University of Glasgow

The Effects of Microbial Relocation from Glaciers to Periglacial Environments

2309271S

SUBMITTED IN FUFUILMENT OF REQUIRMENTS FOR THE DEGREE OF

MSc by RESEARCH

SCHOOL OF EARTH SCIENCES

COLLEGE OF SCIENCE AND ENGINEERING

26. 02. 2024

Declaration

I certify that the thesis presented here for examination for an MSc by Research degree of The University of Glasgow is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it) and that the thesis has not been edited by a third party beyond what is permitted by the University's PGR Code of Practice.

Acknowledgments

I'd like to acknowledge my supervisors, Karen Cameron and John Moreau, for their support and feedback throughout this project. I would also like to express my deepest gratitude to James Bradley and Arwyn Edwards for the provision of samples, as well as Kara Marsden Andy Mitchell, and Helen Glanville, for their methodological and practical support. I am also thankful to Charlotte Slaymark, Susan Ballie, and Leandro Soares for their training and assistance in the lab. Special thanks to Iain Neil the head of the master's programme for his continued support. Lastly, I would be remiss in not mentioning my partner for their belief in me, but also for providing their freezer for the storage of my samples.

Table of Contents

List of Abbreviations

Abbreviation	Meaning
AA	Arctic Amplification
BONCAT	Biorthogonal Non- Canonical Amino Acid Tagging
CH_4	Methane
CO_2	Carbon Dioxide
EC	Ecosystem Carbon
ESP	Extracellular Polymeric Substance
GPP	Gross Primary Production
GC	Gas Chromatography
HMA	High Mountain Asia
IPCC	International Panel Climate Committee
MFG	Microbial Functional Gene
N_2O	Nitrous Oxide
NCQ	Non-Photochemical Quenching
NEP	Net Ecosystem Production
PAR	Photosynthetically Available Radiation
PCR	Polymerase Chain Reaction
PPM	Parts per Million
R	Respiration
SLE	Sea level Equivalents
SMA	Standard Methods Agar
SOC	Soil Organic Carbon
TOC	Total Organic Carbon

Abstract

Glacier microbial communities, residing on the surface and within subglacial regions, play significant roles in biogeochemical cycling. However, the implications of global deglaciation and the subsequent displacement of these communities into surrounding ecosystems remain insufficiently understood, especially within the context of a warming climate. Here, microcosm incubation experiments were utilised to explore how cryoconite and subglacial communities from Svalbard respond to three temperature conditions: Svalbard's summertime supraglacial conditions (1^{o}C), the average summertime temperatures of the periglacial region (7^{o}C), and the extreme temperatures recorded in Svalbard during the summer of 2019 (21^{o}C). Viability analysis using live/dead staining revealed the resilience of both cryoconite and subglacial communities, displaying a high viability of 80% and 70% at 21^{O}C, respectively. Additionally, gas chromatography analysis measured CO_2 concentrations of 4000ppm in the headspace of cryoconite-containing microcosms, indicating sustained metabolic activity even under warmer conditions of 7^{o}C and 21^{o}C. These findings bear significant importance in understanding the potential role of glacial communities in nutrient cycling and greenhouse gas emissions within newly deglaciated soils. However, low concentrations of CH_4 and N_2O detected in this study highlight the necessity of refined laboratory techniques and in-situ measurements, as priority research areas to comprehensively assess metabolic activity in glacial samples.

1.0 Introduction

Glacial ecosystems are home to rich and diverse microbial communities (Margesin et al., 2019). However, due to unprecedented rates of glacial retreat since the early 2000s, these communities have been experiencing a higher frequency of being flushed with meltwater and expelled into neighbouring periglacial environments (i.e., the terrestrial surfaces enduring prolonged sub-zero conditions often located near a glacier) (Garcia- Lopez and Cid, 2017). The impact of this process on downstream ecosystems, and the fate of the relocated glacial microbes is an area requiring further exploration. Glacial communities exhibit distinct characteristics depending on their origin (Hodson et al., 2008). For example, microbial communities in cryoconite holes (i.e., microbial hotspots inhabiting water filled depressions) have adapted to the sunlit and oxygenated surface whilst subglacial communities have colonised the dark and anoxic bed (Hotaling, Hoood, and Hamilton, 2017). However, several studies have showcased the functional potential of both supra-and subglacial communities in nutrient cycling, highlighting their potential to disrupt regional biogeochemistry and carbon storage (Skidmore, Foght, and Sharp, 2000). Upon relocation, microbes are faced with several abiotic stressors, including record Arctic temperature extremes, and rising daily average temperatures driven by climate warming (Serreze and Barry, 2011). Consequently, comprehensive research is imperative to fully understand the effects of microbial relocation from glaciers to periglacial environments, within the context of a warming climate. This necessitates the utilisation and development of laboratory studies, allowing for the manipulation of environmental variables. Therefore, this study compares two gas sampling methodologies to improve headspace analysis during the incubation of glacial samples.

Objectives:

1. Assess the resilience of glacial communities to typical Arctic periglacial temperatures and extreme conditions observed in recent Arctic summers.

2. Examine the impact of short-term (36 days) exposure to both average and extreme Arctic periglacial temperatures on community biogeochemical cycling and greenhouse gas emissions.

3. Develop new methodologies for microcosms gas sampling of glacial sediments.

2.0 Project Background

This project is centred around life within the cryosphere, focusing on glacial and periglacial ecology, and how the relationship between these two ecosystems is changing. An in-depth analysis on the potential fate of glacial microbes upon relocation from the glacier to the periglacial environment is conducted, alongside assessments of their impact on carbon (C) and nitrogen cycling (N). Two different methodologies for measuring gas production during the incubation of glacial sediments are evaluated, including headspace replenishment and non-headspace replenishment methods. This study intends to further our knowledge of these topic areas through the incubation of cryoconite and subglacial sediments at different temperatures (1^{o}C, 7^{o}C, 21^{o}C) and measure the response through cell viability and greenhouse gas (GHG) production. Additionally, this study aims to establish the necessity of headspace replenishment of a closed system.

2.1 The Cryosphere

The cryosphere is an all-encompassing term for a component of Earth's system, which can be loosely described as environments at which temperatures prevail below freezing point and water exists in solid form (Wang, Liu, and Zhang, 2019). Owing to this broad definition, these cold environments include a diverse range of ecosystems, from terrestrial (e.g., glaciers, ice sheets & permafrost etc.), marine (e.g., sea ice, marine permafrost, icebergs etc.) and aerial (e.g., precipitation) (Ding et al., 2021). Influencing approximately 68 million km^2 (Haeberli and Whitman, 2015), as well as covering more than 60% of The Northern Hemisphere with snow during the winter months (Hornberger and Winter, 2009), the cryosphere provides many provisional (e.g., freshwater), regulatory (e.g., climate), supporting (e.g., habitation), and cultural services (e.g., recreation) (Su et al., 2019). As seen in Figure 1, the cryosphere is predominantly localised to higher latitudes, with the Antarctic and Greenland ice sheets cumulatively covering 9.2% of Earth's land area, and forming the largest section of the cryosphere (Vaughan et al., 2013). More specifically the cryosphere is an important hydrological reservoir, storing 70% of Earth's freshwater (McConnell, 2006), and is a crucial component of Earth's energy budget (Knight, 2013).

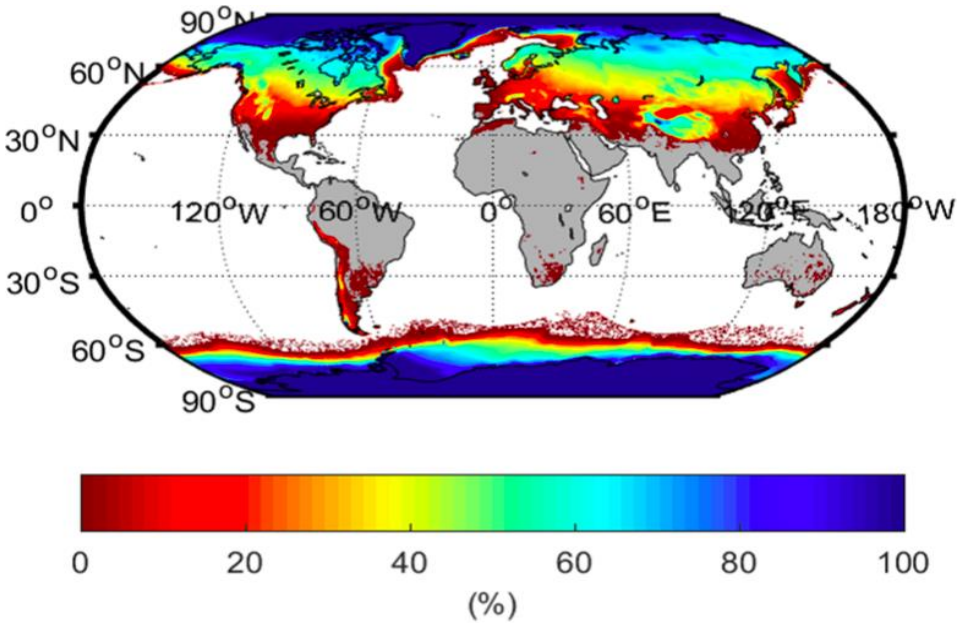

Figure 1: Global Cryosphere Extent Analysis during 1981-2010 Climatology Period. *A detailed depiction of the geographical distribution of cryosphere components, indicating the percentage of each grid cell that is occupied by one or more cryosphere components. The highest latitudinal regions can be seen as focal points of extensive cryosphere presence, shedding light on the intricate spatial dynamics governing cryosphere extent on a global scale. Figure from Peng et al. (2021).*

2.2 Arctic Amplification and Glacial Retreat

The cryosphere is highly sensitive to climate, and as a result, it is undergoing rapid changes in response to anthropogenic forcings (Elser et al., 2020). Glacial decline serves as evidence of such change, with unprecedented rates of retreat having been recorded over the 21st-century, as seen in Figure 2 (Meredith et al., 2019). A glacier is a large body of flowing ice, which is formed through the process of firnification (i.e., the crystallisation of snow into ice) and is sustained by having a higher net rate of accumulation than ablation (Zemp, Hoelzle, and Haeberli, 2009). Glaciers can be found on almost every continent constituting 10% of Earth's terrestrial surface (Meredith et al.,2019), making these ecosystems a prominent facet of the cryosphere. The relationship between anthropogenic global warming and the loss of glacial environments can be observed in temporal data, with trends indicating further decline under different carbon emission scenarios (Bosson, Huss, and Osipova, 2019). Whilst rising air temperatures are an issue faced globally the process of Arctic Amplification, a phenomenon where high-latitude environments experience accelerated warming in

comparison to the global average (Serreze and Barry, 2011), makes Arctic environments particularly vulnerable. For example, CMIP5 (Coupled Model Intercomparison Project Phase 5) models predict that if CO_2 emissions were to quadruple, Arctic mean surface warming would increase twice as much as global mean surface warming (Previdi and Polvani, 2021). The causes of this are predominately linked to changes in surface albedo feedback from sea ice loss (Winton, 2006). Documents such as The Intergovernmental Panel on Climate Change (IPCC) climate reports, designed to outline the critical implications of climate change and deglaciation in areas such as the Arctic, continue to overlook the consequences on glacial ecology (IPCC, 2021). This is not reflective of the literature which recognises the importance of microbial communities within cold environments and acknowledges global warming as an imminent threat to these communities and their habitat (Anesio et al., 2009; Edwards et al., 2014; Boetius et al., 2015). With glaciers and ice sheets melting at a rate of ~780 Gt yr^{-1} (Slater et al., 2021), it necessitates a comprehensive understanding of the fate of glacial communities within a warming climate.

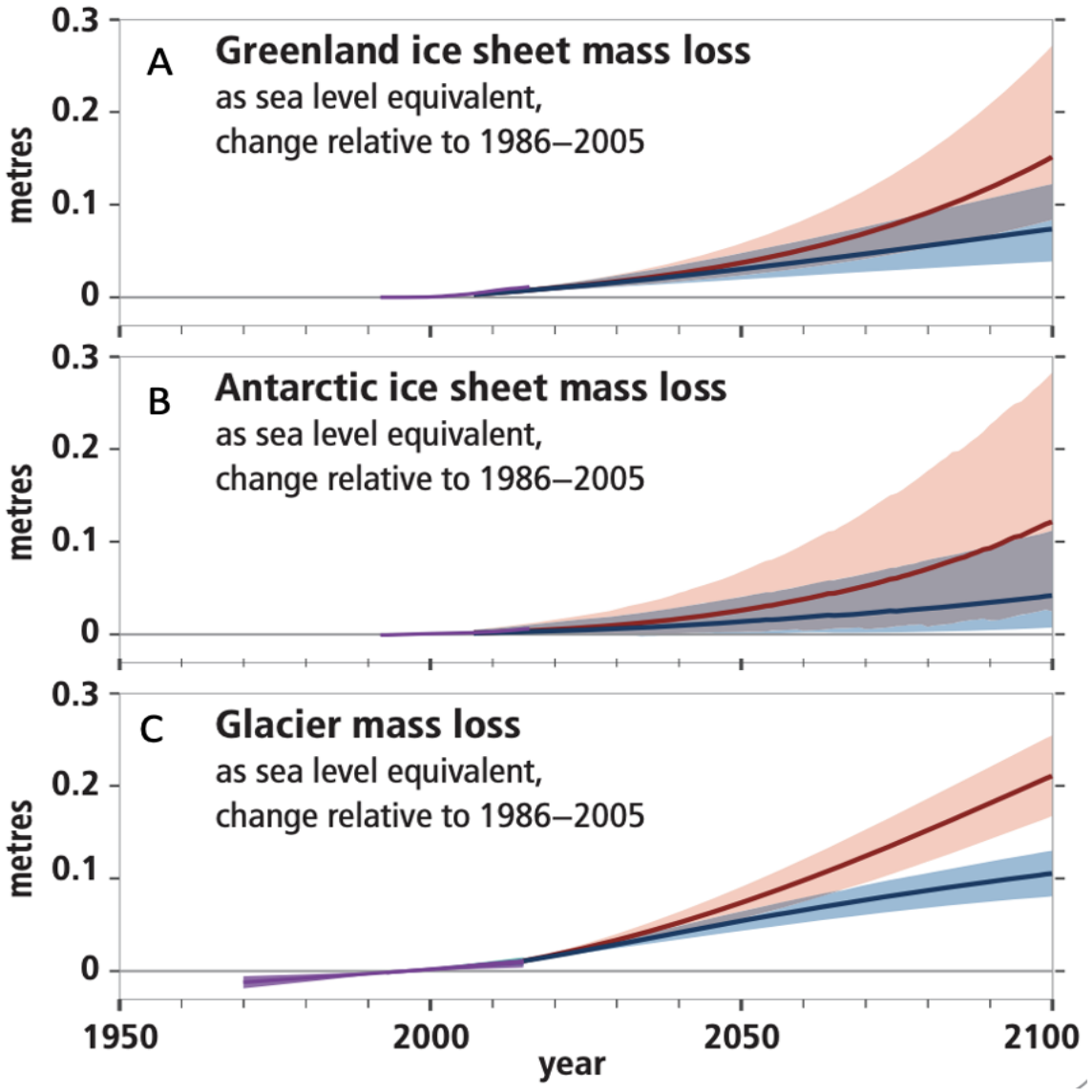

Figure 2: Projections of Glacier and Ice Sheet Mass Loss under RCP2.6 and RCP8.5. *The graphs (a) Greenland Ice Sheet, (b) Antarctic Ice Sheet, and (c) glaciers, depict projected mass loss in sea level equivalents (meters). The purple line represents observed and modelled historical ice mass loss. Two emission scenarios- low (RCP2.6, red line) and high (RCP8.5, blue line) greenhouse gas emissions- are presented. The graphs illustrate an anticipated increase in ice mass loss for both scenarios, with a notably accelerated melt under the RCP8.5 scenario. Figure from IPCC (2019).*

2.3 The Discovery of Life in Glaciers

Previously, the terrestrial cryosphere was thought to be void of life, as reflected in the words of Naval Officer Richard Byrd (1938)- “From disease I had little to fear. Antarctica is a paradise in that respect. It is the germless continent.” Over a century ago, the discovery of life in ice dismantled the ‘Goldilocks ideology’ that a set of perfect environmental conditions must be met for life to prevail (e.g., temperature) (Forster, 1887). It is now an established paradigm that glacier ice provides a habitat in which many microbes thrive. Many of these microorganisms are termed ‘psychrophilic’. Psychrophiles are cold-loving microorganisms that optimally function at temperatures between 10^{o}C to -20^{o}C, with metabolic activity having been detected at temperatures as low as -30^{o}C (Margesin and Collins, 2019). These organisms comprise a diverse community, including bacteria, archaea, fungi, viruses, and algae. Figure 3 showcases this diversity within bacteria (Boetius et al., 2015), highlighting their distribution in different cryosphere ecosystems. It can be seen that even when comparing similar habitats, such as snow, different regions support unique community compositions, shaped by geographical and environmental factors. Cold communities are important, with more than 85% of species within the biosphere permanently subject to temperatures below 5^{o}C (Rampelotta, 2014), requiring microbial communities to develop physiological responses to survive (Anesio and Laybourn-Parry, 2012). These mechanisms include changes to the cell membrane, lipids, proteins, and exopolysaccharides, allowing life to exist below freezing point (Tribelli and Lopez, 2018). These adaptations have enabled psychrophiles to successfully colonise large areas of Earth, playing a critical role in the functioning of many ecosystems (Elster et al., 2017; Hamdan, 2018; Anani et al., 2023). In fact, unique to many other biomes, the cryosphere is almost exclusively microbially driven (Anesio et al., 2017).

