demonstration, using Desulfobacter sp., of two pools of acetate with different biological availabilities in marine pore water. *Marine Biology*, *83*(3). https://doi.org/10.1007/BF00397459

Passow, U. (2002). Transparent exopolymer particles (TEP) in aquatic environments. In *Progress in Oceanography* (Vol. 55, Issues 3–4). https://doi.org/10.1016/S0079-6611(02)00138-6

Paul, E. A. (2006). Soil microbiology, ecology and biochemistry: Third edition. In *Soil Microbiology, Ecology and Biochemistry: Third Edition*. https://doi.org/10.1016/C2009-0-02816-5

Paul, M. J. (2013). *Photosynthetic Carbon Dioxide Fixation* (W. J. Lennarz & M. D. B. T.-E. of B. C. (Second E. Lane (eds.); pp. 497–502). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-378630-2.00050-5

Pautler, B. G., Dubnick, A., Sharp, M. J., Simpson, A. J., & Simpson, M. J. (2013). Comparison of cryoconite organic matter composition from Arctic and Antarctic glaciers at the molecular-level. *Geochimica et Cosmochimica Acta*, *104*, 1–18. https://doi.org/https://doi.org/10.1016/j.gca.2012.11.029

Peeters, K., & Willems, A. (2011). The gyrB gene is a useful phylogenetic marker for exploring the diversity of Flavobacterium strains isolated from terrestrial and aquatic habitats in Antarctica. *FEMS Microbiology Letters*. https://doi.org/10.1111/j.1574-6968.2011.02326.x

Pegg, D. E. (2007). Principles of cryopreservation. In *Methods in molecular biology (Clifton, N.J.)* (Vol. 368, pp. 39–57). https://doi.org/10.1007/978-1-59745-362-2_3

Perkins, R. G., Bagshaw, E., Mol, L., Williamson, C. J., Fagan, D., Gamble, M., & Yallop, M. L. (2017). Photoacclimation by arctic cryoconite phototrophs. *FEMS Microbiology Ecology*. https://doi.org/10.1093/femsec/fix018

Perkins, R., Williamson, C., Lavaud, J., Mouget, J.-L., & Campbell, D. A. (2018). Time-dependent upregulation of electron transport with concomitant induction of regulated excitation dissipation in Haslea diatoms. *Photosynthesis Research*, *137*(3), 377–388. https://doi.org/10.1007/s11120-018-0508-x

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., & Amann, R. (1998). Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Applied and Environmental Microbiology*, *64*(11). https://doi.org/10.1128/aem.64.11.4299-4306.1998

Petchey, O. L., & Gaston, K. J. (2006). Functional diversity: Back to basics and looking forward. In *Ecology Letters* (Vol. 9, Issue 6, pp. 741–758). https://doi.org/10.1111/j.1461-0248.2006.00924.x

Peters, V., & Conrad, R. (1995). Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Applied and Environmental Microbiology*, *61*(4), 1673–1676.

Pham, V. H. T., & Kim, J. (2012). Cultivation of unculturable soil bacteria. *Trends in Biotechnology*, *30*(9), 475–484. https://doi.org/https://doi.org/10.1016/j.tibtech.2012.05.007

Pinhal, S., Ropers, D., Geiselmann, J., & De Jong, H. (2019). Acetate metabolism and the inhibition of bacterial growth by acetate. *Journal of Bacteriology*, *201*(13). https://doi.org/10.1128/JB.00147-19

Pittino, F., Maglio, M., Gandolfi, I., Azzoni, R. S., Diolaiuti, G., Ambrosini, R., & Franzetti, A. (2018a). Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. *Annals of Glaciology*, *59*(77), 1–9. https://doi.org/DOI: 10.1017/aog.2018.16

Pittino, F., Maglio, M., Gandolfi, I., Azzoni, R. S., Diolaiuti, G., Ambrosini, R., & Franzetti, A. (2018b). Bacterial communities of cryoconite holes of a temperate alpine glacier show both seasonal trends and year-to-year variability. *Annals of Glaciology*, *59*(77). https://doi.org/10.1017/aog.2018.16

Ploug, H., Kühl, M., Buchholz-Cleven, B., & Jørgensen, B. B. (1997). Anoxic aggregates-an ephemeral phenomenon in the pelagic environment? *Aquatic Microbial Ecology*, *13*(3), 285–294.

Poage, M. A., Barrett, J. E., Virginia, R. A., & Wall, D. H. (2008). The influence of soil

geochemistry on nematode distribution, Mcmurdo Dry Valleys, Antarctica. *Arctic, Antarctic, and Alpine Research*, *40*(1), 119–128. https://doi.org/10.1657/1523-0430(06-051)[POAGE]2.0.CO;2

Poli, A., Anzelmo, G., & Nicolaus, B. (2010). Bacterial exopolysaccharides from extreme marine habitats: Production, characterization and biological activities. In *Marine Drugs*. https://doi.org/10.3390/md8061779

Poniecka, E.A., Bagshaw, E. A., Tranter, M., Sass, H., Williamson, C. J., & Anesio, A. M. (2018). Rapid development of anoxic niches in supraglacial ecosystems. *Arctic, Antarctic, and Alpine Research*, *50*(1). https://doi.org/10.1080/15230430.2017.1420859

Poniecka, Ewa A, Bagshaw, E. A., Sass, H., Segar, A., Webster, G., Williamson, C., Anesio, A. M., & Tranter, M. (2020). Physiological Capabilities of Cryoconite Hole Microorganisms . In *Frontiers in Microbiology* (Vol. 11, p. 1783). https://www.frontiersin.org/article/10.3389/fmicb.2020.01783

Porazinska, D. L., Fountain, A. G., Nylen, T. H., Tranter, M., Virginia, R. A., & Wall, D. H. (2004). The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. *Arctic, Antarctic, and Alpine Research*, *36*(1), 84–91. https://doi.org/10.1657/1523-0430(2004)036[0084:TBABOC]2.0.CO;2

Poretsky, R., Rodriguez-R, L. M., Luo, C., Tsementzi, D., & Konstantinidis, K. T. (2014). Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0093827

Pörtner, H., Roberts, D. C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., Algeria, A., Nicolai, M., Okem, A., Petzold, J., Rama, B., & Weyer, N. M. (2019). Summary for Policymakers. In: IPCC Special Report on the Ocean and Cryosphere in a changing Climate. In *IPCC Report 2019* (Issues vii, 973). https://doi.org/http://www.ipcc.ch/publications_and_data/ar4/wg2/en/spm.html

Price, P. B. (2000). A habitat for psychrophiles in deep Antarctic ice. *Proceedings of the National Academy of Sciences*, *97*(3), 1247–1251. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC15584/pdf/pq001247.pdf

Price, P. B., & Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4631 LP – 4636. https://doi.org/10.1073/pnas.0400522101

Priscu, J. C., Tulaczyk, S., Studinger, M., Kennicutt, M. C., Christner, B. C., & Foreman, C. M. (2009). Antarctic subglacial water: Origin, evolution, and ecology. In *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems*. https://doi.org/10.1093/acprof:oso/9780199213887.003.0007

Puttaswamygowda, G. H., Olakkaran, S., Antony, A., & Kizhakke Purayil, A. (2019). *Chapter 22 - Present Status and Future Perspectives of Marine Actinobacterial Metabolites* (V. B. T.-R. D. in A. M. and B. Buddolla (ed.); pp. 307–319). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-816328-3.00022-2

Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, *82*(3), 222–237. https://doi.org/https://doi.org/10.1016/j.aquabot.2005.02.006

Ramamoorthy, S., Sass, H., Langner, H., Schumann, P., Kroppenstedt, R. M., Spring, S., Overmann, J., & Rosenzweig, R. F. (2006). Desulfosporosinus lacus sp. nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *International Journal of Systematic and Evolutionary Microbiology*, *56*(12), 2729–2736. https://doi.org/10.1099/ijs.0.63610-0

Raymond, J. A. (2016). Dependence on epiphytic bacteria for freezing protection in an Antarctic moss, Bryum argenteum. *Environmental Microbiology Reports*. https://doi.org/10.1111/1758-2229.12337

Rivkina, E. M., Friedmann, E. I., McKay, C. P., & Gilichinsky, D. A. (2000). Metabolic activity of Permafrost Bacteria below the freezing point. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.66.8.3230-3233.2000

Roe, A. J., O'Byrne, C., McLaggan, D., & Booth, I. R. (2002). Inhibition of Escherichia coli growth by acetic acid: A problem with methionine biosynthesis and homocysteine toxicity. *Microbiology*, *148*(7). https://doi.org/10.1099/00221287-148-7-2215

Rosenberg, E., De Long, E. F., Lory, S., Stackebrandt, E., & Thompson, F. (2014). The prokaryotes: Other major lineages of bacteria and the archaea. In *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*. https://doi.org/10.1007/978-3-642-38954-2

Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E., & Thompson, F. (2014). The prokaryotes: Firmicutes and tenericutes. In *The Prokaryotes: Firmicutes and Tenericutes* (Vol. 9783642301209). https://doi.org/10.1007/978-3-642-30120-9

Rosselló-Mora, R., & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiology Reviews*. https://doi.org/10.1016/S0168-6445(00)00040-1

Russell, J. B., & Diez-Gonzalez, F. (1998). The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology*, *39*. https://doi.org/10.1016/s0065-2911(08)60017-x

Samui, G., Antony, R., Mahalinganathan, K., & Thamban, M. (2017). Spatial variability and possible sources of acetate and formate in the surface snow of East Antarctica. *Journal of Environmental Sciences*, *57*, 258–269. https://doi.org/https://doi.org/10.1016/j.jes.2017.02.003

Samui, G., Antony, R., & Thamban, M. (2018). Chemical characteristics of hydrologically distinct cryoconite holes in coastal Antarctica. *Annals of Glaciology*, *59*(77). https://doi.org/10.1017/aog.2018.30

Samui, G. D. (2019). *Biogeochemistry of selected supraglacial ecosystems in coastal Antarctica in Marine Science* (Issue August). Goa University, India.