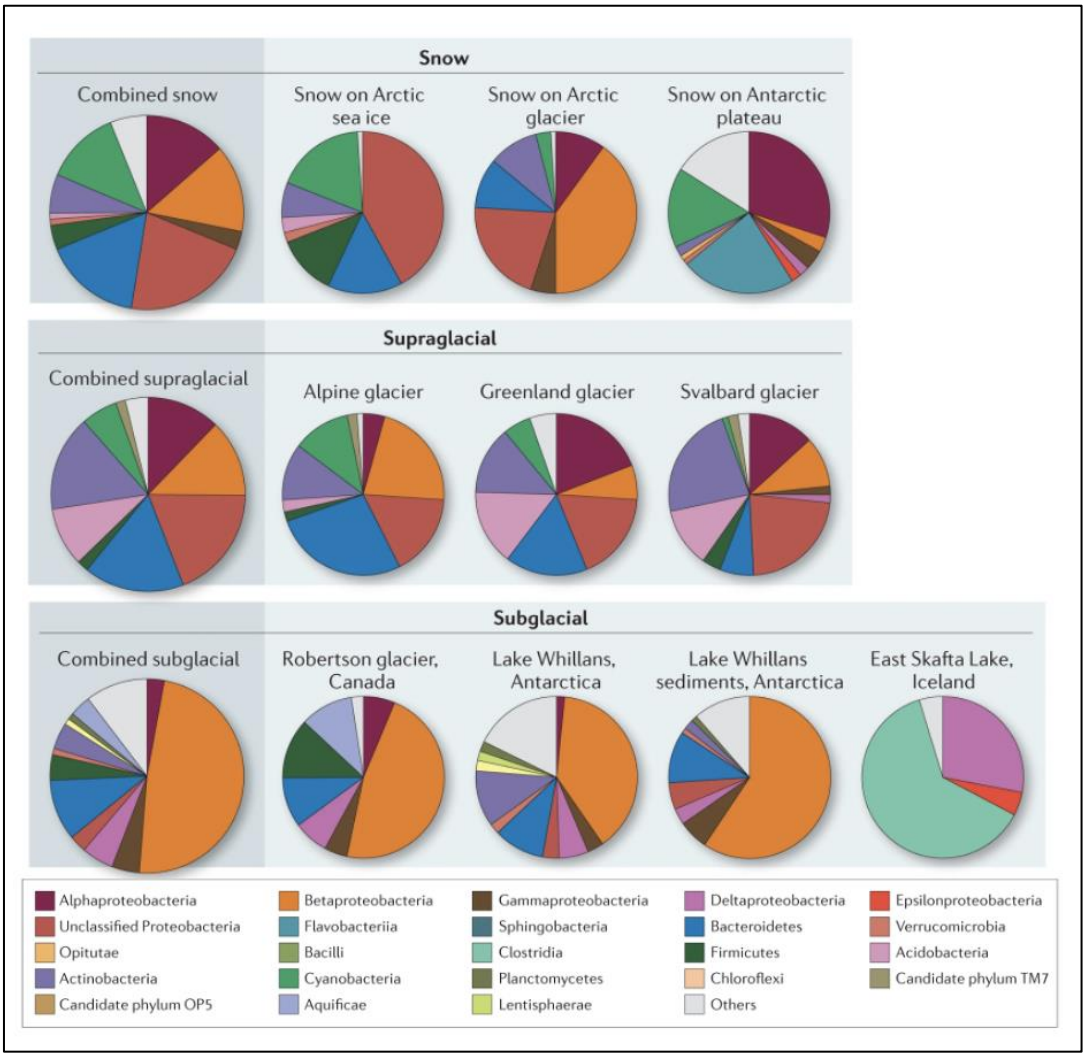

Figure 3: Bacterial Community Composition across Cryosphere Habitats. *The Figure showcases 13 distinct cryosphere ecosystems across varying geographically regions categorised within snow, supraglacial and subglacial environments. Each ecosystem's bacterial composition is visually represented visually via pie charts and a key detailing the bacterial species and their corresponding colours in is provided. Figure from Boetius et al. (2015).*

2.4 The Importance of Cold Microorganisms

2.4.1 Understanding Life's Adaptability to the Extremes

Cold microorganisms are crucial components of Earth's ecosystems, with wide implications for ecosystem dynamics, biogeochemical cycles, and our understanding of life's adaptability to extreme conditions (Siddiqui et al., 2013). These adaptations include a diverse range of strategies, as seen in Figure 4 such as antifreeze compounds (Muñoz et al., 2017). Antifreeze glycoproteins are an example of such compounds commonly found in Arctic organisms, known to lower the freezing point of cellular fluids which allows microbes to remain liquid

even at sub-zero temperatures (Kawahara, 2017); and their unique structure allows for glycoproteins to bind to ice crystals and prevent them from forming within cells (Baskaran et al., 2021). Efficient enzymes and nutrient uptake are also examples of such adaptations that help to facilitate nutrient cycles (Cavicchiolu et al., 2011). Psychrophiles have evolved to produce enzymes that are better adapted to optimally function at low temperature which improves the efficiency of biogeochemical reactions and allows psychrophiles to have access to nutrients in forms that are less available to other microorganisms. Additionally, osmolytes (e.g., betaine, proline, and trehalose) are molecules used by cold organisms to regulate temperature as well as osmotic stress often encountered due the thawing of ice which can contain high concentrations of solutes, such as salts (Bowman, 2017). Several adaptations have been observed within the cellular membranes of psychrophiles, with increased unsaturated fatty acids and shorter fatty acid chains being two commonly reported (Akagi, 1971; Moyer and Morita, 2007; Russell, 2007). Unsaturated fatty acids contain double bonds, which introduce “kinks” into the fatty acid chains preventing them from being packed closely together allowing for increased fluidity even at low temperatures (Shah, 2016). Shorter fatty acid chains have numerous advantages, but also help to increase fluidity by allowing lipids to move within the lipid bilayer more easily (Hassan et al., 2020).

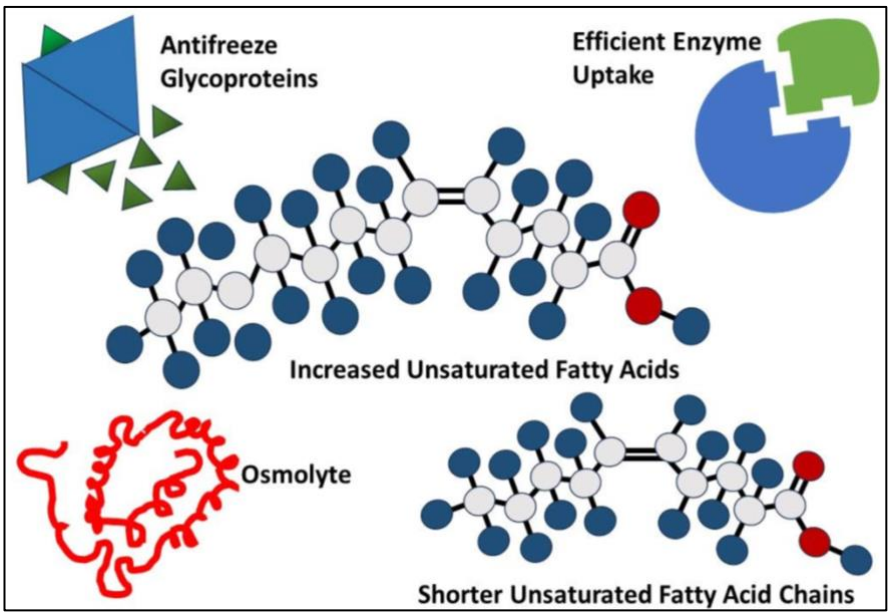

Figure 4: Adaptations of Cold Microorganisms. A visual representation of five characteristic seen in cold-adapted microorganisms including antifreeze glycoproteins, efficient enzyme function, osmolytes, increased number of unsaturated fatty acids, and short unsaturated fatty acid chains.

2.4.2 The Importance of Psychrophiles as Bioindicators

Because of their sensitivity to climate, cold microorganisms are valuable bioindicators, as changes in their distribution, abundance, or activity provide information about climate and glacial retreat. There is ongoing research into how psychrophiles are responding to climate change, however, evidence of their sensitivity to climate-related factors, such as nutrient availability, temperature, moisture availability, light levels, and salinity changes, are emerging (Gracía-López et al., 2016; Fell, Carrivick, and Lee., 2017; Gracía-López et al; Cvetkovska et al., 2022). For example, Buonigorno et al. (2019) has found that increased nutrient availability as a direct result of glacial retreat is disrupting the intricate balance of fjord ecosystems in Svalbard. Here, a growth in sulphate reducers was measured, in the species Desulfobacteraceae and Desulfobulbaceae, as total organic matter becomes more abundant. Buonigorno et al. (2019) predicts that if sulphate reduction surpasses sulphate oxidation, excess sulphate can precipitate into iron sulphate minerals, decreasing the downstream transport of iron and potentially influencing the productivity of phytoplankton populations. This, in turn, can have consequences for a vital atmospheric carbon sink, highlighting the importance of gaining a comprehensive understanding of the complex interactions between cold organisms and their changing habitat. Furthermore, research in the Austrian Alps have showcased psychrophiles sensitivity to increases in temperature, such as in the work of Wilhem et al. (2013). Here a relationship was observed between rising temperatures in glacially fed streams and microbial community composition, highlighting a potential link between global rising temperatures and reduced biodiversity. Moreover, changes to moisture content have become a prevalent reality for organisms in the cryosphere with changes to precipitation patterns, enhanced glacial melt, and thawing of permafrost being driven by climate. Psychrophiles are highly sensitive to these changes, as showcased by Altshuler et al. (2017) that found increased water availability was a key factor in enhancing the productivity of permafrost communities during thaw. This can be attributed to several factors such as the ‘reawakening’ of dormant communities that become active in response to water availability, the mobilization of nutrients, the mediation of temperature and reactions for metabolism, as well as the hydration of cells (Altshuler et al., 2017). Therefore, the sensitivity of these communities to climate can have cascading effects on global biogeochemical cycles.

2.4.3 Biotechnological applications

Beyond their ecological significance, cold microorganisms have practical applications in biotechnology and medicine. Bioremediation serves as a key example of this, with some psychrophiles possessing the ability to degrade organic contaminants such as hydrocarbons and persistent pollutants (Gregson et al., 2020). This unique capability is because they can produce enzymes capable of breaking down organic molecules. This has remarkable applications to oil spill clean-ups, potentially helping to mitigate environmental impacts of oil contaminated in cold seas (Margesin and Feller, 2010). Furthermore, compounds produced by psychrophiles may have pharmaceutical applications in the development of novel drugs and antibiotics. Some of these compounds have immunomodulatory properties that modulate the immune systems response and could be relevant to autoimmune diseases and immunotherapy for cancer treatment (Skjånes et al., 2021). Psychrophiles even has implications for astrobiology as scientist explore life in cold extra-terrestrial environments (Seckbach, 2000). This is because the mechanisms in which psychrophiles have adapted to survive in the harshest regions of Earth can inform astrobiologists about the potential mechanisms for life to prevail on other planets.

2.5 Glacier Ecology

In the intricate ecosystem of a glacier there are five distinct ecological zones. As depicted in Figure 5, three of these are located within the glacier itself including: the supraglacial surface, englacial interior, and the subglacial bedrock/ ice interface (Boetius et al., 2015). They differ in crucial factors like sunlight, ionic strength, pressure, pH, and redox potential, resulting in distinct habitats (Yarzábal, 2020). The supra-and-subglacial environments are host to biological hotspots (Hodson et al., 2008), that serve important roles in local biogeochemistry, whilst englacial ice has been found to support significantly lower biomass and metabolic activity. Additionally, there are two ecological zones connecting the glacier with its surrounding environment: proglacial streams and the surrounding periglacial landscape (Hodson et al., 2008).

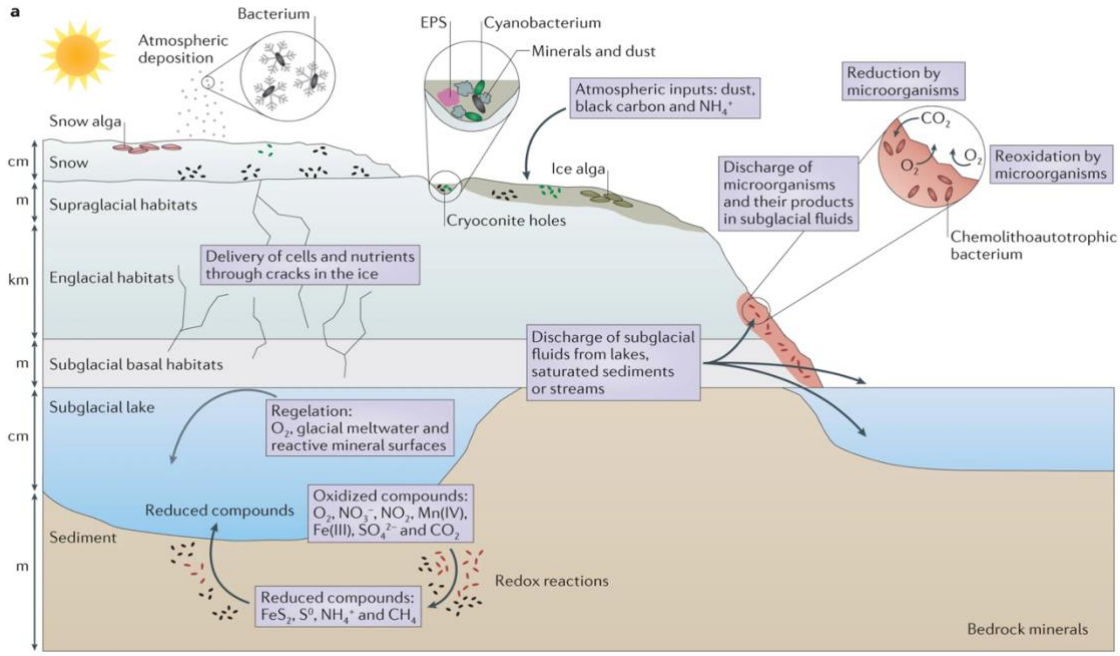

Figure 5: Glacial Habitats and Microbial Processes. *This cross-sectional diagram of a glacier outlines three ecological zones: supraglacial (cm-m), englacial (km), and subglacial environments (cm-m). The supraglacial environment features snow and ice habitats, including cryoconite holes, illustrating their interactions with the atmosphere. Hydrological networks are depicted, delivering cells and nutrients through englacial ice to the subglacial habitats. Microbial communities discharged from subglacial environment are shown within subglacial fluids and sediments. The diagram also demonstrates the interactions of subglacial basal habitats with subglacial lakes and bedrock sediment. Figure from Boetius et al (2015).*

2.5.1 The Supraglacial Environment

The glacier surface is host to a diverse community of bacteria, archaea, fungi, viruses, and algae (Anesio et al., 2017). Their abundance and diversity fluctuate geographically and between supraglacial habitats of snow and ice (Boetius et al., 2015; Musilova et al., 2015). During the summer months, the presence of liquid water and sunlight supports significant phototrophic activity at the surface, with glacier algae emerging as key primary producers (Williamson et al., 2019). The consistent observation of limited diversity in these communities has been a recurring focus in over 20 studies. Typically, these studies highlight the presence of only three key species, collectively referred to as 'glacier algae': Ancylonema nordenskiöldii, Mesotaenium berggrenii, and Cylindrocystis brebissonii (Williamson et al, 2019). These organisms are biogeochemically significant to supraglacial ecosystems, due to their role in nutrient cycling and the fixing of atmospheric carbon (Havig and Hamilton, 2019). For example, Takeuchi et al. (2019) identified a positive correlation between algae

and the abundance of bacteria, linked to nutrient production, showcasing the importance of algae to microbial community dynamics. However, in response to light stress, glacier algae have developed different light absorbing pigments (Lutz et al., 2014) that during algae bloom paint the surface with shades of green (Chlorophyll), red (Carotenoids), and yellow (xanthophylls) (Anesio et al., 2017). This process is termed 'biological darkening', and through the lowering of surface albedo, is responsible for driving accelerated glacial melt (Williamson et al., 2020). Cook et al. (2020) aimed to quantify the magnitude of this on the south-western sector of the Greenland Ice Sheet during the summer of 2017. Utilising a combination of techniques, including field spectroscopy and satellite remote sensing data, it was estimated that algal growth led to an additional 4.4-6.0 Gt of runoff from bare ice. Thus, it can be seen that supraglacial communities not only shape local ecosystems but significantly impact broader glacial dynamics.

2.5.2 Cryoconite Holes

Cryoconite holes are an example of a supraglacial habitat that forms on bare ice, and have been recognised as microbial hotspots, harbouring multiple trophic levels (Cameron, Hodson, and Osborn, 2012). Cryoconite granule formation is the product of autogenic ecosystem engineering, whereby allochthonous debris (Abakumov et al., 2021), which has become entangled through the filamentous properties of cyanobacteria, is stabilised through the cohesion of extracellular polymeric substances (EPS) that act as biogenic 'cements' (Cook, 2016). These quasi-spherical granules harbour microbial communities within their microstructures but are also responsible for the creation of a microenvironment referred to as cryoconite holes (Zawierucha et al., 2021). As illustrated by Figure 6, cryoconite holes are cylindrical depressions (mm-cm in depth) created through accelerated melting beneath an accumulation of cryoconite that, compared to the surrounding environment, has a lower albedo (McIntyre, 1984). A fraction of the microbial community found within the cryoconite granule is therefore suspended within the surrounding water, creating a rich aquatic environment containing bacteria, algae, fungi, and microfauna such as rotifers and tardigrades (Poniecka and Bagshaw, 2021).

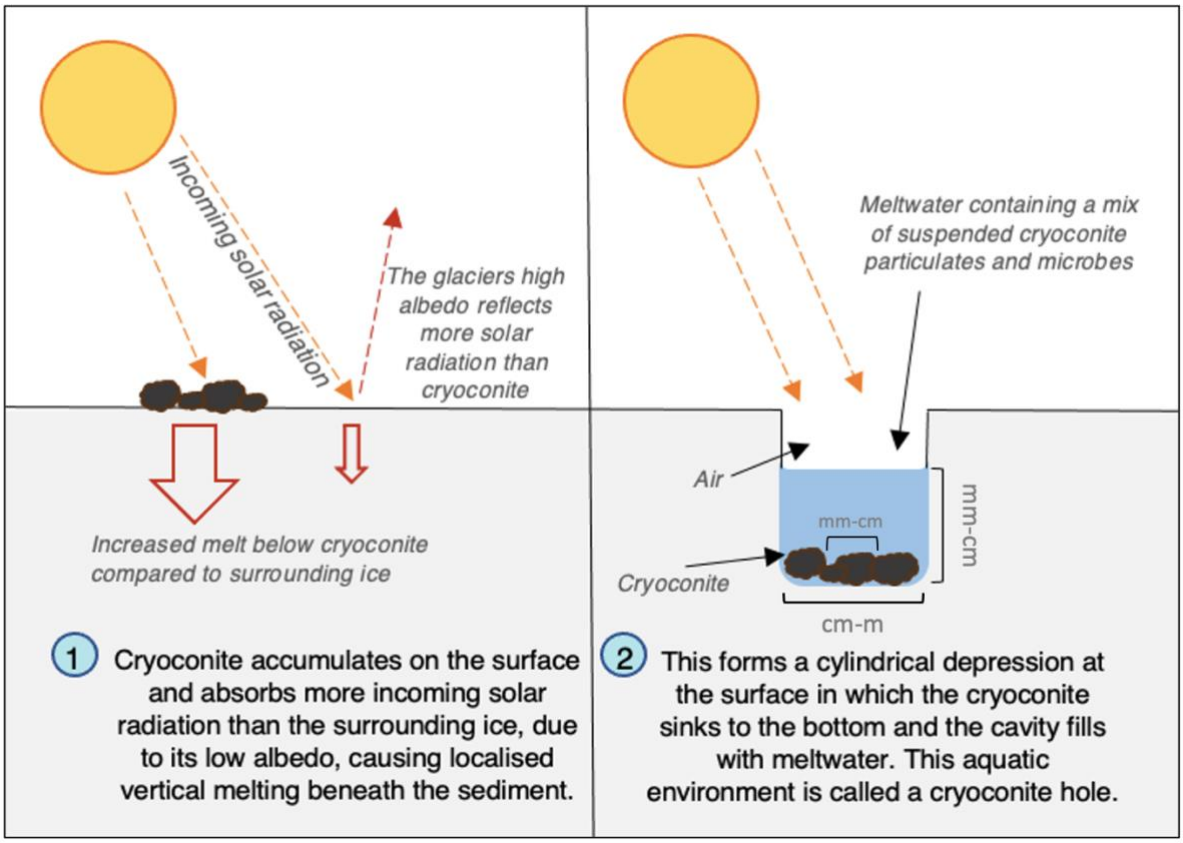

Figure 6: Cryoconite hole formation. *(1) cryoconite granules accumulate on the glacial surface, absorbing more solar radiation that the surrounding area, because of lower albedo, resulting in vertical melting. (2) Cylindrical depressions form due to accelerated melting resulting in an aquatic environment as the cavity fills with water and microbes become suspended from the sunken cryoconite.*

2.5.3 The Englacial Environment

Sandwiched between the surface and the bed, the englacial zone comprises the largest section of ice (Kim et al., 2022). The inaccessibility of this zone presents an enormous challenge to researchers, consequently leading to high levels of uncertainty in our knowledge, however, it is assumed that metabolic functioning within this region is negligible on a global scale (Hodson et al., 2008).

2.5.4 The Subglacial Environment

The subglacial environment, found between the glacier bed and ice, stands as a distinct domain with conditions such as light deprivation, scarce nutrients, low oxygen levels, and high pressure caused by the glacier ice above (Hamilton et al., 2013). Like the englacial

environment, the subglacial zone is a relatively inaccessible ecosystem, however, data collected from meltwater, ice cores, and sediments at the margin of the glacier have demonstrated the successful colonisation of microorganisms within this region (Turchettii et al., 2008). Subglacial microbial communities encompass diverse taxa and are broadly identified by their metabolic activity, being categorised as either autochthonous or allochthonous (Kaštovská et al., 2006). Autochthonous microbes originate from the subglacial environment and are seen to harness a variety of essential substrates to fuel their metabolic processes. Chemolithotrophs for example, derive energy from inorganic compounds like sulphur, iron, and hydrogen (Boyd et al., 2014). Conversely, allochthonous microorganisms remain in a dormant state, until metabolically favourable conditions are met upon removal from the glacier (Kaštovská et al., 2006). This group includes phototrophic microbes such as cyanobacteria and algae, unable to photosynthesize beneath the glacier due to the absence of light (Foght et al., 2004). Yet, despite their inactivity, these organisms play a pivotal role upon exposure to sunlight, becoming essential colonisers of newly exposed landscapes and contributing significantly to ecosystem development (Marshall and Chalmers, 2006). Additionally, these communities passively provide an organic carbon source for the active autochthonous communities. Other key sources of organic carbon, and essential nutrients, are entrapped within ice and released during thaw, or derive from supraglacial meltwater transported to the bed (Hotaling, Hood, and Hamilton, 2017). Furthermore, methanogenic archaea form a substantial component of subglacial communities, and play a significant role in subglacial carbon cycles. These archaea produce methane (CH_4) through pathways utilising hydrogen or carbon dioxide (CO_2), profoundly impacting meltwater geochemistry and contributing to biogenic gas production (Wadham et al., 2010). It can therefore be seen that despite the harsh and limiting conditions, subglacial environments host a diverse consortium of microbial communities and metabolic pathways.

2.5.5 Proglacial Streams

Sourced from glacial meltwater, proglacial streams (i.e., streams leaving the glacier) are the main pathway in which macronutrients and microbial communities are delivered into the surrounding catchment (Dubnick et al., 2017). Hydrological shifts induced by anthropogenic climate change have intensified these pathways, as microenvironments are more frequently

flooded and glacial microbes are discharged into the periglacial environment (Delaney and Adhikari, 2020). Drawing on both climate and hydrology models, microbial relocation from the glacier into the surrounding areas is expected to increase under future warming scenarios (Liu et al., 2022). Therefore, proglacial streams facilitate an important biogeochemical connection between glaciers and the neighbouring landscape under a warming climate. Figure 7 visually depicts the intricate physical and biogeochemical linkages between the glacier and its surrounding environment through proglacial streams, emphasizing the vital role these habitats play in the cycling of Nitrogen, Carbon, and Phosphorus (Ren et al., 2019).

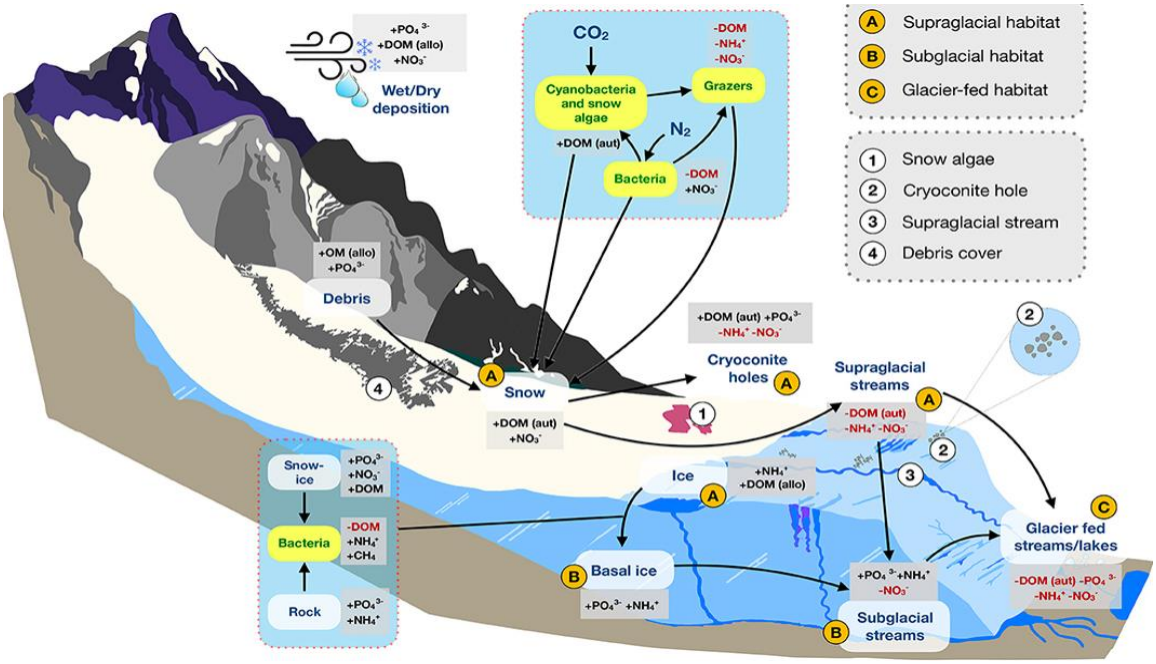

Figure 7: Habitants and nutrient dynamics of mountain glacier ecosystems. *Both major (A-C) and smaller (1-4) components of the mountain cryosphere are highlighted. Nutrient movement pathways are represented by arrows with plus signs (+) indicating nutrient sources and minus signs (-) indicating nutrient sinks. Organisms are represented by yellow boxes with green text. Abbreviations: allo (allochonous), aut (autochthonous), DOM (dissolved organic matter), PO_4^{3-} (phosphate), NH_4^+ (ammonium), CH_4 (methane), and NO_3^- (nitrate). Figure from Ren et al (2019).*

2.5.6 Periglacial Landscape

The periglacial environment describes the vast landscape surrounding a glaciated region, consisting of heterogeneous geomorphologies such as moraines, rock, fields, floodplains, and erosion channels (Murton, 2021). Covering 25% of Earth's surface (Ruiz-Fernández, Oliva, and Hughes, 2019), glacier forefields are rapidly expanding, having increased by 30%

in Europe's upland Alpine regions between the 1970s and 2003 (Paul, Frey, and Bris, 2011). This expansion is projected to continue, with global glacier mass loss between 2015 and 2100 expected to reach ~18% for the lowest emission scenario (RCP2.6) and ~36% for the highest emission scenario (RCP8.5). This corresponds to ~94 mm and ~200 mm of sea level rise, respectively (Shahgendanova, 2021). These newly deglaciated areas of land are typically oligotrophic, and subjected to harsh and fluctuating environmental conditions, such as freeze-thaw cycles (Bradley et al., 2016). Research at the Damma glacier in Switzerland suggests that, by comparison to atmospheric deposition (e.g., rain, snow, dust etc.), glacially derived microbes (e.g., supraglacial, englacial, and subglacial) are the major source of inoculate in recently exposed soils (Rime, Hartmann, and Frey, 2016). Successional characteristics appear to vary between microbial communities associated with different regions of the glacier. For example, chemoautotrophs (e.g., *Acidothiobacillus*, *Thiobacillus*, and *Gallonella*) associated with subglacial environments are more commonly found in younger soils (Mapelli et al., 2011), and supraglacial microbes such as cyanobacteria and various species of fungi are more prevalent in older soils (Sattin et al., 2009). Research on the colonisation of these environments, especially in the context of understanding biological and nutrient dynamics is still underexplored, however. The expanding periglacial landscape has the capacity to significantly impact biogeochemical cycles by giving rise to a novel terrestrial biosphere and serving as a dynamic ecosystem host (Bradley, 2021). Understanding more deeply the fate of glacial microbes upon removal from the glacier, and their role post-relocation will help to inform us about the ecosystem-level changes anticipated under a warming climate.

2.6 Periglacial Carbon Cycling

Whilst the characterisation of plant and microbial communities present within glacial forefields has been reasonably well documented (Kaštovská et al., 2005; Tscherko et al., 2005; Nemergut et al., 2007), studies examining their contribution to nutrient cycling upon glacier retreat are limited. Following deglaciation, microbial communities are seen to be active and are thought to be responsible for the development of initial nutrient pools (Strauss, Garcia-Pichel, and Day, 2012), however, their role in carbon and nitrogen turnover remains unclear. In cold regions ecosystem carbon (EC) is stored predominately in soil organic carbon (SOC), unlike in warmer regions where EC tends to be present in biomass

(Smittenberg et al., 2012). As anthropogenic warming increases, shifts in the distribution of carbon within cold ecosystems are expected, as observed by Cebrián and Duarte (1995), but predicting whether the periglacial environment will become a source or sink is challenging. Circumarctic permafrost (defined as soils that have remained frozen for a minimum of two years) is a large carbon reservoir, storing twice as much as the atmosphere (Moore and Waddington, 1998). Proxy data used to reconstruct the contribution of permafrost to atmospheric carbon during the last deglaciation, reflect nonlinear soil emissions (Meissner et al., 2021). This suggests that several biosphere-atmosphere feedbacks are involved in the cycling of permafrost carbon under enhanced retreat. The seeding of deglaciated soils with glacial material is a potential driver of these fluctuations, a process that has long attracted the attention of soil scientists who are faced with the challenge of predicting how higher-latitude soils will fare under the conditions of climate change. Consequently, several chronosequence studies exist showcasing a relationship between cryoconite accumulation and accelerated soil formation (Brankatschk et al., 2011; Franzetti et al., 2020). For example, Hallang et al. (2020) suggest that the inoculation of periglacial soils with cryoconite facilitates the settlement of higher plants, which leads to carbon fixation through photosynthesis in the short term and net carbon loss through respiration in the long term. Several approaches using ecological models have been developed in an attempt to predict the transformation of carbon within high Arctic soils, such as SHIMMER created by Bradley et al. (2016). Considering factors such as temperature, light, snow depth, allochonous inputs, and microbial community dynamics, SHIMMER predicts that the Midre Lovénbreen catchment will become a net source of carbon over the next 120 years of exposure. This is highlighted in Figure 8 which shows heterotrophic respiration increasing with soil age. It was found that allochonous inputs, from supra-and-subglacial ecosystems, were the most significant factor in controlling carbon flux; supporting the hypothesis that glacially derived microbes will influence periglacial carbon cycles. However, a current limitation of ecological models, such as SHIMMER, is that the quantification of allochonous inputs is currently underdeveloped as it does not consider the quantity, timing, and composition of these inputs. More research is thus required on the inoculation of these communities, as highlighted by Zazovskya et al. (2022), who state that the role of new cryoconite in these processes has not yet been properly evaluated.