Sandle, T. (2019). *Chapter 11 - Special Types of Environmental Monitoring* (T. B. T.-B. C. for P. and H. Sandle (ed.); pp. 179–197). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-814911-9.00011-0

Sanyal, A., Antony, R., Samui, G., & Thamban, M. (2018). Microbial communities and their potential for degradation of dissolved organic carbon in cryoconite hole environments of Himalaya and Antarctica. *Microbiological Research*. https://doi.org/10.1016/j.micres.2018.01.004

Sass, A. M., Eschemann, A., Kühl, M., Thar, R., Sass, H., & Cypionka, H. (2002). Growth and chemosensory behavior of sulfate-reducing bacteria in oxygen—sulfide gradients. *FEMS Microbiology Ecology*, *40*(1), 47–54.

Sass, H., Cypionka, H., & Babenzien, H. D. (1997). Vertical distribution of sulfate-reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic Lake Stechlin. *FEMS Microbiology Ecology*. https://doi.org/10.1016/S0168-6496(96)00096-7

Sathiyanarayanan, G., Yi, D. H., Bhatia, S. K., Kim, J. H., Seo, H. M., Kim, Y. G., Park, S. H., Jeong, D., Jung, S., Jung, J. Y., Lee, Y. K., & Yang, Y. H. (2015). Exopolysaccharide from psychrotrophic Arctic glacier soil bacterium Flavobacterium sp. ASB 3-3 and its potential applications. *RSC Advances*. https://doi.org/10.1039/c5ra14978a

Sattler, B., & Storrie-Lombardi, M. C. (2009). L.I.F.E. in Antarctic Lakes. *Polar Microbiology: The*

Ecology, Biodiversity and Bioremediation Potential of Microorganisms in Extremely Cold Environments, 95.

Säwström, C., Mumford, P., Marshall, W., Hodson, A., & Laybourn-Parry, J. (2002). The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79 N). *Polar Biology*, *25*(8), 591–596.

Schimel, J. P., & Mikan, C. (2005). Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biology and Biochemistry*, *37*(8), 1411–1418. https://doi.org/https://doi.org/10.1016/j.soilbio.2004.12.011

Schmidt, S. K., Nemergut, D. R., Todd, B. T., Lynch, R. C., Darcy, J. L., Cleveland, C. C., & King, A. J. (2012). A simple method for determining limiting nutrients for photosynthetic crusts. *Plant Ecology and Diversity*, *5*(4). https://doi.org/10.1080/17550874.2012.738714

Schramm, A., Santegoeds, C. M., Nielsen, H. K., Ploug, H., Wagner, M., Pribyl, M., Wanner, J., Amann, R., & de Beer, D. (1999). On the occurrence of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge. *Applied and Environmental Microbiology*, *65*(9), 4189–4196.

Schumann, P., Zhang, D.-C., Redzic, M., & Margesin, R. (2012). Alpinimonas psychrophila gen. nov., sp. nov., an actinobacterium of the family Microbacteriaceae isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *62*(11), 2724–2730. https://doi.org/doi:10.1099/ijs.0.036160-0

Segawa, T., Ishii, S., Ohte, N., Akiyoshi, A., Yamada, A., Maruyama, F., Li, Z., Hongoh, Y., & Takeuchi, N. (2014). The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environmental Microbiology*, *16*(10), 3250–3262. http://onlinelibrary.wiley.com/store/10.1111/1462-2920.12543/asset/emi12543.pdf?v=1&t=ihg4p1l4&s=4ac8c5ea0fed9e277bcf82484ac80449621f3f3f

Segawa, T., Matsuzaki, R., Takeuchi, N., Akiyoshi, A., Navarro, F., Sugiyama, S., Yonezawa, T., & Mori, H. (2018). Bipolar dispersal of red-snow algae. *Nature Communications*, *9*(1), 3094. https://doi.org/10.1038/s41467-018-05521-w

Shin, S. C., Kim, S. J., Hong, S. G., Ahn, D. H., Lee, Y. M., Lee, H., Lee, J., & Park, H. (2012). Genome sequence of Pseudomonas sp. Strain PAMC 25886, isolated from alpine glacial cryoconite. In *Journal of Bacteriology* (Vol. 194, Issue 7). https://doi.org/10.1128/JB.00057-12

Siegert, M. J., Clarke, R. J., Mowlem, M., Ross, N., Hill, C. S., Tait, A., Hodgson, D., Parnell, J., Tranter, M., Pearce, D., Bentley, M. J., Cockell, C., Tsaloglou, M.-N., Smith, A., Woodward, J., Brito, M. P., & Waugh, E. (2012). Clean access, measurement, and sampling of Ellsworth Subglacial Lake: A method for exploring deep Antarctic subglacial lake environments. *Reviews of Geophysics*, *50*(1). https://doi.org/10.1029/2011RG000361

Singh, P, Hanada, Y., Singh, S. M., & Tsuda, S. (2014). Antifreeze protein activity in Arctic cryoconite bacteria. *FEMS Microbiology Letters*, *351*(1), 14–22. https://doi.org/10.1111/1574-6968.12345

Singh, P, Singh, S. M., & Dhakephalkar, P. (2014). Diversity, cold active enzymes and adaptation strategies of bacteria inhabiting glacier cryoconite holes of High Arctic. *Extremophiles*, *18*(2), 229–242. https://doi.org/10.1007/s00792-013-0609-6

Singh, Purnima, & Singh, S. M. (2012). Characterization of yeast and filamentous fungi isolated from cryoconite holes of Svalbard, Arctic. *Polar Biology*. https://doi.org/10.1007/s00300-011-1103-1

Singh, Purnima, Singh, S. M., Tsuji, M., Prasad, G. S., & Hoshino, T. (2014). Rhodotorula svalbardensis sp. nov., a novel yeast species isolated from cryoconite holes of Ny-Ålesund, Arctic. *Cryobiology*, *68*(1), 122–128. https://doi.org/https://doi.org/10.1016/j.cryobiol.2014.01.006

Singh, Purnima, Tsuji, M., Singh, S. M., & Takeuchi, N. (2020). Contrasting Patterns of Microbial Communities in Glacier Cryoconite of Nepali Himalaya and Greenland, Arctic. *Sustainability*, *12*(16), 6477. https://doi.org/10.3390/su12166477

Skidmore, M., Anderson, S. P., Sharp, M., Foght, J., & Lanoil, B. D. (2005). Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Applied and Environmental Microbiology*, *71*(11), 6986–6997. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1287656/pdf/0569-05.pdf

Smeets, P. C. J. P., Kuipers Munneke, P., van As, D., van den Broeke, M. R., Boot, W., Oerlemans, H., Snellen, H., Reijmer, C. H., & van de Wal, R. S. W. (2018). The K-transect in west Greenland: Automatic weather station data (1993–2016). *Arctic, Antarctic, and Alpine Research*. https://doi.org/10.1080/15230430.2017.1420954

Smith, S. (2019). phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. *Journal of Open Source Software*, *4*(38).

https://doi.org/10.21105/joss.01442

Smith, H. J., Foster, R. A., McKnight, D. M., Lisle, J. T., Littmann, S., Kuypers, M. M. M., & Foreman, C. M. (2017). Microbial formation of labile organic carbon in Antarctic glacial environments. *Nature Geoscience*, *10*(5), 356–359. https://doi.org/10.1038/ngeo2925

Sommaruga, R., & Casamayor, E. O. (2009). Bacterial "cosmopolitanism" and importance of local environmental factors for community composition in remote high-altitude lakes. *Freshwater Biology*, *54*(5). https://doi.org/10.1111/j.1365-2427.2008.02146.x

Sommers, P., Darcy, J. L., Gendron, E. M. S., Stanish, L. F., Bagshaw, E. A., Porazinska, D. L., & Schmidt, S. K. (2018). Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes. *FEMS Microbiology Ecology*, *94*(1). https://doi.org/10.1093/femsec/fix167

Speirs, L. B. M., Rice, D. T. F., Petrovski, S., & Seviour, R. J. (2019). The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. In *Frontiers in Microbiology* (Vol. 10). https://doi.org/10.3389/fmicb.2019.02015

Spring, S., Lapidus, A., Schröder, M., Gleim, D., Sims, D., Meincke, L., del Rio, T. G., Tice, H., Copeland, A., Cheng, J. F., Lucas, S., Chen, F., Nolan, M., Bruce, D., Goodwin, L., Pitluck, S., Ivanova, N., Mavromatis, K., Mikhailova, N., … Han, C. (2009). Complete genome sequence of Desulfotomaculum acetoxidans type strain (5575 T). *Standards in Genomic Sciences*, *1*(3), 242–253. https://doi.org/10.4056/sigs.39508

Srivastava, N., Gupta, B., Gupta, S., Danquah, M. K., & Sarethy, I. P. (2019). Analyzing Functional Microbial Diversity: An Overview of Techniques. *Microbial Diversity in the Genomic Era*. https://doi.org/10.1016/B978-0-12-814849-5.00006-X

Stal, L. J., & Moezelaar, R. (1997). Fermentation in cyanobacteria. In *FEMS Microbiology Reviews* (Vol. 21, Issue 2). https://doi.org/10.1016/S0168-6445(97)00056-9

Stanier, R. Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriological Reviews*, *35*(2). https://doi.org/10.1128/mmbr.35.2.171-205.1971

Stanish, L. F., Bagshaw, E. A., McKnight, D. M., Fountain, A. G., & Tranter, M. (2013). Environmental factors influencing diatom communities in Antarctic cryoconite holes. *Environmental Research Letters*, *8*(4). https://doi.org/10.1088/1748-9326/8/4/045006

Stanish, Lee F, Bagshaw, E. A., McKnight, D. M., Fountain, A. G., & Tranter, M. (2013).

Environmental factors influencing diatom communities in Antarctic cryoconite holes. *Environmental Research Letters*, *8*(4), 45006.

Steinbock, O. (1936). Uber Kryokonitlocher und ihre biologische Bedeutung. *Zeitschrift Fur Gletscherkunde*, *24*.

Stewart, E. J. (2012). Growing Unculturable Bacteria. *Journal of Bacteriology*, *194*(16), 4151 LP – 4160. https://doi.org/10.1128/JB.00345-12

Stibal, M, Anesio, A. M., Blues, C. J. D., & Tranter, M. (2009). Phosphatase activity and organic phosphorus turnover on a high Arctic glacier. *Biogeosciences*, *6*(5), 913–922.

Stibal, Marek, Hasan, F., Wadham, J. L., Sharp, M. J., & Anesio, A. M. (2012). Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates. *Extremophiles*, *16*(2). https://doi.org/10.1007/s00792-011-0426-8

Stibal, Marek, Šabacká, M., & Kaštovská, K. (2006). Microbial communities on glacier surfaces in Svalbard: Impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microbial Ecology*, *52*(4), 644–654. https://doi.org/10.1007/s00248-006-9083-3

Stibal, Marek, Šabacká, M., & Žárský, J. (2012). Biological processes on glacier and ice sheet surfaces. *Nature Geoscience*, *5*(11), 771–774.

Stibal, Marek, Schostag, M., Cameron, K. A., Hansen, L. H., Chandler, D. M., Wadham, J. L., & Jacobsen, C. S. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environmental Microbiology Reports*, *7*(2), 293–300. https://doi.org/10.1111/1758-2229.12246

Stibal, Marek, & Tranter, M. (2007). Laboratory investigation of inorganic carbon uptake by cryoconite debris from Werenskioldbreen, Svalbard. *Journal of Geophysical Research: Biogeosciences*, *112*(G4).

Stibal, Marek, Tranter, M., Benning, L. G., & Řehák, J. (2008). Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environmental Microbiology*, *10*(8), 2172–2178. https://doi.org/10.1111/j.1462-2920.2008.01620.x

Stibal, Marek, Wadham, J. L., Lis, G. P., Telling, J., Pancost, R. D., Dubnick, A., Sharp, M. J., Lawson, E. C., Butler, C. E. H., Hasan, F., Tranter, M., & Anesio, A. M. (2012). Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting

organic carbon sources. *Global Change Biology*, *18*(11). https://doi.org/10.1111/j.1365-2486.2012.02763.x

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M. M. B., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., & Midgley, P. M. (2013). Climate change 2013 the physical science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change. In *Climate Change 2013 the Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. https://doi.org/10.1017/CBO9781107415324

Süß, J., Herrmann, K., Seidel, M., Cypionka, H., Engelen, B., & Sass, H. (2008). Two distinct Photobacterium populations thrive in ancient Mediterranean sapropels. *Microbial Ecology*. https://doi.org/10.1007/s00248-007-9282-6

Takeuchi, N., Kohshima, S., & Seko, K. (2001). Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. *Arctic, Antarctic, and Alpine Research*, *33*(2), 115–122.

Taylor, B. L., Zhulin, I. B., & Johnson, M. S. (1999). Aerotaxis and other energy-sensing behavior in bacteria. *Annual Reviews in Microbiology*, *53*(1), 103–128.

Tedesco, M., Fettweis, X., Van den Broeke, M. R., Van de Wal, R. S. W., Smeets, C., van de Berg, W. J., Serreze, M. C., & Box, J. E. (2011). The role of albedo and accumulation in the 2010 melting record in Greenland. *Environmental Research Letters*, *6*(1), 14005.

Telling, J, Boyd, E. S., Bone, N., Jones, E. L., Tranter, M., MacFarlane, J. W., Martin, P. G., Wadham, J. L., Lamarche-Gagnon, G., Skidmore, M. L., Hamilton, T. L., Hill, E., Jackson, M., & Hodgson, D. A. (2015). Rock comminution as a source of hydrogen for subglacial ecosystems. *Nature Geoscience*, *8*(11), 851–855. https://doi.org/10.1038/ngeo2533

Telling, J, Stibal, M., Anesio, A. M., Tranter, M., Nias, I., Cook, J., Bellas, C., Lis, G., Wadham, J. L., & Sole, A. (2012). Microbial nitrogen cycling on the Greenland Ice Sheet. *Biogeosciences*, *9*(7), 2431–2442.

Telling, Jon, Anesio, A. M., Tranter, M., Fountain, A. G., Nylen, T., Hawkings, J., Singh, V. B., Kaur, P., Musilova, M., & Wadham, J. L. (2014). Spring thaw ionic pulses boost nutrient availability and microbial growth in entombed Antarctic Dry Valley cryoconite holes. *Frontiers in Microbiology*, *5*. https://doi.org/10.3389/fmicb.2014.00694

Telling, Jon, Anesio, A. M., Tranter, M., Irvine-Fynn, T., Hodson, A., Butler, C., & Wadham, J.

(2011). Nitrogen fixation on Arctic glaciers, Svalbard. *Journal of Geophysical Research: Biogeosciences*, *116*(G3).

Telling, Jon, Anesio, A. M., Tranter, M., Stibal, M., Hawkings, J., Irvine-Fynn, T., Hodson, A., Butler, C., Yallop, M., & Wadham, J. (2012). Controls on the autochthonous production and respiration of organic matter in cryoconite holes on high Arctic glaciers. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, *117*(G1).

Tessarolli, L. P., Bagatini, I. L., Bianchini-Jr., I., & Vieira, A. A. H. (2017). Bacterial degradation of dissolved organic matter released by Planktothrix agardhii (Cyanobacteria). *Brazilian Journal of Biology*, *78*(1). https://doi.org/10.1590/1519-6984.07616

Thomas, J. D. (1997). The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. In *Freshwater Biology* (Vol. 38, Issue 1). https://doi.org/10.1046/j.1365-2427.1997.00206.x

Tranter, M, Sharp, M. J., Lamb, H. R., Brown, G. H., Hubbard, B. P., & Willis, I. C. (2002). Geochemical weathering at the bed of Haut Glacier d'Arolla, Switzerland—a new model. *Hydrological Processes*, *16*(5), 959–993.