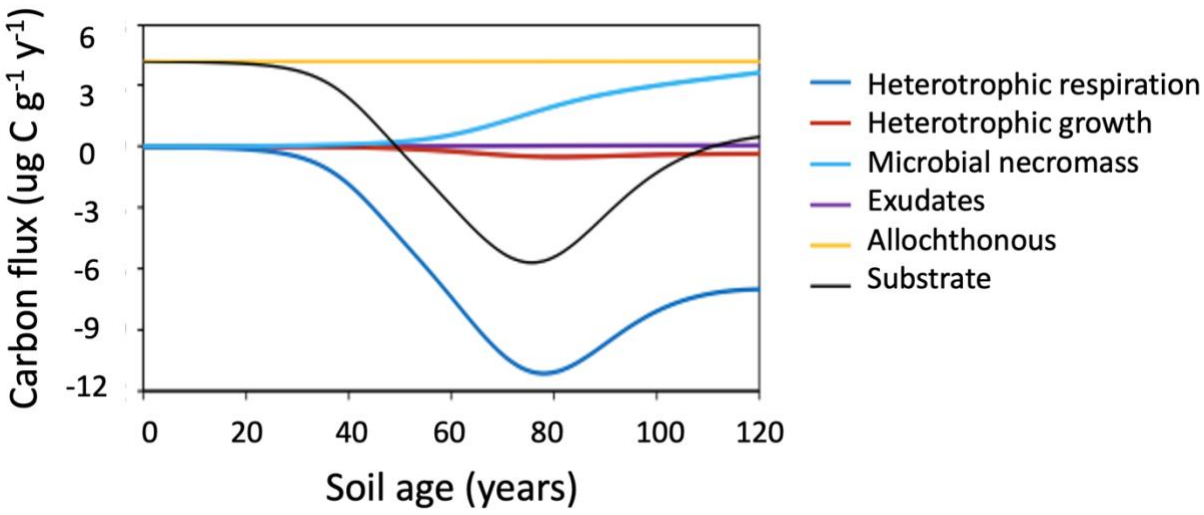

Figure 8: Carbon Flux Dynamics in the Midtre Lovénbreen Catchment over 120 Years. *The Figure illustrates the contributions of microbial necromass (light blue), exudates (purple), and allochthonous sources (yellow) to the substrate pool (black). Concurrently, heterotrophic growth (red) and respiration (dark blue) deplete this pool. Figure from Bradley et al. (2016).*

In addition to CO_2, CH_4 is a potent GHG that plays a fundamental role in the carbon cycle and global warming. Controlled by both anthropogenic (e.g., landfills and rice paddies) and natural inputs (e.g., wetlands and geological sources), CH_4 has contributed to approximately 16–25% of atmospheric warming since the pre-industrial era (Rosentreter et al., 2021). Data spanning from 2007 to 2019 indicates an annual increase of 7.3 ± 0.6 parts per billion (ppb) (IPCC, 2021). Under climate change, rising average temperatures are thought to enhance CH_4 exports from natural origins, especially within cryospheric regions (Zhang et al., 2021). Large exports of CH_4 have been measured from beneath the Greenland ice sheet during a melt season and have been found entrapped within subglacial sediments, suggesting glacial communities are important sources of CH_4 (Lamarche-Gagnon et al., 2019). Methanogens are among the viable communities that have been identified beneath glaciers, generating energy by producing methane from H_2 and/ or, simple organic compounds, such as acetate and methanol (Ma et al., 2018). These processes describe methanogenesis, which is a form of anaerobic respiration in the last stage of organic matter degradation (Welte and Depenmeier, 2014). Climate change is expected to influence methanogenic communities as increased temperatures alter the rate of deposition, influencing the amount and type of substrates available (Wadham et al., 2008). In general, endogenous CH_4 production from

organic carbon was positively correlated with temperature, suggesting that biogenic CH_4 is likely to generate a positive feedback system under global warming (Tandong et al 2002 ; Zhu et al., 2023). This can be seen in the work of Stibal et al. (2012) that exposed subglacial samples from the Arctic and Antarctica to temperatures ranging from 1-10°C and found that CH_4 production incrementally increased at each temperature (see Figure 9). However, Ma et al. (2018) observed that hydrogen-metabolising and acetate-metabolising methanogens may have different responses to temperature, with more ex-situ research exposing methanogens to different substrates and temperatures being required.

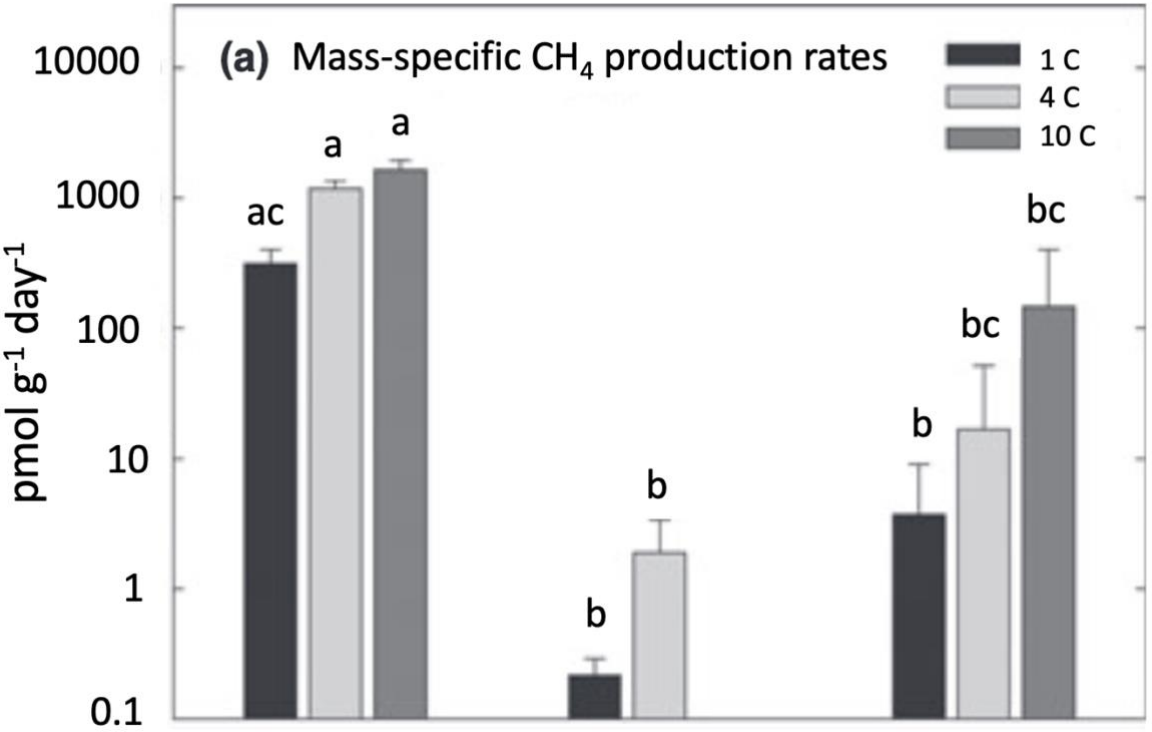

Figure 9: Methane (CH_4) Production Rates in Subglacial Sediment. *The Figure presents CH_4 emissions from the Lower Wright Glacier, Antarctica (LW), Russell Glacier, Greenland (RU), and the John Evans Glacier, Canadian Arctic (JE) at temperatures of 1°C, 4°C, and 10°C observed over a 720-day period. Similar lettered values indicate no significant difference. Figure from Stibal et al. (2012).*

2.7 Periglacial Nitrogen Cycling

Several studies have measured an abundance of microbial functional genes (MFG) relating to nitrogen cycling in glacier forefield soils (Kandeler et al., 2006; Zeng et al., 2016; Feng et al., 2023). As one of the central biogeochemical cycles in soil ecosystems, the nirogen cycle is driven by soil microorganisms (Wang et al., 2022). Here, MFG are involved in the biological processes of nitrification, denitrification, dissimilatory nitrate reduction to ammonium, N_2 fixation, and ammonification (Hu et al., 2021). Nitrogen fixation has been identified as an important process in the initial stages of soil development when bioavailable nitrogen is thought to be low (Bradley et al., 2016). Töwe et al. (2010) explored this in the forefield of The Damma Glacier (Switzerland) finding that in less developed soils, plants depended more on microbial inputs of nitrogen through fixation, than in more developed soils (Töwe et al., 2010). This suggests that as the periglacial environment expands, the residing microbial communities are important dictators and facilitators of initial nitrogen pools, and consequently soil development. Several studies have demonstrated cryoconite's nitrogen cycling abilities, serving as important biogeochemical hotspots and influencing productivity downstream (Bagshaw et al., 2013). The structure and microbial composition of cryoconite have been demonstrated to be highly conducive to nitrogen cycling, as shown in Figure 10 (Sagewa et al., 2014), with nitrification, assimilation, and denitrification occurring on their surface and within their anoxic core. Marker genes for nitrogen fixation (nifH), nitrogen mineralisation (chiA, aprA), nitrification (amoA AOB, amoA AOA) and denitrification (nirS, nirK and nosZ), have all been detected in samples from Central Asia, Svalbard, Antarctica, and Greenland (Telling et al., 2011; Cameron, Hodson, and Osborn., 2012; Segawa et al., 2014). Similarly, research on The Russell Glacier (Canada) in 2009 provided the first genetic evidence of nitrogen cycling within the subglacial environment (Boyd et al., 2011), with MFGs, such as amoA, narG and nifH, having been detected. At present, research has investigated the nitrogen cycle within glacial communities, but it has yet to explore how these processes could change upon relocation from the glacial environment. For example, MFGs could express differently within the periglacial environment as environmental stressors, such as increased light exposure or temperature, result in different genes being switched on and off. It would thus be of interest to assess whether nitrogen is cycled differently by glacial communities under

conditions representative of the periglacial environment, informing predictions of soil development and GHG release upon enhanced glacial retreat.

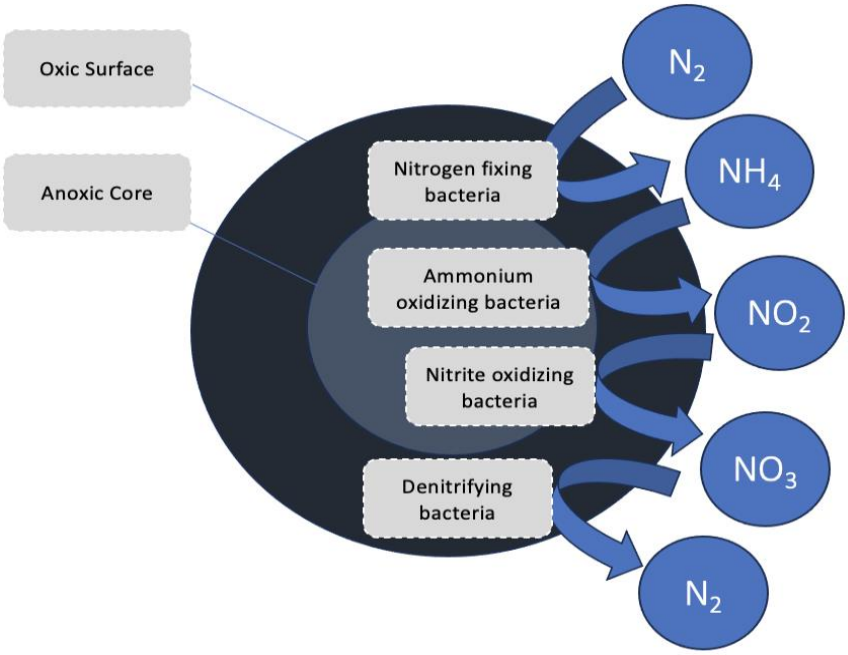

Figure 10: Nitrogen Cycling in Supraglacial Cryoconite Granules. *The diagram illustrates the intricate process of nitrification and denitrification within cryoconite granules, involving Nitrogen-fixing, Ammonium oxidizing, Nitrite oxidizing, and Denitrifying bacteria. It showcases the oxic surface (depicted by a dark blue ring) and the anoxic core (illustrated as a light blue circle). Abbreviations include: N_2 (dinitrogen) NH_4 (Ammonium), NO_2 (Nitrite), NO_3 (nitrate). Figure adapted from Segawa et al. (2014).*

2.8 Factors Influencing Periglacial Communities.

2.8.1 Temperature

Glacial communities are subject to frequent climate disturbances and environmental pressures (Chen, Liu, and Li, 2022), yet their resilience to physical changes upon relocation is unknown. Temperature has been proposed as one of the main physical factors controlling cold communities (Zarsky et al., 2013; Ambrosini et al., 2017). Therefore, understanding the response of microbial communities to changes in climate is essential to our understanding of ecosystem functioning. The disparity between temperatures of the glacier and periglacial environment is greatest over the summer months and can be expected to grow under

future warming scenarios (López-Moreno et al., 2016). For example, Svalbard has been identified as an area particularly sensitive to climate change, warming at a rate that is seven times faster than the rest of the world (Isaksen et al., 2022). In July 2020, a temperature of 21°C broke the record for the highest air temperature reached in Svalbard, a region which usually sits around 7°C at this time of year (Hauber et al., 2011). Førland et al. (2011) generated predictions for seasonal temperatures in Svalbard over the next 100 years using Empirical-Statistical Downscaling (a technique used to refine coarse resolution climate data by establishing statistical relationships between large-scale climate variable and local conditions) and regional climate models. Figure 11 shows these predications, demonstrating an expected rise in temperature overtime in Svalbard during all seasons. This should be considered when investigating the fate of relocated microbial communities, as the response of new microorganisms to the warming periglacial landscape will be different to that of previous communities inhabiting a much cooler periglacial environment. To date, studies aimed at measuring cryoconites resilience to elevated temperature have cultivated select microbes and measured their ability to grow under controlled temperatures. Poniecka et al's. (2021) research is an example of this approach, having extracted a mix of bacteria, fungi, and yeast isolates from cryoconite and exposing them to incrementally increasing temperatures ranging from 1- 40. This provided an insight into the resilience and varying robustness of different cryoconite organisms to temperature stress, for instance some bacterial isolates demonstrated growth at temperatures exceeding 22°C, while numerous yeast isolates failed to grow above this threshold. While cultivating microbes is a common practice, its limitations include providing optimal nutrient conditions and focusing solely on cultivable species. Observing changes in viability at the community level would be beneficial to measure and compare responses of different glacial communities to higher temperatures. This approach enables a comprehensive examination of the community response as a whole, capturing a broader spectrum of microbial diversity beyond what cultivation methods may reveal. Currently, no research has been conducted on the tolerance range of subglacial communities to this. Therefore, more research is needed to understand whether glacial communities are adaptable enough to cope with Arctic warming or whether dramatic changes to ecosystem functioning can be expected as a result of anthropogenic impacts.

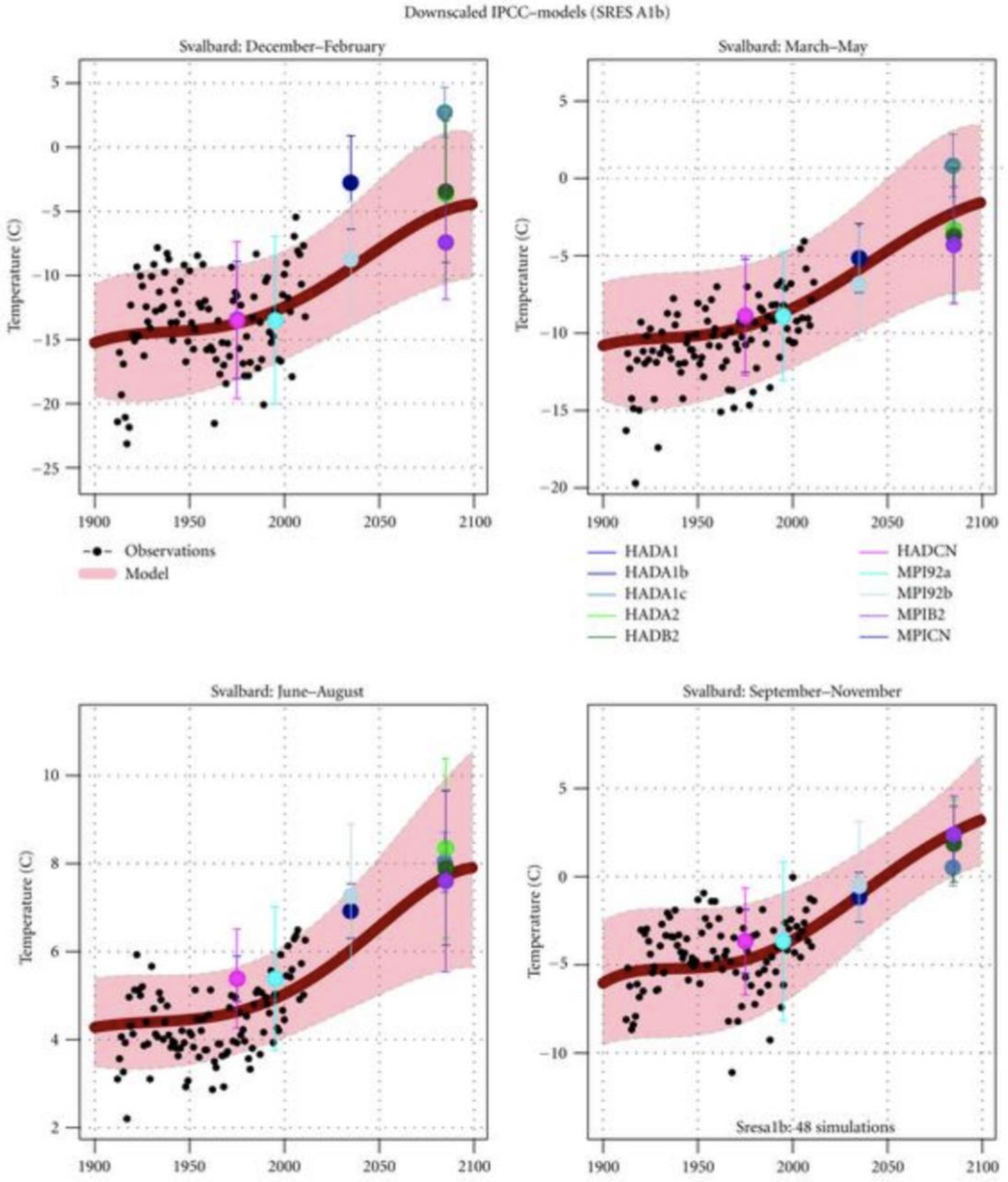

Figure 11: Temperature Projections for Longyearbyen, Svalbard. *Results generated from Empirical Statistical Downscaling (ESD) and Regional Climate Model (RCM) downscaling for (a) winter, (b) spring, (c) summer, and (d) autumn. Observed values are indicated by black dots, with the 50% median shown as a red line. 5% and 95% intervals for ESD are indicated by the pink region. Median values for RCM are indicated by coloured dots with the 5% and 95% intervals indicated by colour-matched vertical lines. Figure from Førland et al. (2011).*

2.8.2 Light

Light plays a crucial role in shaping the dynamics of supraglacial communities, influencing growth, productivity, diversity, and distribution. Phototrophic organisms populating the

glacier surface heavily depend on photosynthetically available radiation (PAR) for primary production. However, beyond a certain threshold light can lead to adverse effects, such as light stress. Given the prolonged duration and high intensity of light on the surface of glaciers during the summer months, numerous studies have delved into understanding how supraglacial communities adapt to harness this light alongside protecting against cellular damage. For instance, the research conducted by Williamson et al. (2020) has significantly advanced our comprehension of how algae communities react to intense light, unveiling adaptations like the pigment purpurogallin carboxylic acid-6-O-Beta-D. This serves as a protective mechanism by shading chloroplasts and converting surplus light into heat.

Understanding the adaptive strategies employed by cryoconite is essential for comprehending how light influences microbial relocation to periglacial environments. In Antarctica, the low temperatures ensure that cryoconite holes are shielded by an ice lid throughout the summer, effectively attenuating up to 99% of incoming PAR. In contrast, regions like the Arctic, with slightly milder temperatures, lack a sustained ice lid during the melt season. Bagshaw et al. (2016) identified that Arctic communities relied on different strategies to maintain ecosystem productivity, such as being frequently redistributed by meltwater (Bagshaw et al., 2016). Therefore, upon removal into newly deglaciated soils, cryoconite organisms will more heavily rely on behavioural and physiological strategies to mitigate light stress. Chlorophyll fluorescence analysis showed that phototrophic communities located within cryoconite holes on Longyearbyen in Svalbard deployed a mixture of responses (Perkins et al., 2017). Physiological adaptations included forms of downregulation such as non-photochemical quenching, which describes the process of dissipating excessive absorptions of light into heat to protect against physical damage (Anesio, et al., 2017). Behavioural strategies were also utilised to optimise productivity through cell motility, changing its location on the granule from areas of high to low light.

Currently, nothing is known about the tolerance of subglacial communities to light stress. Formed predominately from microorganisms that function in the absence of light, subglacial communities could be less equipped to adapt to new levels of solar radiation upon emergence from the glacier. Despite the dark conditions found at the glacier bed, there is evidence of photoautotrophs (Cameron et al., 2020), which could thrive upon relocation and are consequently thought to be important colonisers (Kaštovská et al., 2005). Viability

analysis would be insightful in monitoring community change when faced with greater light exposure.

2.9 Methodologies for Gas Sampling

Ex-situ experiments in glaciology serve as valuable complements to in situ fieldwork, enriching our comprehension of natural processes. For example, Perkin et al. (2017) coupled in-situ measurements of variable chlorophyll fluorescence and ex situ microscopy analysis to better assess cryoconite's photoautotroph community and photo physiology. Ex-situ experiments also function independently as tools for precise manipulation of specific environmental variables within controlled laboratory setting. Microcosms, representing small, controlled environments, are frequently employed to replicate and study intricate ecosystem interactions (Fraser and Keddy, 1997)- a particularly effective approach in glaciology, where harsh or inaccessible environments, like the subglacial environment, pose challenges to directly study. For example, Boyd et al. (2011) utilised microcosms to emulate nitrification rates in subglacial communities by creating anaerobic incubations comprising 7.5g of wet sediment and 75mm of pore water. They conducted qPCR analysis to estimate the abundance of nitrifying bacteria, shedding light on subglacial microbial processes and the nuances of microcosms. Boyd et al. (2011) discovered that nitrification rates from sediment incubated from the Russell Glacier were at the upper limit of what had been measured in situ. This underscores the disparity between laboratory and field findings, emphasizing the crucial need for more research aimed at bridging this gap in understanding. Compared to other environmental samples, especially temperate soils, microcosms containing glacial samples have undergone fewer tests and experiments. Consequently, there is less knowledge about the specific factors that should be considered when setting up glacial microcosms. For instance, the practice of headspace replenishment, which involves replacing the headspace within closed microcosms with fresh air or gas, is a common technique when incubating temperature soils (Walworth, Woolard, and Harris., 2003; Davis et al., 2005; Tang et al., 2016). This replenishment is necessary to regulate pressure, address gas accumulation, and prevent oxygen depletion (Davis et al., 2005). While it is generally accepted that glacial communities exhibit slower rates of microbial activity compared to temperate soils, the assumption that frequent headspace replenishment may not be

necessary should be considered within the context of the experiment's specific objectives and duration. In instances where short-term responses or specific gas dynamics are under investigation, precise control over the headspace may prove essential. Therefore, the decision regarding the necessity of headspace replenishment in glacial microcosm studies should be made on a case-by-case basis, considering the experimental goals and parameters. Therefore, more studies working with glacial samples should integrate testing of headspace to develop our understanding of this further.

2.10 Initial Study Goal

Originally, this study aimed to explore the intricate relationship between surface and subsurface glacier ecosystems in response to a warming climate. The acceleration of glacial melt, a consequence of global warming, has strengthened hydrological channels such as moulins and crevasses, enabling a greater transfer of organisms from the surface to the subglacial environment (Hodson et al.,2008). This phenomenon has been identified as a potential factor contributing to increased GHG emissions observed from retreating glaciers (Du et al., 2022). To investigate this, we devised a microcosm incubation experiment wherein cryoconite was to be exposed to various conditions characteristic of the subglacial environment, including oxygen-depleted, dark, and high-pressure conditions. To achieve this, an anaerobic chamber was to be set-up to create anoxic incubations, and samples were to be covered in aluminium foil and subjected to 1.5mpa (220 psi) at 1.5ºC. To analyse the response of cryoconite, live/dead staining would be employed in combination with BONCAT to evaluate changes in community viability and metabolic activity, respectively. BONCAT is a technique used to selectively label and track actively metabolizing microbial cells within a complex community. Given that BONCAT represents a relatively recent technique in polar sample applications, we sought to enhance current laboratory methodologies for studying metabolic activity in sediments by collaborating with Dr James Bradley (Queen Mary University, London) and building upon his preliminary work.

Furthermore, gas chromatography was to be employed to measure changes in CO_2, CH_4, and N_2O, in the incubation headspace. qPCR analysis of 16S rRNA genes aimed to track changes in bacterial community composition over the experiment's duration, which was intended to span 96 days with regular sampling points.

Unfortunately, the project faced significant setbacks due to COVID-19 implications and unforeseen circumstances. Initial travel delays, a consequence of COVID-19 restrictions, led to the rescheduling of fieldwork until later in the melt season. This delay resulted in the inability to collect samples due to the onset of winter weather conditions, preventing access to the sampling site. As an alternative, we sought to find collaborators who could supply alternative material, which further added to delays. Upon successfully obtaining samples, a major setback occurred with the unplugging of the freezer storing them. The freezer remained unplugged for an unknown period (likely weeks), leading to the warming of all samples, with consequences for the viability and validity of these samples for these investigations. This incident therefore required a reassessment of the project's design and timelines. When the revised project commenced, an irreparable breakage in the gas chromatography machine was discovered, requiring us to source an alternative machine from an external institute. Although eventually sourced in Bangor, this process involved collecting appropriate vials from Wales and transporting the samples to the university. Additionally, during preliminary research on microscopy techniques, issues arose with the microscope. A suitable replacement was obtained from another department. However, due to other students also facing setbacks due to COVID, the booking system became extremely limited, with sampling slots available weeks apart. Consequently, the project's scope and goals were adapted, as detailed in section 2.11. Due to constraints in sample availability and limited laboratory time, BONCAT, 16S rRNA analysis, and anabolic chamber analyses were removed, as outlined in the appendix. Microcosm numbers additionally had to be limited on account of reduced sample availability.

2.11 Revised Study Goal:

There is a growing interest in the sensitivity of cold ecosystems to climate change and the responses of microbial communities. However, the role of glacial microorganisms in processes associated with climate change, such as deglaciation, is currently unclear (Margesin and Colins, 2019). Ascertaining a better knowledge of changes to the function

and dynamics of microbial communities under a warming climate allows for an earlier and more appropriate reaction. Questions remain about the processes associated with the inoculation of periglacial landscapes with glacial microbes and the impact of this on

biogeochemical cycling within the early stages of succession. Furthermore, it remains unclear whether glacial microorganisms are adaptable enough to respond to the expected rates of climate change within the Arctic, or if changes to viability and functioning at the community scale will lead to ecosystem-level changes. This study aims to investigate the influence of elevated temperatures on microbial metabolic processes and viability within cryoconite and subglacial communities. Specifically, we will assess their response to extreme conditions observed in Svalbard during the summer of 2020 (21°C), average temperatures found at the glacier surface (1°C), and periglacial environments during the summer months (7°C). Utilising laboratory incubation techniques and gas chromatography analysis to measure biogenic greenhouse gas emissions, our objective is to enhance our understanding of the potential contributions of relocated glacial communities to global biogeochemical cycles under climate warming. Beyond exploring the metabolic consequences of heat stress, a primary goal is to investigate the resilience of these two distinct glacial communities. With a specific focus on assessing variations in viability over a 5-week period, employing live/dead staining and microscopy analysis, we aim to comprehend how temperature might impact the capacity of microbes to colonise newly deglaciated soils.

Hypotheses

1. Cryoconite communities demonstrate greater resilience to temperature increases compared to subglacial communities.

2. Elevated temperatures will lead to decreased viability in both cryoconite and subglacial communities.

3. Rising temperatures will lead to an increase in the concentration of CO_2, N_2O, and CH_4 in the studied environment.

4. Replenishing the headspace will be effective in preventing the accumulation of gases within microcosms.

3.0 Methods and Materials

3.1 Sample Details

All samples used for this research were kindly provided by James Bradley (Queen Mary University, London) and Arwyn Edwards (Aberystwyth University, Wales) who collected cryoconite and subglacial till on glaciers located in West Svalbard (Figure 12). Table 1 outlines the sample type and source. Samples were transported at 4^{o}C and stored at -18^{o}C.

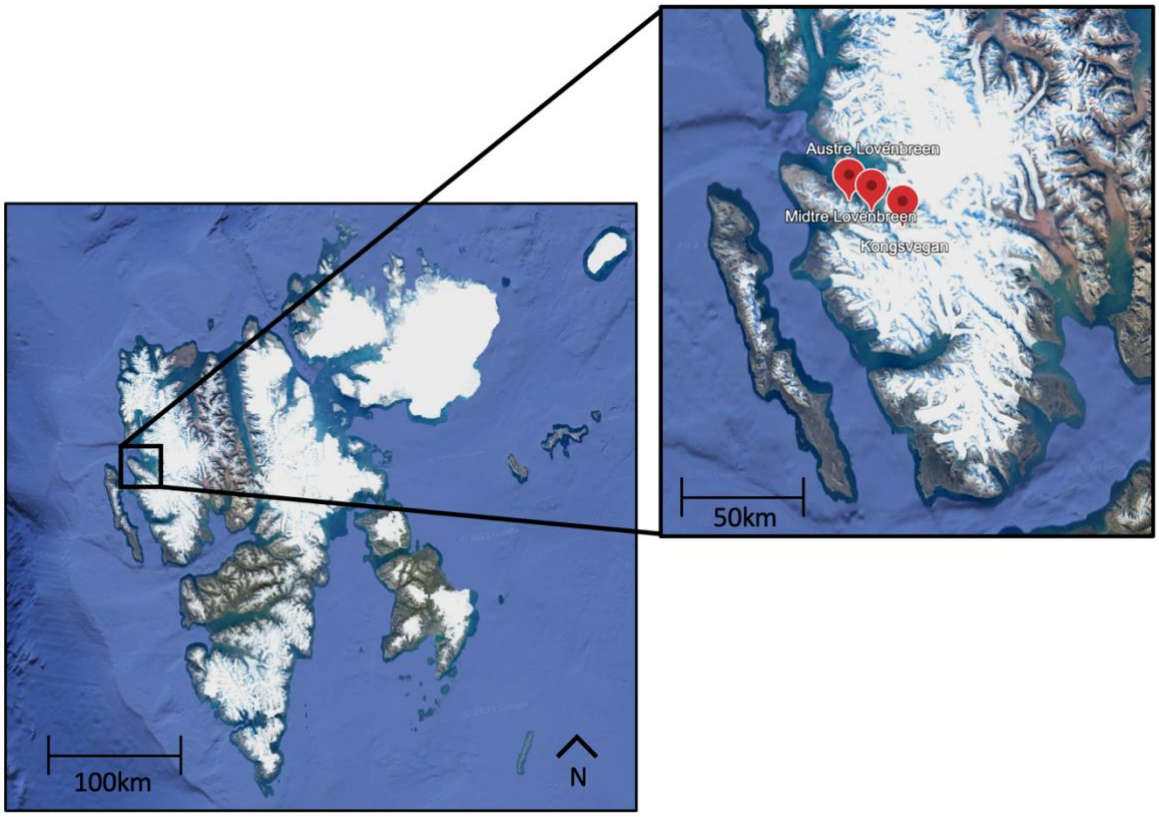

Figure 12: Geographical Sampling Locations in Svalbard. *This map illustrates the precise geographical positions of sample collection sites—Austre Lovenbreen, Midtre Lovenbreen, and Kongsvegan glaciers—marked by red pins. The locations are highlighted to provide contextual reference within the landscape of Svalbard and their spatial relationship to each other. Data sourced from Google Earth (2023).*

Table 1. Sample type, source, and year of collection. *Sample ID allocation used to identify cryoconite (C) and subglacial (S) samples collected from different locations.*

Sample ID	Sample Type	Sample Source	Year Collected
C1	Cryoconite	Austre Lovénbreen (78.909629^0E, 11.937759^0N)	2013
C2	Cryoconite	Midtre Lovénbreen (78.915384^0E, 11.768171^0N) and Foxanna (78.133254^0E, 16.2000279^0N)	2017
S1	Subglacial Till	Midtre Lovénbreen (78.915384^0E, 11.768171^0N)	2017
S2	Subglacial Till	Kongsvegen (78.754022^0E, 16.2000279^0N)	2010

3.2 Incubator Set-Up and Temperature Conditions

To set up conditions that were representative of summer temperatures in Svalbard at the glacier surface (1^OC), in the periglacial environment (7^OC), and in the periglacial summer extreme (21^OC), 3 incubators were used. Incubators were cleaned with 70% ethanol and left to stand at their selected temperature for seven days prior to starting the experiment. Lights (410nm) were fitted to each incubator to provide 24/7 light throughout the experiment.

3.3 Sample Preparation

Prior to being weighed into microcosms, all samples were thawed at room temperature. C2 and S2 samples were then autoclaved at 121^OC for 90mins to achieve a kill control group. 2g of sample was set up in quadruplicate for each sampling time point (T1, T2, T3, T5), and temperature conditions (1^OC, 7^OC, and 21^OC). This was repeated for dark conditions where bottles were covered in aluminium foil. In total, there were 96 microcosms for C1 and S1 samples, and 96 kill control microcosms, made up from samples C2 and S2 as seen in in table 2. C2 and S2 samples were chosen for kill control because they had been subject to thaw during the turning off of the incubator described in section 2.10, and thus were not homogenised with the other samples (C1 and S1) and used in the living conditions.

Table 2: Experimental Conditions for Samples. *This table outlines the varied conditions to which samples were subjected. It details the temperatures (1°C, 7°C, 21°C) and light exposure (Light or Dark) for each sample. Additionally, it indicates any supplementary treatments applied (e.g., killed).*

Sample ID	Temperature Condition (*°C)*	Light Condition	Additional Treatment
C1	1, 7, 21	Light & Dark	N/A
C2	1, 7, 21	Light & Dark	Killed
S1	1, 7, 21	Light & Dark	N/A
S2	1, 7, 21	Light & Dark	Killed

3.3.1 Sample Preparation Limitations

Originally, the plan was to expose all subglacial and cryoconite samples to both light and dark conditions across the three temperature conditions. However, due to unforeseen sample limitations (please refer to the appendix), it became necessary to eliminate one of the conditions. Consequently, the decision was made to omit the subglacial till under light conditions at 1^{O}C. This choice was justified by the consideration that samples at 1^{o}C were intended to mimic the conditions typically encountered within a glacier, where light does not penetrate. In contrast, cryoconite is naturally found in both light and dark conditions within the glacial environment. This study includes comparisons between subglacial till at 1^{o}C under dark conditions and subglacial till at 7^{o}C and 21^{o}C under light and dark conditions, to represent subglacial till transported to the periglacial environment. These comparisons serve as preliminary indicator of the influence of microbial relocation on factors such as microbial viability and gas production. However, caution is advised in interpreting these comparisons, as subglacial samples at 1^{o}C were not subjected to light as the other two temperature conditions were.

3.4 Sampling Approach

Once assembled, microcosms were left to settle for seven days as an open system until airtight butyl caps were crimped onto each microcosm following the first sampling time point. Figure 13 depicts a diagram of the microcosm structure including the headspace to sample ratio (60mL: 2g). Sediments were naturally saturated in ~1-2ml of meltwater and therefore no additional water was added into microcosms. While logistical constraints

during the COVID-19 pandemic prevented the establishment of an anaerobic chamber, it was hoped that microcosms would become anoxic during the longer incubations. The sampling approach followed a destructive method in which microcosms were eliminated for analysis. At each time point, 4 light and dark replicates of each sample (C1, C2, S1, and S2) were analysed for each temperature condition. Over a 5-week incubation period, sampling took place on 7, 14, 21, and 32 days (T1, T2, T3, and T5). This sampling approach was used to capture any potential rapid fluctuations in microbial activity.