Tranter, Martyn. (2014). Biogeochemistry: Microbes eat rock under ice. *Nature*, *512*(7514), 256–257. http://www.nature.com/nature/journal/v512/n7514/pdf/512256a.pdf

Tranter, Martyn, Fountain, A. G., Fritsen, C. H., Lyons, W. B., Priscu, J. C., Statham, P. J., & Welch, K. A. (2004). Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. *Hydrological Processes*, *18*(2), 379–387. https://doi.org/10.1002/hyp.5217

Ueki, A., Akasaka, H., Suzuki, D., & Ueki, K. (2006). Paludibacter propionicigenes gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *56*(1), 39–44. https://doi.org/10.1099/ijs.0.63896-0

Uetake, J., Tanaka, S., Segawa, T., Takeuchi, N., Nagatsuka, N., Motoyama, H., & Aoki, T. (2016). Microbial community variation in cryoconite granules on Qaanaaq Glacier, NW Greenland. *FEMS Microbiology Ecology*, *92*(9), fiw127. https://doi.org/10.1093/femsec/fiw127

Ul-Hassan, A., & Wellington, E. M. (2009). *Actinobacteria* (M. B. T.-E. of M. (Third E. Schaechter (ed.); pp. 25–44). Academic Press. https://doi.org/https://doi.org/10.1016/B978-

012373944-5.00044-4

Vartoukian, S. R., Palmer, R. M., & Wade, W. G. (2010). Strategies for culture of 'unculturable' bacteria. *FEMS Microbiology Letters*, *309*(1), 1–7. https://doi.org/10.1111/j.1574-6968.2010.02000.x

Vick-Majors, T. J., Priscu, J. C., & Amaral-Zettler, L. A. (2014). Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *ISME Journal*. https://doi.org/10.1038/ismej.2013.190

Vincent, W F, Gibson, J. A. E., Pienitz, R., Villeneuve, V., Broady, P. A., Hamilton, P. B., & Howard-Williams, C. (2000). Ice shelf microbial ecosystems in the high Arctic and implications for life on snowball Earth. *Naturwissenschaften*, *87*(3), 137–141.

Vincent, Warwick F., & Laybourn-Parry, J. (2009). Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems. In *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199213887.001.0001

Wadham, J. L., Bottrell, S., Tranter, M., & Raiswell, R. (2004). Stable isotope evidence for microbial sulphate reduction at the bed of a polythermal high Arctic glacier. *Earth and Planetary Science Letters*, *219*(3–4). https://doi.org/10.1016/S0012-821X(03)00683-6

Wadham, J. L., Hawkings, J. R., Tarasov, L., Gregoire, L. J., Spencer, R. G. M., Gutjahr, M., Ridgwell, A., & Kohfeld, K. E. (2019). Ice sheets matter for the global carbon cycle. In *Nature Communications* (Vol. 10, Issue 1). https://doi.org/10.1038/s41467-019-11394-4

Wadham, J. L., Tranter, M., Tulaczyk, S., & Sharp, M. (2008). Subglacial methanogenesis: A potential climatic amplifier? *Global Biogeochemical Cycles*, *22*(2). https://doi.org/10.1029/2007GB002951

Wadham, Jemma Louise, Hawkings, J., Telling, J., Chandler, D., Alcock, J., O'Donnell, E., Kaur, P., Bagshaw, E., Tranter, M., Tedstone, A., & Nienow, P. (2016). Sources, cycling and export of nitrogen on the Greenland Ice Sheet. *Biogeosciences*, *13*(22). https://doi.org/10.5194/bg-13-6339-2016

Wand, U., Schwarz, G., Brüggemann, E., & Bräuer, K. (1997). Evidence for physical and chemical stratification in Lake Untersee (central Dronning Maud Land, East Antarctica). *Antarctic Science*, *9*(01), 43–45.

Webster-Brown, J. G., Hawes, I., Jungblut, A. D., Wood, S. A., & Christenson, H. K. (2015). The

effects of entombment on water chemistry and bacterial assemblages in closed cryoconite holes on Antarctic glaciers. *FEMS Microbiology Ecology*, fiv144.

Webster, G., John Parkes, R., Cragg, B. A., Newberry, C. J., Weightman, A. J., & Fry, J. C. (2006). Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. *FEMS Microbiology Ecology*. https://doi.org/10.1111/j.1574-6941.2006.00147.x

Weidick, A. (1995). Satellite image atlas of glaciers of the world: Greenland. *US Geological Survey Professional Paper*, *1386 C*.

Weiland-Bräuer, N., Fischer, M. A., Schramm, K. W., & Schmitz, R. A. (2017). Polychlorinated biphenyl (PCB)-degrading potential of microbes present in a cryoconite of Jamtalferner glacier. *Frontiers in Microbiology*, *8*(JUN). https://doi.org/10.3389/fmicb.2017.01105

Weisleitner, K., Perras, A. K., Unterberger, S. H., Moissl-Eichinger, C., Andersen, D. T., & Sattler, B. (2020). Cryoconite Hole Location in East-Antarctic Untersee Oasis Shapes Physical and Biological Diversity. *Frontiers in Microbiology*, *11*, 1165. https://doi.org/10.3389/fmicb.2020.01165

Werner, U., Bird, P., Wild, C., Ferdelman, T., Polerecky, L., Eickert, G., Jonstone, R., Hoegh-Guldberg, O., & de Beer, D. (2006). Spatial patterns of aerobic and anaerobic mineralization rates and oxygen penetration dynamics in coral reef sediments. *Marine Ecology Progress Series*, *309*, :93-105.

Weston, N. B., & Joye, S. B. (2005). Temperature-driven decoupling of key phases of organic matter degradation in marine sediments. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(47), 17036 LP – 17040. https://doi.org/10.1073/pnas.0508798102

WGMS. (2016). Http://Wgms.Ch/Products_ref_glaciers/Midtre-Lovenbreen-Svalbard/.

Wharton, R. A., McKay, C. P., Simmons, G. M., & Parker, B. C. (1985). Cryoconite Holes on Glaciers. *BioScience*. https://doi.org/10.2307/1309818

Wharton, R. A., Vinyard, W. C., Parker, B. C., Simmons, G. M., & Seaburg, K. G. (1981). Algae in cryoconite holes on Canada Glacier in Southern Victorialand, Antarctica. *Phycologia*, *20*(2), 208–211. https://doi.org/10.2216/i0031-8884-20-2-208.1

White, R. E. (2006). Principles and Practice of Soil Science: The Soil as a Natural Resource. In *Blackwell Publishing*. https://doi.org/10.1002/msj.20251

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Widdel, F., Kohring, G.-W., & Mayer, F. (1983). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. *Archives of Microbiology*, *134*(4), 286–294. https://doi.org/10.1007/BF00407804

Williams, T. J., Wilkins, D., Long, E., Evans, F., Demaere, M. Z., Raftery, M. J., & Cavicchioli, R. (2013). The role of planktonic Flavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. *Environmental Microbiology*, *15*(5), 1302–1317. https://doi.org/10.1111/1462-2920.12017

Williamson, C. J., Cook, J., Tedstone, A., Yallop, M., McCutcheon, J., Poniecka, E., Campbell, D., Irvine-Fynn, T., McQuaid, J., Tranter, M., Perkins, R., & Anesio, A. (2020). Algal photophysiology drives darkening and melt of the Greenland Ice Sheet. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(11). https://doi.org/10.1073/pnas.1918412117

Wilson, S. L., Grogan, P., & Walker, V. K. (2012). Prospecting for ice association: Characterization of freeze-thaw selected enrichment cultures from latitudinally distant soils. *Canadian Journal of Microbiology*. https://doi.org/10.1139/W2012-010

Woese, C. R. (1987). Bacterial evolution. In *Microbiological Reviews*. https://doi.org/10.1128/mmbr.51.2.221-271.1987

Wolfe, A. J. (2005). The Acetate Switch. *Microbiology and Molecular Biology Reviews*, *69*(1), 12 LP – 50. https://doi.org/10.1128/MMBR.69.1.12-50.2005

Wolin, E. A., Wolin, M. J., Wolfe, & R. S. (1963). Formation of methane by bacterial extracts. *The Journal of Biological Chemistry*.

Wüst, P. K., Horn, M. A., & Drake, H. L. (2009). Trophic links between fermenters and methanogens in a moderately acidic fen soil. *Environmental Microbiology*, *11*(6). https://doi.org/10.1111/j.1462-2920.2009.01867.x

Xu, K., Lavaud, J., Perkins, R., Austen, E., Bonnanfant, M., & Campbell, D. A. (2018). Phytoplankton σPSII and Excitation Dissipation; Implications for Estimates of Primary Productivity . In *Frontiers in Marine Science* (Vol. 5, p. 281). https://www.frontiersin.org/article/10.3389/fmars.2018.00281

Yallop, M. L., Anesio, A. M., Perkins, R. G., Cook, J., Telling, J., Fagan, D., MacFarlane, J., Stibal, M., Barker, G., & Bellas, C. (2012). Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. *The ISME Journal*, *6*(12), 2302–2313.

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*(D1), D643–D648. https://doi.org/10.1093/nar/gkt1209

Yoon, S. S., Hennigan, R. F., Hilliard, G. M., Ochsner, U. A., Parvatiyar, K., Kamani, M. C., Allen, H. L., DeKievit, T. R., Gardner, P. R., Schwab, U., Rowe, J. J., Iglewski, B. H., McDermott, T. R., Mason, R. P., Wozniak, D. J., Hancock, R. E. W., Parsek, M. R., Noah, T. L., Boucher, R. C., & Hassett, D. J. (2002). Pseudomonas aeruginosa anaerobic respiration in biofilms: Relationships to cystic fibrosis pathogenesis. *Developmental Cell*, *3*(4). https://doi.org/10.1016/S1534-5807(02)00295-2

Zawierucha, K, Kolicka, M., Takeuchi, N., & Kaczmarek, Ł. (2015). What animals can live in cryoconite holes? A faunal review. *Journal of Zoology*, *295*(3), 159–169.