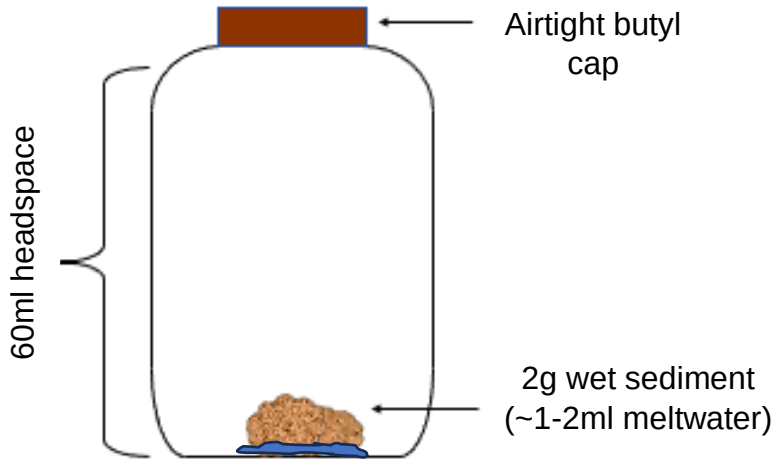

Figure 13: Microcosm set-up. *Diagram showing the microcosmic incubation of 2g of sample, 60ml headspace, and closed system through an airtight butyl cap.*

3.5 Fluorescence Microscopy

3.5.1 Sample Preparation

Relative viability counts were obtained using the live/ dead Baclight viability kit. A 3uL mixture of fluorescent nucleic acid stains, Syto 9 and Propidium iodide, was added to 1g of sample with 1 mL of MilliQ water. Samples were vortexed for five minutes to separate aggregates and a 1mL aliquot was extracted. It should be noted that no further disaggregation steps were followed due to the challenges associated with removing cells from their attachment sites. Although this is sufficient for relative viability counts, this technique has inherent biases and reduces the effectiveness of imaging slides which can reduce the accuracy of counts. Aliquots were incubated in the dark for 15 minutes. After this, samples were filtered onto a 47mm and 0.45um (COBETTER MCE Membrane Filter)

pore-sized filter and transferred onto slides with a coverslip for fluorescence microscopy analysis. Because cell counts were relative, filter volumes were chosen to get the best image resolution and avoid overloading with sediments.

3.5.2 Microscopy Analysis

Slides were imaged using the Lecia DMi8 widefield microscope using the Lecia Application Suite (LAS X) software. On 40x magnification, GFP and Y5 channels were used to capture 3, 332.80um^2 images per slide. Syto 9, with the excitation/ emission maxima of 480/500nm, binds to DNA and fluoresces green (live) under GFP channels; whilst propidium iodide binds to damaged cell membranes and fluoresces red (dead) under Y5 channels at the excitation/ emission maxima 490/635nm. Images were exported as TIFF files and analysed on the software FIJI with the live/ dead package. Three replicates were collected per microcosm. Viability counts were converted into the percentage of living microbes using equation 1:

(1)

$$\left(\left(\frac{Living\ Cell\ Count}{Total\ Cell\ Count}\right) \times 100\right)$$

3.6 Time Series Analysis Using Gas Chromatography

The biogeochemical activity of each sample was investigated through gas concentration measurements. N_2O, CO_2, and CH_4 were measured from 20mL samples that had been extracted from the 60mL headspace, using a syringe and needle. Extracted gas was immediately transferred into pre-evacuated vials and stored in the dark at room temperature, until being transported to Bangor University for gas chromatography analysis (Thermo Scientific Q Exactive Plus Orbitrap LC-MS.MS System). Following the destructive sampling method, microcosms were removed from the experiment after sampling.

3.6.1 Calculating CO_2 Fluxes from Headspace Concentrations

Once headspace concentrations of CO_2 had been measured using GC, they were converted into an estimated flux using a method originally developed by Franzblubbers (1996). This allows for GC measurements to be better assimilated into climate research that report

terrestrial emissions of GHGs as fluxes which can be utilised in predictive climate models for estimating ecosystem carbon budgets. Equation 2 was used to convert static concentrations into rates:

(2)

Step 1:

$$\text{Cm } (\mu g\ CO_2\text{-C L headspace}^{-1}) = \frac{Cv \times M \times P}{R \times T}$$

Step 2:

$$\text{mg } CO_2\text{-C kg soil}^{-1} = \frac{\mu g\ CO_2\text{-C L headspace}^{-1} \times \text{headspace volume (0.06L)}}{\text{weight of sample (2g)}}$$

Step 3:

$$\text{mg } CO_2\text{-C kg soil}^{-1}\ day^{-1} = \frac{\text{mg } CO_2\text{-C kg soil}^{-1}}{\text{incubation period (days)}}$$

Where:

Cm= μg CO_2-C L headspace^{-1}
Cv= ppm
M= molecular weight of C (12 μg/ mol)
P= Barometric pressure (1atm)
R= Universal gas constant (0.0820575L)
T= Incubation temperature in ºC (1, 7, or 21)

3.6.2 Calculating the Temperature Sensitivity of Glacial Samples Using the Q10 Coefficient

Once CO_2 fluxes (mg CO_2-C kg soil^{-1} day^{-1}) had been calculated using GC concentrations a Q10 coefficient was calculated to indicate the sensitivity of glacial samples to temperature. The Q10 coefficient works by comparing CO_2 fluxes calculated from exposure to temperatures 10^{o}C apart. Therefore, fluxes measured for cryoconite under dark conditions, representing respiration rates, and incubated at 1^{o}C and 21^{o}C, were used in equation 3 to calculate the Q10 coefficient:

(3)

$$\boldsymbol{Q10 = \left(\frac{R2}{R1}\right)^{\frac{10}{T2-T1}}}$$

Where-

R2= The rate of the process at temperature 1

R2= The rate of process at temperature 2

T2= Temperature 2

T1= Temperature 1

3.6.3 Gas Sampling Approaches

In addition to the gas analysis approach described in 3.6, a second gas sampling approach was implemented to test whether there was a build-up of gas over time that impacted function. This was achieved by serial sampling from four microcosms over a 36-day period (at time points 7, 14, 21 and 36 days), where the collected microcosm gas was replenished with air collected from the back of a sterilised positive fume hood, before returning the microcosms to the incubators for further incubation. The microcosms used for this test contained microcosms exposed to dark conditions at $1^{O}C$.

3.6.4 Light and Dark Incubations

Cryoconite's Net Ecosystem Production (NEP), defined as the difference between gross primary production (GPP) and total ecosystem respiration (*R*), is an important indicator of biological activity and local biogeochemistry. Consequently, CO_2 measurements paired with light and dark microcosms were used to estimate NEP and R, respectively, for each sampling time point, allowing GPP to be determined by the difference (Hodson et al., 2010) as seen in equation 4:

(4)

$$NEP = GPP - R$$

3.7 Statistical Analysis

Analysis of Variance (ANOVA) was conducted on SPSS to evaluate whether increases or decreases in either viability or gas concentration were significant between each time point.

4.0 Results

This study focused on microcosmically incubating cryoconite, and subglacial samples, obtained from both supra- and subglacial environments of glaciers in Western Svalbard. The microcosms were maintained at temperatures representing the average summer conditions of the glacial surface (1°C), periglacial environments (7°C), and periglacial summer extremes (21°C). Additionally, the samples underwent different light exposure, being kept in either light or dark conditions. The incubation period spanned five weeks, during which samples were systematically analysed at four distinct sampling points (weeks one, two, three, and five). This approach allowed for an examination of cell viability and total gas concentrations. The primary objective was to explore the impact of periglacial temperatures on both community viability and biogeochemical activity within the samples.

4.1 Viability Counts Using Fluorescence Microscopy

Figure 14 shows fluorescence microscopy images with a resolution of 332.80µm displaying positively stained cells fluorescing in both cryoconite and subglacial samples. These images were used to establish relative viability counts using the Fiji software. Figure 14a and 14b highlight selected cryoconite and subglacial samples, respectively, indicating a high number of viable cells through cyan-coloured dots. Figures 14c and 14d present a lower abundance of dead cells in the corresponding cryoconite and subglacial samples, represented by magenta fluorescing dots and chains. This contrast allows for a quantitative assessment of the relative viability of cells. The counts were transformed into percentage viable data for statistical analysis, providing a basis for evaluating the microbial viability within these glacial samples.

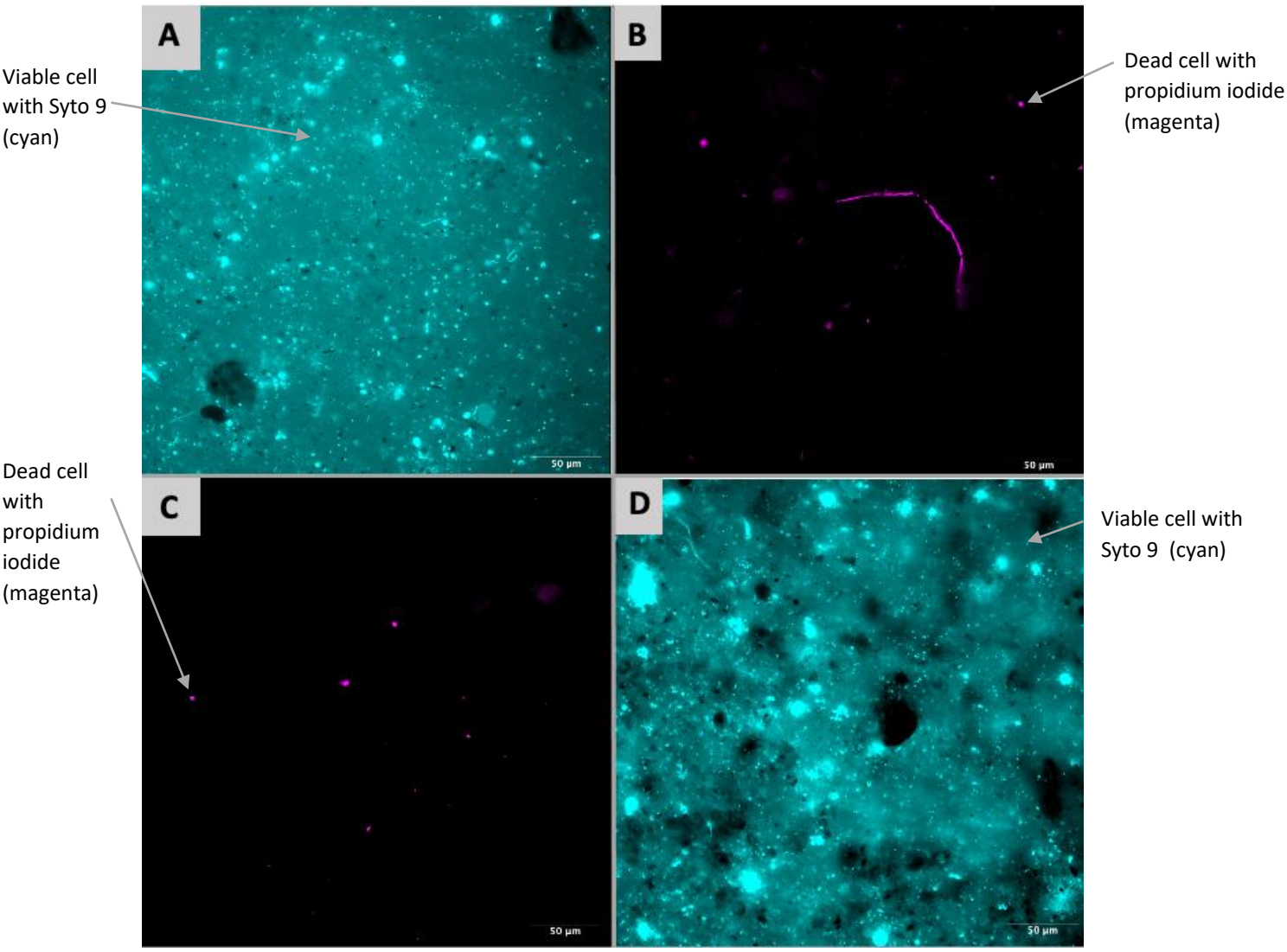

Figure 14. Relative Viability Counts using Live/ dead BacLight Viability Kit. *A. Cryoconite sample positively stained for Syto 9 and viewed at 40x magnification under GFP channel. B. Cryoconite sample positively stained for propidium iodide and viewed at 40x magnification under Y5 channel. C. Subglacial Sediment positively stained for propidium iodide and viewed at 40x. magnification under Y5 channel. D. Subglacial Sediment positively stained for Syto 9 and viewed at 40x magnification under Syto 9 channel.*

4.2. Fluctuations in viability and headspace gas concentrations

Table 4 outlines the findings from a one-way analysis of variance (ANOVA) that was conducted to determine the significance of fluctuations in community viability (see figure 16) and gas concentrations (see figure 21) across various sampling points. Multiple sampling intervals were strategically established throughout the experiment to capture potential fluctuations in communities exposed to distinct temperatures over time. This was rooted in the assumption that these communities would exhibit varying levels of viability and activity across different time points and temperatures.

Table 4: ANOVA from Microbial Viability and Headspace Gas Concentrations: This table presents significant (p<0.05) findings from ANOVA analysis on live/ dead viability counts and headspace gas concentrations (CO_2, N_2O, CH_4) between time points (T1-T5) and temperatures (1^OC, 7^OC, and 21^OC). The leftmost common delineates the test type, situated next to the sampling week. Sampling weeks that saw a significant change at the p=0.05 are symbolised by a single asterisk (*) and a double asterisk (**) for p=0.01. Non-significant results are reported as *(n.s).* Not applicable (N/A) refers can be found in subglacial at 1^OC in the light which was not tested due to study limitations.

Test	Sampling Point (weeks)	1 ^OC				7 ^OC				21 ^OC			
		Cryoconite		Subglacial		Cryoconite		Subglacial		Cryoconite		Subglacial	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Percentage Living	1 to 2	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	**	*n.s*	*n.s*	*	*n.s*	*n.s*
	2 to 3	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	**	**	**	*n.s*	*n.s*	*n.s*
	3 to 5	*n.s*	*n.s*	N/A	**	*n.s*	*n.s*	**	**	**	*n.s*	*n.s*	*n.s*
CO_2 Concentrations	1 to 2	**	*n.s*	N/A	*n.s*	**	*n.s*	*n.s*	*n.s*	**	**	*	*n.s*
	2 to 3	*n.s*	*n.s*	N/A	**	*n.s*	*n.s*	*n.s*	**	**	**	*n.s*	*n.s*
	3 to 5	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	**	**	*n.s*	*n.s*
CH_4 Concentrations	1 to 2	*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
	2 to 3	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
	3 to 5	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	**	*n.s*
N_2O Concentrations	1 to 2	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
	2 to 3	**	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
	3 to 5	*n.s*	*n.s*	N/A	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*

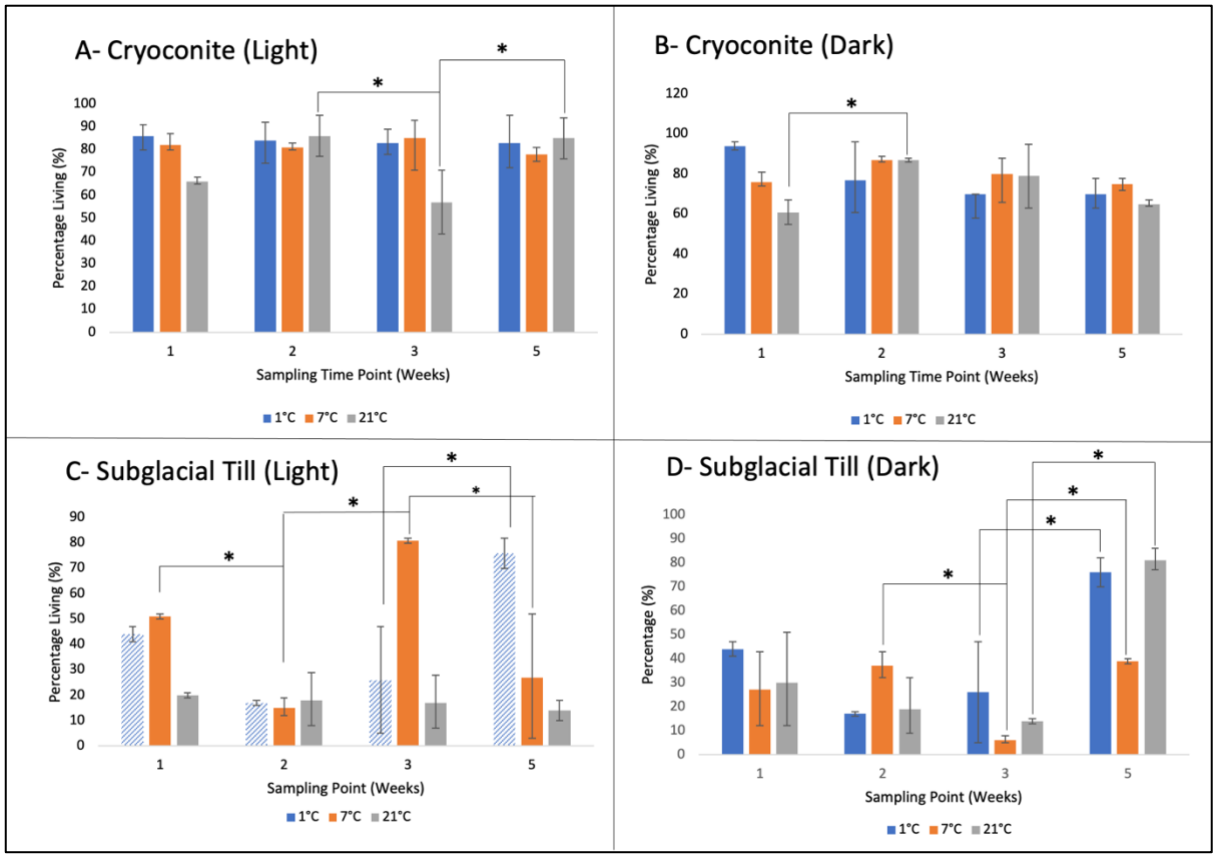

Figure 15: Temporal Variations in Percentage Living. *This Figure depicts the temporal dynamics of percentage living (%) for* ***(a)*** *Cryoconite in the light* ***(b)*** *Cryoconite in the dark* ***(c)*** *subglacial till in the light, and* ***(d)*** *subglacial till in the dark across different temperatures: 1°C (blue bar), 7°C (orange bar), and 21°C (grey bar). Significant changes obtained through ANOVA are denoted by asterisks (*).*