Zawierucha, Krzysztof, Buda, J., Fontaneto, D., Ambrosini, R., Franzetti, A., Wierzgoń, M., & Bogdziewicz, M. (2019). Fine-scale spatial heterogeneity of invertebrates within cryoconite holes. *Aquatic Ecology*, *53*(2), 179–190. https://doi.org/10.1007/s10452-019-09681-9

Zawierucha, Krzysztof, Buda, J., & Nawrot, A. (2019). Extreme weather event results in the removal of invertebrates from cryoconite holes on an Arctic valley glacier (Longyearbreen, Svalbard). *Ecological Research*, *34*(3). https://doi.org/10.1111/1440-1703.1276

Zdanowski, M. K., Bogdanowicz, A., Gawor, J., Gromadka, R., Wolicka, D., & Grzesiak, J. (2017). Enrichment of Cryoconite Hole Anaerobes: Implications for the Subglacial Microbiome. *Microbial Ecology*, *73*(3), 532–538. https://doi.org/10.1007/s00248-016-0886-6

Zhang, D.-C., Busse, H.-J., Liu, H.-C., Zhou, Y.-G., Schinner, F., & Margesin, R. (2011). Sphingomonas glacialis sp. nov., a psychrophilic bacterium isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *61*(3), 587–591. https://doi.org/doi:10.1099/ijs.0.023135-0

Zhang, D.-C., Redzic, M., Liu, H.-C., Zhou, Y.-G., Schinner, F., & Margesin, R. (2012). Devosia psychrophila sp. nov. and Devosia glacialis sp. nov., from alpine glacier cryoconite, and an

emended description of the genus Devosia. *International Journal of Systematic and Evolutionary Microbiology*, *62*(3), 710–715.

Zhang, D.-C., Redzic, M., Schinner, F., & Margesin, R. (2011). Glaciimonas immobilis gen. nov., sp. nov., a member of the family Oxalobacteraceae isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *61*(9), 2186–2190. https://doi.org/doi:10.1099/ijs.0.028001-0

Zhang, D.-C., Schumann, P., Redzic, M., Zhou, Y.-G., Liu, H.-C., Schinner, F., & Margesin, R. (2012). Nocardioides alpinus sp. nov., a psychrophilic actinomycete isolated from alpine glacier cryoconite. *International Journal of Systematic and Evolutionary Microbiology*, *62*(2), 445–450. https://doi.org/doi:10.1099/ijs.0.031047-0

List of Appendices

Appendix A – Supplementary material specific to Chapter 3 (Material and methods)

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Appendix A

Materials and methods

A.1 DNA sequencing

Supplementary table 1 contains a list of the unique sequences of reverse primers which were used to barcode individual samples in microbial community structure analysis via 16S rDNA sequencing (Section 3.5.3.2. in Chapter 3).

Supplementary table 1. List of sequences of barcodes used for 16S rDNA sequencing of microbial community.

Barcode name	Barcode sequence (5'-3')	Barcode name	Barcode sequence (5'-3')
0	TCCCTTGTCTCC	50	GATGTTCGCTAG
1	ACGAGACTGATT	51	CTATCTCCTGTC
2	GCTGTACGGATT	52	ACTCACAGGAAT
3	ATCACCAGGTGT	53	ATGATGAGCCTC
4	TGGTCAACGATA	54	GTCGACAGAGGA
5	ATCGCACAGTAA	55	TGTCGCAAATAG
6	GTCGTGTAGCCT	56	CATCCCTCTACT
7	AGCGGAGGTTAG	57	TATACCGCTGCG
8	ATCCTTTGGTTC	58	AGTTGAGGCATT
9	TACAGCGCATAC	59	ACAATAGACACC
10	ACCGGTATGTAC	150	ACGCGAACTAAT
11	AATTGTGTCGGA	151	AGCTATGTATGG
12	TGCATACACTGG	152	ACGGGTCATCAT
13	AGTCGAACGAGG	153	GAAACATCCCAC
14	ACCAGTGACTCA	154	CGTACTCTCGAG
15	GAATACCAAGTC	155	TCAGTTCTCGTT
16	GTAGATCGTGTA	156	TCGTGCGTGTTG
17	TAACGTGTGTGC	157	GTTATCGCATGG
18	CATTATGGCGTG	158	GATCACGAGAGG
19	CCAATACGCCTG	159	GTAAATTCAGGC
20	GATCTGCGATCC	160	AGTGTTTCGGAC
21	CAGCTCATCAGC	161	ACACGCGGTTTA
22	CAAACAACAGCT	162	TGGCAAATCTAG
23	GCAACACCATCC	163	CACCTTACCTTA
24	GCGATATATCGC	164	TTAACCTTCCTG
25	CGAGCAATCCTA	165	TGCCGTATGCCA
26	AGTCGTGCACAT	166	CGTGACAATAGT
27	GTATCTGCGCGT	167	CGCTACAACTCG
28	CGAGGGAAAGTC	168	TTAAGACAGTCG
29	CAAATTCGGGAT	169	TCTGCACTGAGC
30	AGATTGACCAAC	170	CGCAGATTAGTA
31	AGTTACGAGCTA	171	TGGGTCCCACAT
32	GCATATGCACTG	172	CACTGGTGCATA
33	CAACTCCCGTGA	173	AACGTAGGCTCT
34	TTGCGTTAGCAG	174	AGTTGTAGTCCG
35	TACGAGCCCTAA	175	TCGTCAAACCCG
36	CACTACGCTAGA	176	TAATCGGTGCCA
37	TGCAGTCCTCGA	177	TTGATCCGGTAG
38	ACCATAGCTCCG	178	CGGGTGTTTGCT
39	TCGACATCTCTT	179	TTGACCGCGGTT
40	GAACACTTTGGA	466	GTTTGGCCACAC
41	GAGCCATCTGTA	467	TCAGGTTGCCCA
42	TTGGGTACACGT	468	TCATTCCACTCA
43	AAGGCGCTCCTT	469	GTCACATCACGA
44	TAATACGGATCG	470	CGACATTTCTCT
45	TCGGAATTAGAC	471	GGACGTTAACTA
46	TGTGAATTCGGA	472	TAGCAGTTGCGT
47	CATTCGTGGCGT	473	CACGCTATTGGA
48	TACTACGTGGCC	474	AACTTCACTTCC
49	GGCCAGTTCCTA	475	CCAGTGGATATA

A.2 Substrate test

Microbial isolates from cryoconite holes were tested for growth in a liquid media with addition of a single carbon source in the laboratory conditions. Supplementary table 2 contains a list of substrates tested (Section 3.5.4.3.4. in Chapter 3)

Supplementary table 2. List of carbon substrates.

Substrate	Concentration	Substrate	Concentration
Carboxylic acids		*Amino acids*	
Formate	20 mM	Peptone	0.25%
Acetate	15 mM	Casamino Acids	0.25%
Propionate	10 mM	Yeast extract	0.5%
Butyrate	10 mM	Serine	15 mM
Hexonoate	5 mM	Alanine	10 mM
Malonate	15 mM	Arginine	10 mM
Succinate	15 mM	Glutamate	10 mM
Fumarate	15 mM	Proline	10 mM
Malate	15 mM	Isoleucine	6.25 mM
Pyruvate	15 mM	Lysine	5 mM
Lactate	15 mM	Cysteine	10 mM
Glycolate	20 mM	Methionine	10 mM
Salicylate	2.5 mM	Phenylalanine	5 mM
DPA	5 mM		
Glyoxylate	20 mM		
Ketoglutarate	10 mM	*Polyhydric alcohols*	
Citrate	10 mM	Sorbitol	1 mM
		Mannitol	5 mM
		Inositol	5 mM
Carbohydrates			
Fructose	5mM	*Alcohols*	
Cellulose	0.1%	Erythritol	10 mM
Starch	1%	Glycerol	10 mM
Sucrose	1 mM	Glycol	10 mM
Maltose	1 mM	Methanol	10 mM
Cellobiose	0.2 mM	Ethanol	10 mM
Trehalose	0.5 mM	Butanol	5 mM
Mannose	1 mM	Ethanolamine	15 mM
Galactose	1 mM		
Xylose	1 mM	*Other*	
Arabinose	1 mM	Choline	15 mM
Rhamnose	1 mM	Betaine	15 mM
Glucose	5 mM	Methylamine	20 mM
Gluconate	5 mM		
Glucosamine	5 mM		

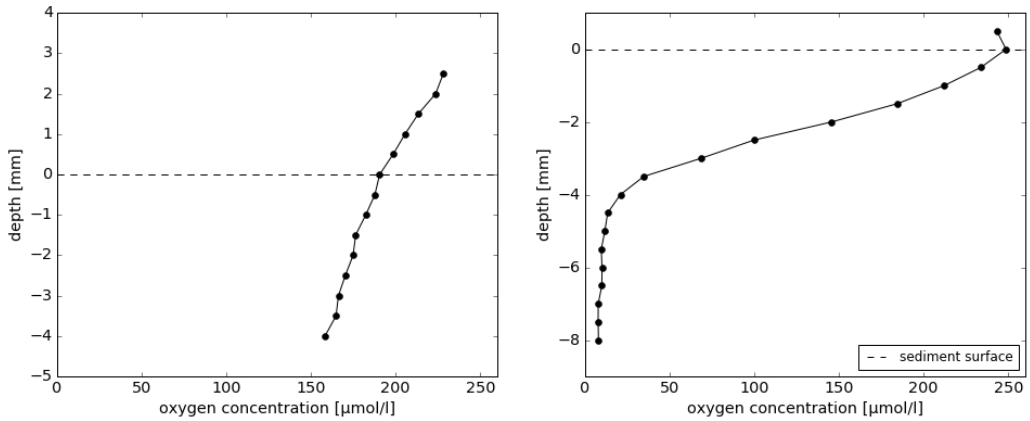
Appendix B

Oxygen in cryoconite holes

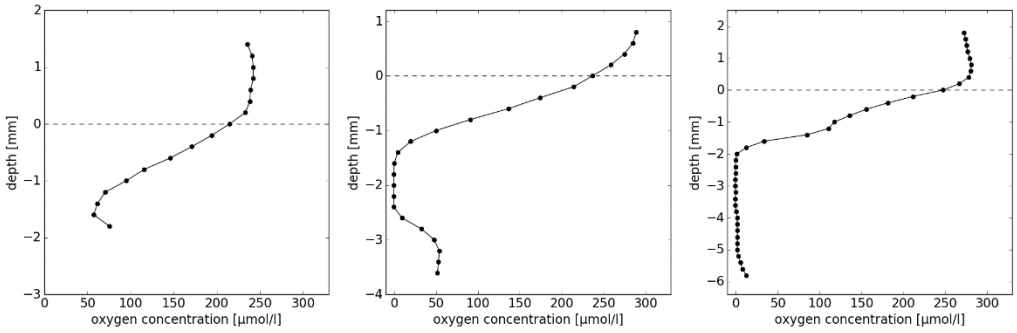
Supplementary material specific to Chapter 4.

B.1 Oxygen profiles

In situ oxygen profiles on Greenland Ice Sheet in 2016 (Supplementary figure 1) and laboratory measurements of oxygen profiles in the cryoconite granules from Greenland margin (Supplementary figure 2).



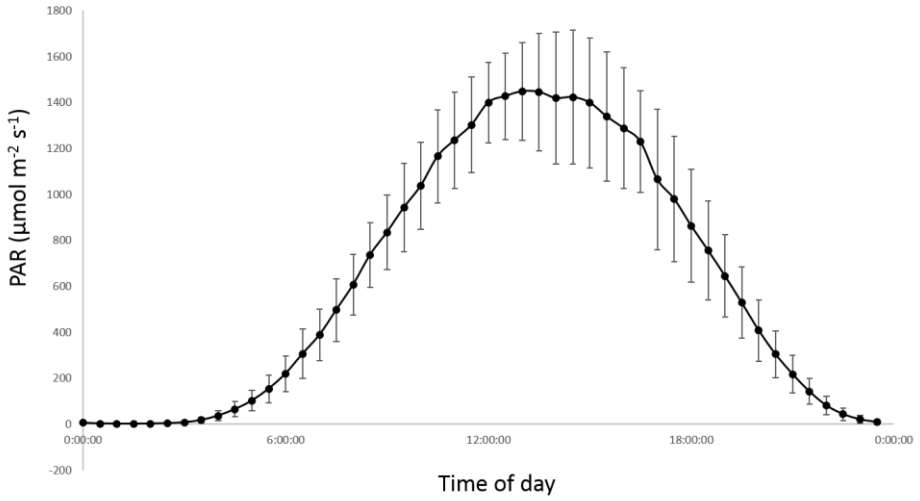
Supplementary figure 1. Oxygen profiles in additional examples of 'classic' and 'partly drained and accumulated' cryoconite debris from camp Black and Bloom (see Methods for definitions)



Supplementary figure 2. Oxygen profile measured using a microsensor within spherical cryoconite granules.

B.1 Photosynthetically Active Radiation

Supplementary figure 3 shows average PAR over 24h period measured *in situ* on Greenland Ice Sheet.



Supplementary figure 3. Typical mean PAR receipt at Camp Black and Bloom over 24 hr in July 2016.

Appendix C

Microbial physiology

Supplementary material specific to Chapter 5.

C.1 Microbial isolates

Supplementary table 3 presents data on the location and material from which the closest relatives of microorganisms in this thesis were isolated. Supplementary figure 4 (four parts) presents raw data of substrate test – microbial isolates (bacteria and yeasts) were grown on single carbon substrate in duplicate (Section 5.2.8). Positive growth of both duplicate isolates is marked as a black square, whereas positive growth of just one of the duplicates is marked with grey square with “1” in the middle.

Supplementary table 3. Closest relative of microbial isolates from cryoconite holes. Closest relative is based on rRNA gene sequencing and similarity is expressed in percent (%). Isolation source and location of closest relative is sourced from Genbank database.

Isolate	Site	Closest relative	%	Closest relative source	Closest relative location
Bacteria					
An02O7	Ant	Flavobacterium sp. R-36976	99	aquatic microbial mat	Antarctica
An4O7	Ant	Flavobacterium sp. R-36976	99	aquatic microbial mat	Antarctica
An15O7	Ant	Arthrobacter agilis strain LV7	99	lake cyanobacterial mat	Antarctica
An15A7	Ant	Tessaracoccus sp. strain AU I5	99	marine macroalgae	Antarctica
An15A8	Ant	Tessaracoccus sp. strain AU I5	99	marine macroalgae	Antarctica
An4A7	Ant	Bacterium CS117	99	cryoconite hole	Antarctica
An4A8	Ant	Bacterium CS117	99	cryoconite hole	Antarctica
An4O8	Ant	Marisediminicola sp. N26	99	permafrost	Antarctica
An15O8	Ant	Marisediminicola sp. N26	99	permafrost	Antarctica
An02O8	Ant	Cryobacterium sp. 1021	99	Glacier No.1	China
Gr15O6	Gr	Frigoribacterium sp. MP117	99	ice water (glacier)	Tibet
Gr15O5	Gr	Frigoribacterium sp. MP117	99	ice water (glacier)	Tibet
Gr02A4	Gr	Antarctic bacterium 2CA	99	glacier sediment	Antarctica
Gr15O4	Gr	Glaciihabitans tibetensis strain TGC-6	99	cryoconite hole	Greenland
Gr4O4P	Gr	Uncultured Bacteroidetes clone IC4058	99	ice core	Svalbard
Gr4O6	Gr	Rugamonas rubra strain HCR18a	99	cryoconite hole	Himalaya
Gr4A5	Gr	Cryobacterium sp. MDB2-A-1	99	glacier	China
Gr02O4	Gr	Cryobacterium psychrotolerans strain MLB-34	99	cryoconite hole	Svalbard
Gr02A6	Gr	Cryobacterium psychrotolerans strain ZS14-85	99	soil	Antarctica
Gr02A5	Gr	Cryobacterium sp. MDB1-44	99	glacier	China
Gr4A4	Gr	Cryobacterium sp. MDB1-44	99	glacier	China
Gr4A6	Gr	Cryobacterium sp. MDB1-44	99	glacier	China
Sv4A3	Sv	Cryobacterium sp. MDB1-44	99	glacier	China
Sv4A2	Sv	Cryobacterium sp. MDB2-A-1	99	glacier	China
Sv15A2	Sv	Cryobacterium sp. MDB2-A-1	99	glacier	China
Sv02A1	Sv	Antarctic bacterium 2CA	99	glacier sediment	Antarctica
Sv4A1	Sv	Antarctic bacterium 2CA	99	glacier sediment	Antarctica
Sv02A3	Sv	Antarctic bacterium 2CA	99	glacier sediment	Antarctica
Sv4O2	Sv	Uncultured bacterium clone LE201D02	99	arctic river	Russia
Sv02O2	Sv	Flavobacterium sp. KJF4-15	99	subarctic fjord	Svalbard
Sv02A2	Sv	Flavobacterium sp. TMS1-10 16S	99	glacier	China
Sv15A1	Sv	Cellulomonas cellasea strain WB102	99	woodchip bioreactor	USA
Sv15O1	Sv	Frigoribacterium sp. Ha8	99	glacier	China
Sv15A3	Sv	Actinobacterium Muzt-D93	99	glacial ice core	China
Sv15O3	Sv	Glaciihabitans tibetensis strain SD-70	99	cryoconite hole	Himalaya
Fungi					
Gr02O5	Gr	Basidiomycota sp. TP-Snow-Y1	91	glacier surface snow	China
Gr4O5	Gr	Basidiomycota sp. TP-Snow-Y1	91	glacier surface snow	China
Gr4O4	Gr	Basidiomycota sp. TP-Snow-Y1	91	glacier surface snow	China
Gr02O4w	Gr	Basidiomycota sp. TP-Snow-Y1	91	glacier surface snow	China
Gr02O6	Gr	Basidiomycota sp. TP-Snow-Y1	91	glacier surface snow	China
Sv02O1	Sv	Basidiomycota sp. TP-Snow-Y1	92	glacier surface snow	China
Sv4O1	Sv	Basidiomycota sp. TP-Snow-Y1	92	glacier surface snow	China
Sv02O3	Sv	Mrakia sp. isolate J-36	99	Russell glacier	Greenland
Sv4O3	Sv	Mrakia robertii isolate J-127	100	Russell glacier	Greenland