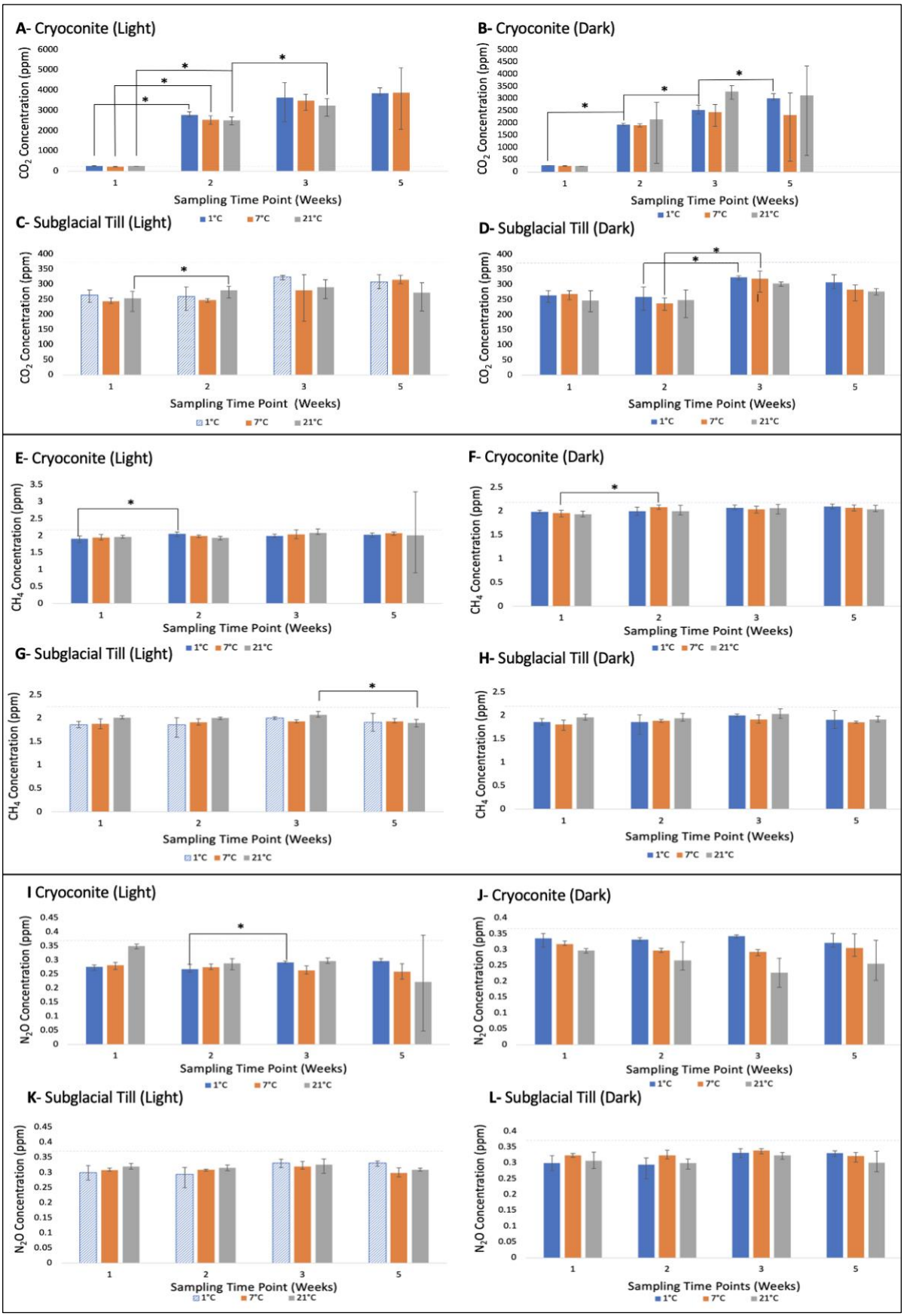

Figure 16: Temporal Variations in Gas Concentrations. This Figure depicts the temporal dynamics of headspace gas concentrations (ppm) for CO_2 **(a-d),** CH_4 **(e-h),** and N_2O **(i-l)** across different temperatures: 1°C (blue bar), 7°C (orange bar), and 21°C (grey bar). Graphs correspond to cryoconite in the light **(a, e, and i),** cryoconite in the dark **(b, f, and j),** subglacial till in the light **(c, g, and k),** and subglacial till in the dark **(d, h, and l).** Significant changes obtained through ANOVA are denoted by asterisks (*).

4.2.1 Viability fluctuations

As seen in figure 15, subglacial till samples kept in the dark at 7 ^{O}C, had a statistically significant decrease (~30%) in viability between T2 and T3 (Figure 16d), followed by a significant increase (~40%) between T3 and T5 (p=0.005). Similarly, significant viability increases of approximately 30% and 60% at T3 and T5 were recorded at 1^{o}C and 21^{o}C, respectively (p=0.001) (p=0.000). In the case of cryoconite samples in the dark at 21 ^{O}C, a significant increase was observed between time point T1 and T2 (p=0.021)(Figure 16b). Fewer significant changes were present in light conditions, however, at 21^{o}C a significant decrease was seen at T2 to T3 for cryoconite (~20%), followed by an increase (~25%) between at T3 and T5 (Figure 16a) (p=0.014). Subglacial samples in the light at 7^{o}C saw a significant decrease at T1 to T2, a significant increase at T2 to T3, and a decrease at T3 to T5 (p=0.001).

4.2.2 Headspace gas concentration fluctuations

Of the three gases measured (CO_2, N_2O, CH_4), it can be seen from figure 16 that CO_2 concentrations exhibited the most pronounced variations, especially within cryoconite microcosms. In light conditions, cryoconite consistently demonstrated significant increases in CO_2 between weeks one and two across all tested temperatures (Figure 16a) (p=0.00). Another increase was observed between weeks two and three at 21°^{C}. In dark conditions at 1°^{C}, cryoconite microcosms exhibited significant increases throughout each sampling point (Figure 16b) (p=0.000). While subglacial till showed fewer fluctuations compared to cryoconite, it still demonstrated significant changes. Specifically, both light and dark microcosms at 1°^{C} exhibited a significant increase in CO_2 concentrations at week three (Figure 16c and d) (p=0.002). Furthermore, the dark subglacial till at 7°^{C} also showed a significant increase at this time point (p=0.003). N_2O concentrations, as outlined in Table 4, did not exhibit significant changes overall, except at week three in cryoconite (light) microcosms at 1°^{C} (Figure 16l) (p=0.001). Similarly, CH_4 displayed limited significant changes, with increases measured in light cryoconite microcosms at 1°^{C} (Figure 16e) and in the dark at 7°^{C} (Figure 16g)(p=0.04).

4.3 The Impact of Temperature on Cryoconite Ecosystem Processes: NEP, Respiration, and Photosynthesis

Carbon flux rates were estimated from headspace concentrations, in a method described in section 3.6.1, to contextualise the activity of Net Ecosystem Production (NEP), respiration (*R*), and photosynthesis (PHS) with wider literature and predictive models. Results show a steady increase in the rate of cryoconite CO_2 emissions at each of the three temperature conditions (Table 4). Because headspace CO_2 concentrations were measured below atmospheric levels and close to the limit of detection at the first sampling time point (after 7 days), CO_2 fluxes were calculated as 0. It can be seen that from 7 to 14 days dark microcosms, representing *R*, increased from 0 to ~1 mg CO_2-C kg $soil^{-1}$ day^{1}. However, the biggest increase was seen in cryoconite at 1°C under light conditions, representing NEP, measuring at 1.23mg CO_2-C kg $soil^{-1}$ day^{1}. Estimated fluxes reveal that the first 14 days had the largest increase in rate of emissions of CO_2 for all microcosms, followed by a noticeable slowing in activity between 14 to 21, and 21 to 36 days (See Figure 17). In addition to estimated fluxes in respiration and NEP, rates of photosynthesis were estimated by subtracting the dark concentrations from the light. It can be seen that photosynthesis was low for each temperature condition, measuring approximately half or less of the respiration and NEP rates. The values missing at 36 days for microcosms at 21°C is due to a mechanical error during GC.

Table 4. Estimated CO_2 flux . *CO_2 flux was calculated from headspace concentrations using a method from Franzlubber et al (1996). Missing fluxes are due to mechanical errors during gas chromatography.*

mg CO_2-C kg $soil^{-1}$ day^{-1}									
DAYS	1 °C			7°C			21°C		
	Light	Dark	L-D	Light	Dark	L-D	Light	Dark	L-D
7	0	0	0	0	0	0	0	0	0
14	1.23	0.85	0.38	1.1	0.83	0.27	1.04	0.89	0.15
21	1.59	1.06	0.53	1.5	1.06	0.44	1.3	1.3	0
36	1.7	1.3	0.4	1.6	1.01	0.59	-	-	-

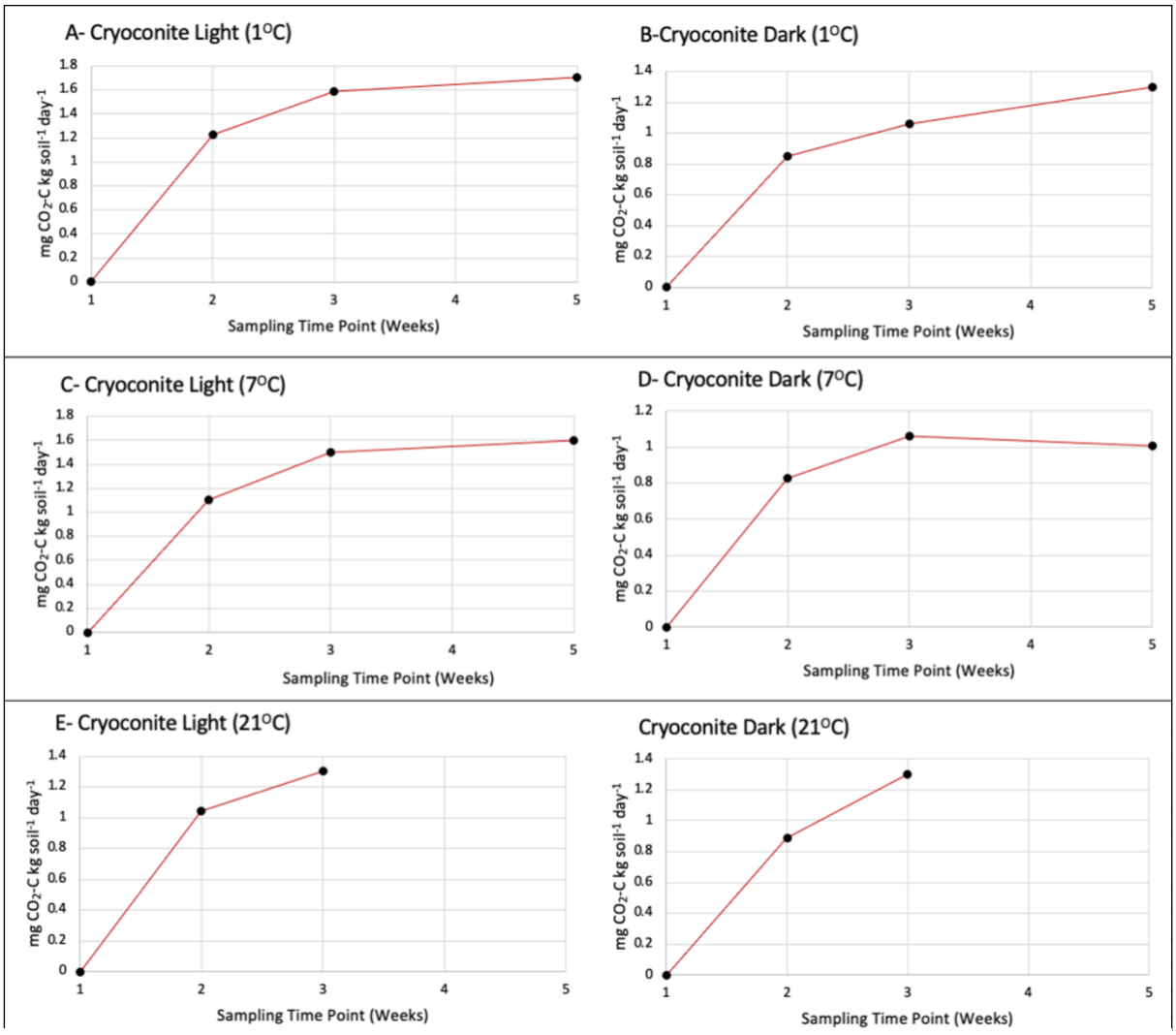

Figure 17: Estimated CO_2 fluxes. *CO_2 fluxes for cryoconite incubated in (a) the light at 1°C, (b) the dark at 1°C, (c) the light at 7°C (d) the dark at 7°C, (e) the light at 21°C, (f) the dark at 21°C.*

4.4 Evaluating the Temperature Sensitivity of Glacial Sediments Using the Q_{10} Coefficient

To evaluate the sensitivity of respiration to temperature, Q_{10} values were derived from the estimated CO_2 flux rates of cryoconite in the dark. Unfortunately, this assessment couldn't be extended to subglacial samples due to the minimal concentrations of headspace CO_2, which hindered the calculation of flux rates. Q_{10} coefficients were calculated for 1°C-21°C (see equation 5) and 7°C-21°C (see equation 6), using the recorded values of 1.06mg and 1.3mg of CO_2-C kg soil^{-1} day^{1} for analysis. Employing the formula provided, a Q_{10} coefficient of 0.935

was calculated for 1°C -21°C and 0.865 for 7-21. These results, being below 1, indicates that cryoconite respiration exhibits a limited response to the measured temperatures.

(5)

1°C -21°C:

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{\frac{10}{21-1}}}$$

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{\frac{10}{20}}}$$

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{0.5}}$$

$$\boldsymbol{Q_{10} = 0.935}$$

7°C -21°C:

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{\frac{10}{21-7}}}$$

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{\frac{10}{14}}}$$

$$\boldsymbol{Q_{10} = \left(\frac{1.06}{1.3}\right)^{0.71}}$$

$$\boldsymbol{Q_{10} = 0.865}$$

4.5 Comparing Gas Sampling Approaches and the Importance of Headspace Replenishment in Closed Incubations

This study trialled two gas sampling approaches to establish whether samples required headspace replenishment as a means of preventing the accumulation of CO_2, N_2O, and CH_4,

within microcosms. A t-test was performed (Figure 18) to determine whether gas concentrations measured at each time point were significantly different between sampling approach 1 (no replenishment) and sampling approach 2 (headspace replenished at each time point). For CO_2 both sampling approaches saw an incremental increase in gas concentration throughout the incubation period. An ANOVA showed that overall sampling approaches did not result in significantly different CO_2 concentrations, except at T1 where sampling approach 2 had a significantly higher concentration at the $p<0.05$ level. For N_2O and CH_4 concentrations varied minimally throughout the experiment, both remaining below average room concentrations, with no significant difference between sampling approaches found.