site	temp	sample	OTUs	Glyoxylate	Ketoglutarate	Citrate	Formate	Acetate	Propionate	Butyrate	Hexonoate	Malonate	Succinate	Fumarate	Malate	Pyruvate	Lactate	Glycolate	Salicylate	DPA	Control		Glucose	Gluconate	Fructose	Glucosamine	Cellulose	Starch	Sucrose	Maltose	Cellobiose	Trehalose	Mannose	Galactose	Xylose	Arabinose	Rhamnose
				CARBOXYLIC ACIDS																			CARBOHYDRATES														
S	0.2°C	A1	Antarctic bacterium 2CA										1	1	1								1	1	1	1											
S	4°C	A1	Antarctic bacterium 2CA															1																				
S	0.2°C	A2	Flavobacterium sp. TMS1-10 16S				1	1																1	1	1											
S	4°C	A2	Cryobacterium sp. MDB2-A-1															1												1	1	1	1	1	1	1	1	1
S	15°C	A2	Cryobacterium sp. MDB2-A-1				1	1			1								1	1			1														
S	0.2°C	A3	Antarctic bacterium 2CA																				1	1	1	1							1	1			
S	4°C	A3	Cryobacterium sp. MDB1-44																																		
G	0.2°C	A4	Antarctic bacterium 2CA				1				1												1	1	1	1											
G	4°C	A4	Cryobacterium sp. MDB1-44		1		1				1		1								1		1	1	1	1											
G	4°C	A5	Cryobacterium sp. MDB2-A-1																																		
G	0.2°C	A5	Cryobacterium sp. MDB1-44																																		
G	4°C	A6	Cryobacterium sp. MDB1-44																																		
G	0.2°C	A6	Cryobacterium sp. MDB1-44				1				1										1																
A	4°C	A7	Bacterium CS117																			1															
A	4°C	A8	Bacterium CS117		1						1					1										1											
S	15°C	O1	Frigoribacterium sp. Ha8																										1		1		1			
S	4°C	O2	Uncultured bacterium clone LE201D02																				1						1	1		1	1				
S	0.2°C	O2	Flavobacterium sp. KJF4-15				1	1			1										1		1														
S	15°C	O3	Uncultured actinobacterium clone IC4013																																		1
G	15°C	O4	Antarctic bacterium 2CD																																1		
G	0.2°C	O4Y	Cryobacterium psychrotolerans strain MLB-34																																		
G	15°C	O5	Frigoribacterium sp. MP117		1																														1	1	1
G	15°C	O6	Frigoribacterium sp. MP117																		1																
G	4°C	O6	Rugamonas rubra strain HCR18a																																1		
A	0.2°C	O7	Flavobacterium sp. R-36976																																		
A	4°C	O7	Flavobacterium sp. R-36976																										1								
A	15°C	O7	Arthrobacter agilis strain LV7																										1			1					
A	15°C	O8	Marisediminicola sp. N26																																1		1
A	4°C	O8	Marisediminicola sp. N26																1				1		1				1								
A	0.2°C	O8	Cryobacterium sp. 1021											1				1					1	1													

Supplementary figure 4. Growth of microbial isolates on single carbon source – black square marks the duplicate growth, grey square with 1 marks growth of one of the duplicates only.

site	temp	sample	OTUs	Serine	Alanine	Arginine	Glutamate	Proline	Isoleucine	Lysine	Cysteine	Methionine	Phenylalanine	Control		Sorbitol	Mannitol	Inositol		Erythritol	Glycerol	Glycol	Methanol	Ethanol	Butanol	Ethanolamine		Choline	Betaine	Methylamine
			AMINO ACIDS													POLYHYDRIC ALCOHOLS					ALCOHOLS							OTHER		
S	0.2°C	A1	Antarctic bacterium 2CA																											
S	4°C	A1	Antarctic bacterium 2CA					1		1														1						
S	0.2°C	A2	Flavobacterium sp. TMS1-10 16S																											
S	4°C	A2	Cryobacterium sp. MDB2-A-1		1			1		1						1	1				1			1						
S	15°C	A2	Cryobacterium sp. MDB2-A-1																		1									
S	0.2°C	A3	Antarctic bacterium 2CA																											
S	4°C	A3	Cryobacterium sp. MDB1-44																											
G	0.2°C	A4	Antarctic bacterium 2CA																											
G	4°C	A4	Cryobacterium sp. MDB1-44																											
G	4°C	A5	Cryobacterium sp. MDB2-A-1																					1						
G	0.2°C	A5	Cryobacterium sp. MDB1-44																											
G	4°C	A6	Cryobacterium sp. MDB1-44																					1						
G	0.2°C	A6	Cryobacterium sp. MDB1-44																											
A	4°C	A7	Bacterium CS117																											
A	4°C	A8	Bacterium CS117																											
S	15°C	O1	Frigoribacterium sp. Ha8																											
S	4°C	O2	Uncultured bacterium clone LE201D02																											
S	0.2°C	O2	Flavobacterium sp. KJF4-15																											
S	15°C	O3	Uncultured actinobacterium clone IC4013				1	1														1								
G	15°C	O4	Antarctic bacterium 2CD																											
G	0.2°C	O4Y	Cryobacterium psychrotolerans strain MLB-34														1													
G	15°C	O5	Frigoribacterium sp. MP117																											
G	15°C	O6	Frigoribacterium sp. MP117									1										1								
G	4°C	O6	Rugamonas rubra strain HCR18a																											
A	0.2°C	O7	Flavobacterium sp. R-36976																											
A	4°C	O7	Flavobacterium sp. R-36976																											
A	15°C	O7	Arthrobacter agilis strain LV7									1																		
A	15°C	O8	Marisediminicola sp. N26		1							1																		
A	4°C	O8	Marisediminicola sp. N26																							1			1	
A	0.2°C	O8	Cryobacterium sp. 1021																											

site	temp	sample	OTUs	Glyoxylate	Ketoglutarate	Citrate	Formate	Acetate	Propionate	Butyrate	Hexonoate	Malonate	Succinate	Fumarate	Malate	Pyruvate	Lactate	Glycolate	Salicylate	DPA	Control		Glucose	Gluconate	Fructose	Glucosamine	Cellulose	Starch	Sucrose	Maltose	Cellobiose	Trehalose	Mannose	Galactose	Xylose	Arabinose	Rhamnose
				CARBOXYLIC ACIDS																			CARBOHYDRATES														
S	0.2°C	O1	Basidiomycota sp. TP-Snow-Y1																									1					1	1		1
S	4°C	O1	Basidiomycota sp. TP-Snow-Y1					1																						1						
S	0.2°C	O3	Mrakia sp. isolate J-36		1		1		1	1										1																
S	4°C	O3	Mrakia robertii isolate J-127																										1							
G	4°C	O4	Basidiomycota sp. TP-Snow-Y1																										1							
G	0.2°C	O4W	Basidiomycota sp. TP-Snow-Y1																																	
G	0.2°C	O5	Basidiomycota sp. TP-Snow-Y1																																	
G	4°C	O5	Basidiomycota sp. TP-Snow-Y1																				1						1							
G	0.2°C	O6	Basidiomycota sp. TP-Snow-Y1																																	

site	temp	sample	OTUs	Peptone	Casamino Acid	Yeast extract	Serine	Alanine	Arginine	Glutamate	Proline	Isoleucine	Lysine	Cysteine	Methionine	Phenylalanine	Control		Sorbitol	Mannitol	Inositol		Erythritol	Glycerol	Glycol	Methanol	Ethanol	Butanol	Ethanolamine		Choline	Betaine	Methylamine
				AMINO ACIDS														POLYHYDRIC ALCOHOLS					ALCOHOLS								OTHER		
S	0.2°C	O1	Basidiomycota sp. TP-Snow-Y1	1	1	1				1	1								1				1						1				
S	4°C	O1	Basidiomycota sp. TP-Snow-Y1			1																											
S	0.2°C	O3	Mrakia sp. isolate J-36												1																		
S	4°C	O3	Mrakia robertii isolate J-127			1																											
G	4°C	O4	Basidiomycota sp. TP-Snow-Y1			1																											
G	0.2°C	O4W	Basidiomycota sp. TP-Snow-Y1																														
G	0.2°C	O5	Basidiomycota sp. TP-Snow-Y1																														
G	4°C	O5	Basidiomycota sp. TP-Snow-Y1			1																											
G	0.2°C	O6	Basidiomycota sp. TP-Snow-Y1																1														

Appendix D

Fermentative potential

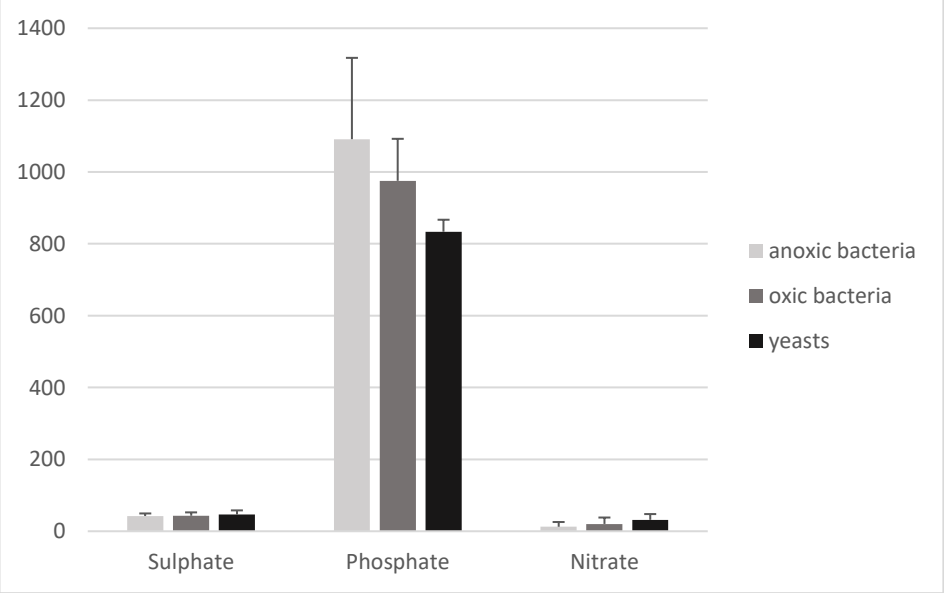
Supplementary material specific to Chapter 6.

D.1 Water chemistry in incubations of pure cultures

Supplementary table 4 presents data on the water chemistry of anaerobic incubations of microbial cultures (Section 6.6). Supplementary figure 5 depicts the data from the Supplementary table 4.

Supplementary table 4. Mean concentrations of major anions and carboxylic acids (µM) detected in anoxic incubations from all sites.

Isolate	Acetate	Lactate	Formate	Propionate	Chloride	Sulphate	Phosphate	Nitrate
anoxic bacteria	**871.6** ± 442.7	**1799.2** ±1420.4	**1782.7** ±853.6	**343.1** ±1000.6	**13100.8** ±2632.7	**42.3** ±7.2	**1091.2** ±226.4	**12.5** ±13.2
oxic bacteria	**145.5** ± 211.5	**236.1** ±699.5	**240.0** ±744.5	**1.7** ±1.7	**12313.3** ±1950.0	**43.1** ±9.4	**975.4** ±116.8	**20.3** ±17.8
yeasts	**38.5** ±31.7	**0.0** ±0.0	**6.0** ±16.9	**0.7** ±1.2	**11320.2** ±1282.1	**46.6** ±11.5	**833.6** ±33.5	**31.8** ±16.0



Supplementary figure 5. Anions concentrations in the fermentation incubations of microbial isolates from cryoconite holes.

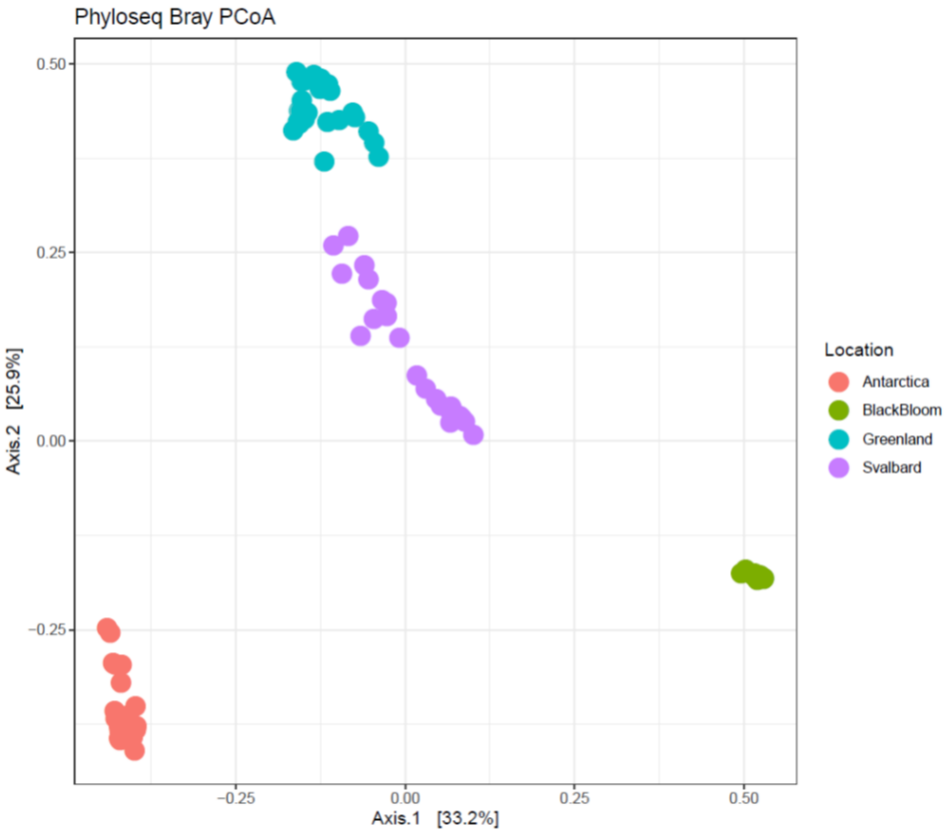
Appendix E

Microbial communities structure

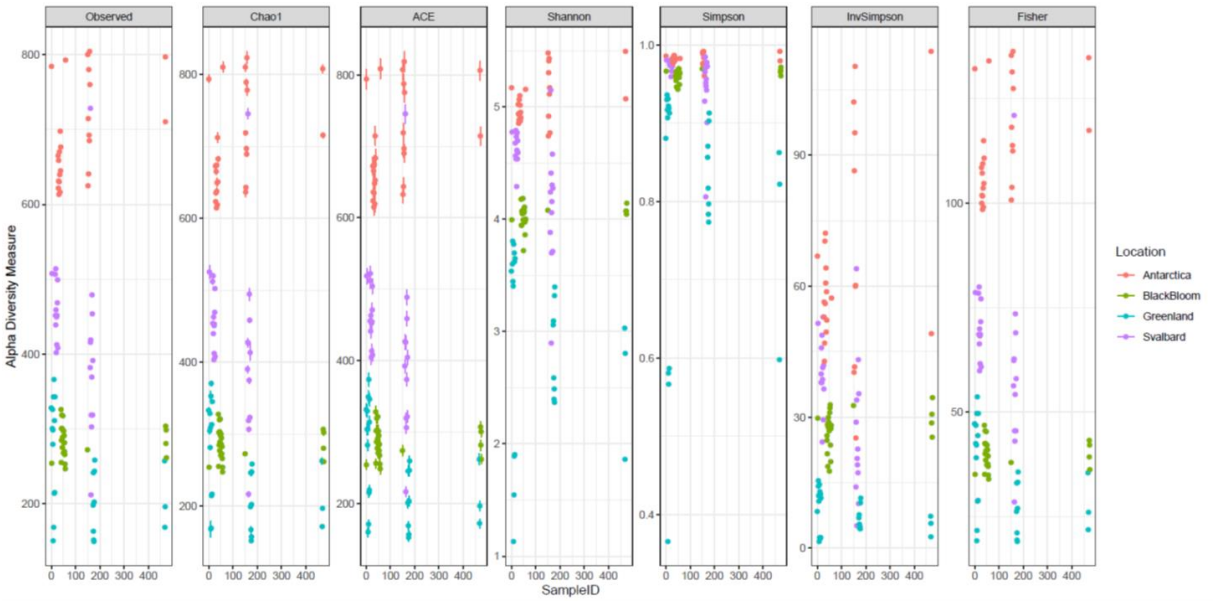
Supplementary material specific to Chapter 7.

E.1 Differences in community structure

Supplementary figure 6 and 7 presents data on the differences of microbial community structure depending on source location of the incubated material.



Supplementary figure 6. PCoA analysis of diversity of microbial community in cryoconite material incubations



Supplementary figure 7. Diversity matrices of community structure from cryoconite material incubations.