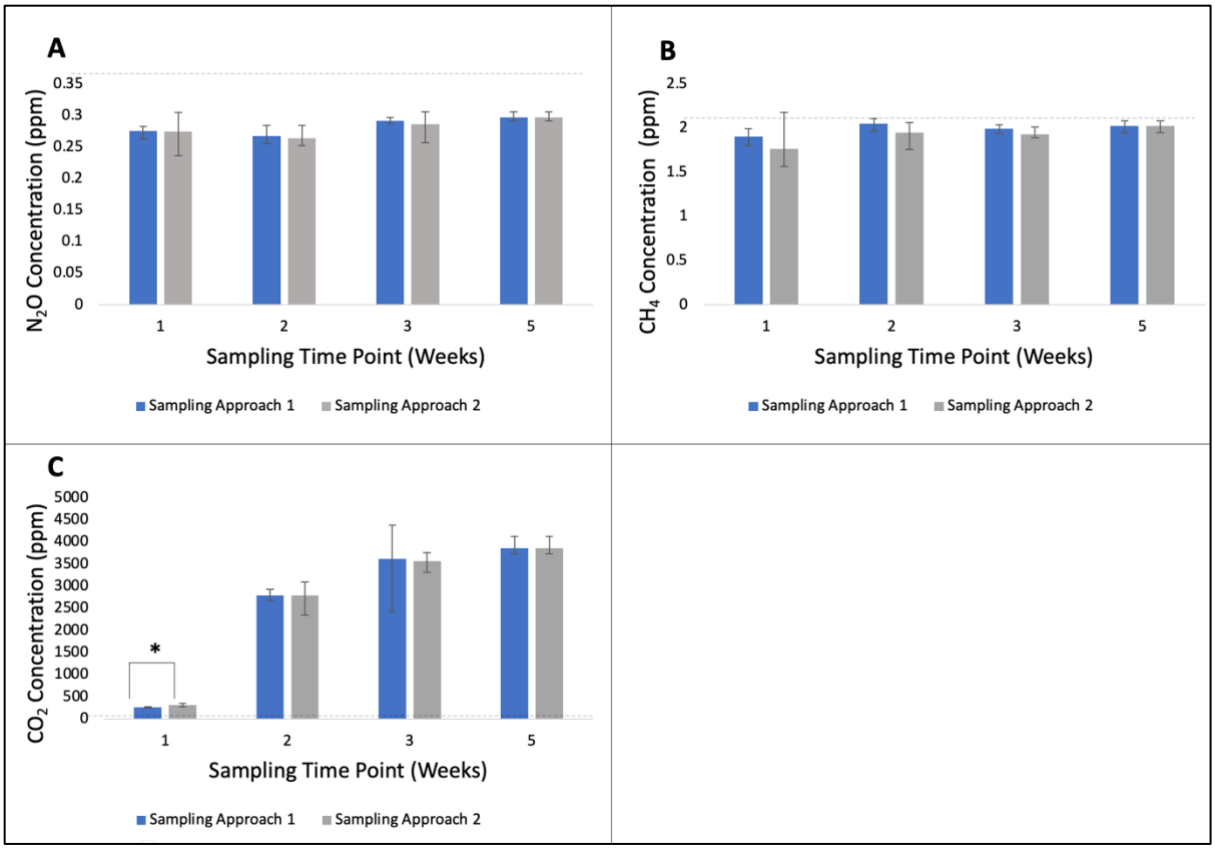

Figure 18: Gas Sampling Approaches. *Bar graph indicating the average (a) N_2O (b) CH_4 and (c) CO_2 concentration found in samples across four-time points, weeks 1, 2, 3, and 5, comparing sampling approach 1 and 2. Data range bars are included, and significance (p=<0.05) is indicated by an asterisk (*).*

5.0 Discussion

The shrinking of glaciers related to climate change is one of the fastest ongoing ecosystem shifts, as enhanced melt impacts periglacial environments (Bosson et al., 2023). Recognised as important reservoirs of nutrients and freshwater, any change in the hydrological regime of glaciers impacts the nutrient dynamics of downstream environments (Milner et al., 2017). Microbial activity in the supraglacial and subglacial environments is key in the storage, transformation, and export of nutrients, influencing the biogeochemistry of cold environments (Bearzot et al., 2023). However, the fate of these microbes in an ever-warming climate and their role in biogeochemical cycling upon release from the glacier is unclear. Current literature has demonstrated the diverse physiological capabilities of these communities (Miteva, Sherdan, and Brenchley, 2004), which would suggest that glacial microbes have the potential to successfully colonise and disrupt nutrient cycling within periglacial environments. To test this hypothesis, this study integrated climate data from Svalbard with the microcosmic incubation of supra- and-subglacial samples collected from local glaciers. Over a five-week period, cryoconite and subglacial samples were incubated at 1°C, 7°C, and 21°C, where cell viability and concentrations of CO_2 N_2O, CH_4 were measured at 4-time points (7-days, 14-days, 21- days, and 36-days). Furthermore, two gas sampling techniques were investigated to test whether headspace replenishment is necessary when studying glacial samples in a closed incubation system for these time periods.

5.1 Cryoconite's Resilience to Warming Climates

We used live/dead staining to measure relative viable cell abundance in cryoconite over five-weeks across varied temperatures. Our findings offer insights into cryoconite's potential to colonise periglacial environments amidst climate warmings. Surprisingly, high viability was consistently observed across the three tested temperatures. While we expected robust viability at 1°C, akin to the typical summer range of supraglacial habitats (0-3°C), we anticipated increased mortality when exposed to warmer climates (7°C and 21°C) post-glacial expulsion. However, cryoconite demonstrated remarkable resilience, maintaining approximately 80% viability throughout the incubation at these elevated temperatures. Notably, our utilisation of live/dead techniques for assessing cryoconite viability in response

to temperature is unprecedented. Therefore, to contextualise our findings we draw parallels with similar studies measuring growth.

Margesin et al. (2002) demonstrated cryoconite's ability to grow across a range of temperatures, with significantly higher microbial growth recorded at 20°C compared to others tested. However, this contrasts with the viability findings in our study and the growth measurements by Poniecka et al. (2020). Poniecka et al. (2020), using The Most Probable Number (MPN) technique and fluorescence analysis, observed notably higher growth at 0.2°C among temperatures tested (ranging from 0.2°C to 20°C) in bacteria cultivated from Antarctic, Greenlandic, and Svalbardian cryoconite. The discrepancy in optimal growth temperatures among these studies could stem from factors including, regional differences in cryoconite composition, adaptability of microbial communities to specific environments, and methodological variations in cultivation and temperature assessments.

Studies like Margesin et al. (2002), employing agar-based techniques, emphasize microbial diversity but introduce a growth bias due to optimal nutrient availability. Similarly, Poniecka et al. (2020) faced constraints with their plate reader method due to sample drying at higher temperatures, limiting their assessment range. Standardised methodologies integrating microscopy and agar-based techniques could offer a more accurate understanding of microbial viability and diversity. However, the high standard deviation evident in certain graphs in the current study, as depicted in Figure 14, highlights the challenges of microscopically studying glacial sediments. Here, an aliquot was extracted from vortexed samples to reduce the amount of conglomerated sediment on slides; however, this is still a major source of variability. The sediment suspended in the aliquot was transported onto slides, comprising of different material types, and varying sediment loads. This makes it challenging to microcosmically analyse samples as sediments visually obstruct cells, proving several fields of view in which cells can be present. This can be mitigated with z-stack imaging. Here, several images can be taken in one location on the slide but at varying depths along the z-axis. Unfortunately, it was not possible to apply this method during the study, due to the enhanced capture and processing times involved, and due to the time constraints of the project. Furthermore, there are alternative techniques available for conducting viable cell counts that do not necessitate fluorescence microscopy. One such method is flow cytometry, a powerful analytical tool used to assess cells within heterogenous populations (Irvine-Fynn and Edwards, 2014).

5.2 Fluctuations in Cryoconite Under Temperature Extremes

This study highlights the extraordinary resilience of cryoconite to temperatures surpassing its natural thermal conditions. Although we didn't pinpoint a precise viability threshold, notable fluctuations became apparent at 21°C, as illustrated in Figure 19a. These findings align with past field and incubation studies examining how alpine soil microbes respond to temperature shifts (Barcenas-Moreno et al., 2009; Donhauser et al., 2020; Rijkers et al., 2022). These studies suggest that cold microbes don't exhibit sudden surges or declines amidst climate shifts, particularly at temperatures typical of warming scenarios (<30°C). Instead, as evidenced by the work of Donhauser et al. (2020), there's a gradual transition in microbial communities favouring species adept at rapid growth, stress tolerance, and adaptability to higher temperatures. This notion is reinforced by Rijkers's et al. (2022) observations, noting a gradual rise in the optimal growth temperature of alpine soil communities under warmer conditions. However, the link between the observed fluctuations, especially at the highest tested temperature (21^{o}C), and community turnover remains uncertain without supplementary analysis exploring shifts in community compositions.

One plausible hypothesis is that the initial viability increase might mirror the proliferation of heat-adapted communities (Donhauser et al., 2020), followed by potential displacements and subsequent resurgences as more adaptive communities establish themselves. Understanding the impact of warming on community composition encounters complexity due to variations in available substrates across samples incubated at different temperatures (Kirschbaum, 2004; Davidson and Janssens, 2006; Donhauser et al., 2020). This complicates untangling warming effects from substrate limitations, potentially distorting our identification of temperature-responsive bacterial species and estimates of warming effects on microbial processes (Hartley et al., 2008; Karhu et al., 2014; Walker et al., 2018). To address these challenges in future studies, integrating community composition analysis while mitigating substrate limitations would be crucial. One approach could involve providing excess substrate to temperature incubations, despite potential alterations in sample organic matter as seen by Dacal et al. (2019). Alternatively, standardising sampling

points based on specific substrate or carbon loss levels could offer insight into aligning different temperature treatments (Whittington, 2019).

When considering the implication of these communities to downstream nutrient dynamics, it Is also important to differentiate between viable cells and their metabolic activity. A study conducted by Blagodatskaya and Kasyanov (2013) reviewed various techniques for measuring the activity levels of microbial communities in bulk soil. The findings revealed that a mere 0.1% to 2% of the total biomass sampled were actively engaged, while a substantial proportion, up to 60%, could be considered potentially active. Regrettably, the significance of this potentially active community often goes unnoticed in research endeavours, despite these organisms demonstrating rapid responses to shifts in available substrates. Notably, supraglacial communities, such as cyanobacteria, lie dormant during winter and become active with the influx of meltwater during the summer months. Blagodatskaya and Kuzyaov's (2013) review found that static analytical methods, primarily centred on the one-time determination of cell components (such as ATP, DNA, RNA, and molecular biomarkers), are effective in detecting total biomass but fall short in assessing community function and activity. In contrast, dynamic approaches, which estimate changes in microbial communities during growth and are based on process rates, particularly substrate utilisation and product formation, have proven more effective. BONCAT, has emerged as a dynamic and promising technique for assessing cellular function. BONCAT operates by tagging cells that are actively synthesizing proteins, enabling estimations of the percentage of community that is in an active state. Figure 19, allows for a direct comparison of BONCAT and live/ dead assays used in the present study, showing microscopy image taken by Bradely et al. (2023) of Greenlandic microbes. Notably, when comparing the two images, it becomes evident that only a fraction of the cells identified as living through live/ dead assay are shown to be metabolically active using BONCAT. Therefore, dynamic approaches such as BONCAT hold promise for enhancing our understanding of whether viable cells are indeed metabolically active. These findings underscore the importance of future research in unravelling the intricate relationship between temperature and microbial responses/processes while navigating the challenges associated with incubations.

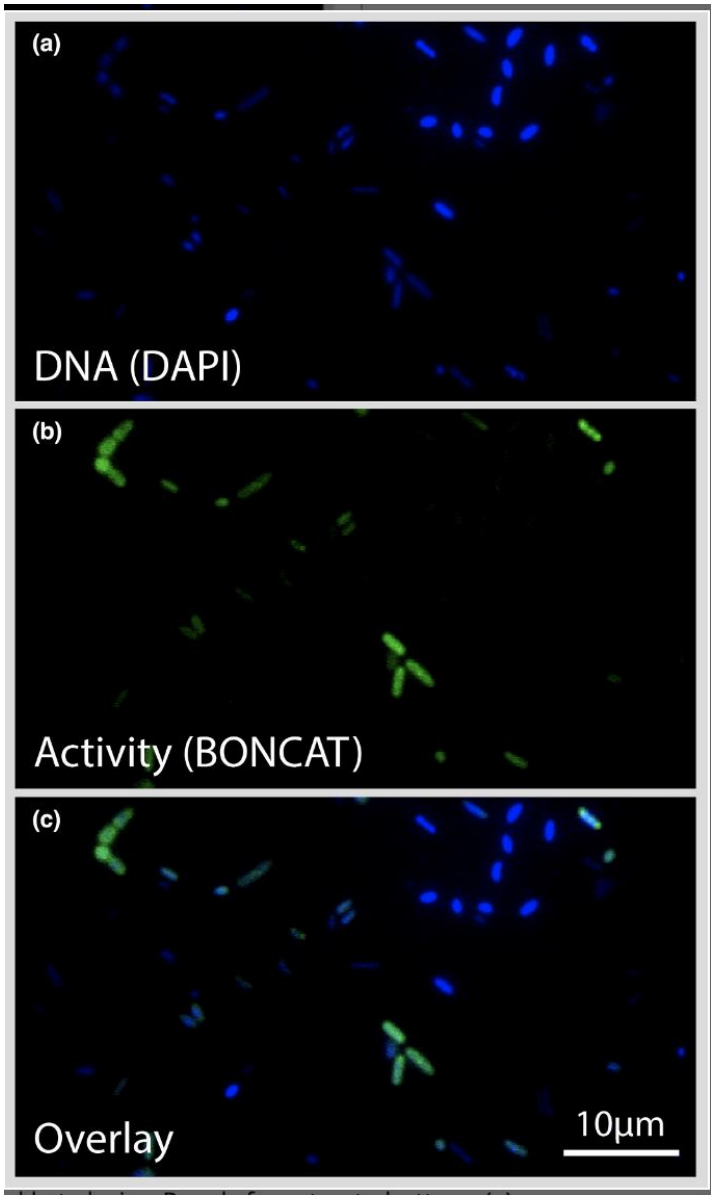

Figure 19. Epifluorescence Microscopy Comparison of Light/Dark and BONCAT for the Visualisation of Single-Cell Activity. (a) *DAPI staining in blue* ***(b)*** *BONCAT in green.* ***(c)*** *Overlay of active cells (green) and inactive cells (blue). Image from: (Bradley et al., 2023).*

5.3 Temperature Sensitivity of Cryoconite Respiration: Implications for CO_2 Release.

Our findings, substantiated by a calculated Q_{10} value (7-21°C) of 0.935, imply that cryoconite is less sensitive to temperature fluctuations compared to observations of other cold ecosystems, such as Arctic soils (Flanagan and Bunnell, 1980; Bekku et al., 2003). Respiration, a common indicator of microbial response to temperature shifts, has garnered significant attention among soil scientists due to its relevance in ecosystem modelling (Bradley et al., 2015). The exploration of potential feedback loops becomes particularly crucial in Arctic regions owing to their rapid warming rates and substantial carbon reservoirs within their soils (Weider et al., 2019). Utilising the Q_{10} method has become prevalent in examining the temperature sensitivity of diverse biological processes, however, the approach assumes a linear relationship between temperature and response (Mundim et al., 2020), which is more nuanced in reality. For instance, in a study by Donhauser et al (2020), a Q_{10} of 2.1 was recorded while investigating respiration in alpine soils between 10°C and 20°C, contrasting with a Q_{10} of 3.4 observed between 0°C and 10°C. Similar non-linearity in temperature response has been echoed in various studies, especially at lower temperature ranges (Bååth, 2016). Our findings support the notion of examining temperature responses within narrower ranges, as our Q_{10} findings differ notably from those in broader temperature spans (Bradley et al., 2016 and Glanville et al., 2012). These nuanced variations underscore the necessity of finer temperature assessments to elucidate at which points temperature exerts the greatest influence on biological activity. Such detailed investigations are necessary in refining climate models, offering deeper insights into how different warming stages might impact biological processes. Our findings highlight the importance of investigating nuances and temperature sensitivity of microbial processes linked to periglacial carbon cycling. For example, considering the temperature sensitivity of extracellular enzyme activity (Zou et al., 2021), would provide a promising avenue for future research. This could be tested by integrating the methods employed in this study with those of Poniecka et al. (2020), conducting substrate tests under varying temperature regimes. The research conducted by Poniecka, et al. (2020) delved into the cryoconite carbon cycle through microplate carbon substrate tests, unveiling the community's proficiency in utilising 58 different carbon sources. These findings highlight cryoconites remarkable carbon-scavenging abilities, likely shaping the initial nutrient pools upon relocation. Such an

approach would provide valuable insights into how enzymatic activities of these communities may respond to warming temperatures in the Arctic.

5.4 Ecosystem Productivity Contrasts: Cryoconite Vs Subglacial Communities in Svalbard

The distinct characteristics of supra-and-subglacial environments, varying in nutrients, oxygen, sunlight, and hydrology, have shaped two distinct microbial communities within glaciers (Boetius et al., 2015). These contrasting conditions resulted in markedly different responses in cryoconite and subglacial samples when subjected to higher temperatures, offering valuable insights into their behaviour upon expulsion from the glacier. During the incubation period, subglacial samples consistently exhibited lower and more erratic viability counts compared to cryoconite. However, an intriguing shift occurred between weeks three and five, notably at temperatures of 1°C and 21°C. Here, viability in dark conditions surged by approximately 60%, reaching levels akin to those observed in cryoconite (~80%). This surge suggests an adaptive response within the community to elevated temperatures. Surprisingly, this adaptation was absent in light conditions, indicating that light played a pivotal role in influencing the sediment's response to temperature. While cryoconite has been extensively studied for its reaction to light, employing various physiological and behavioural strategies to optimize photosynthesis and shield against light-induced stress (Morgan-Kiss et al., 2006; Bagshaw et al., 2016; Perkin et al., 2017), subglacial communities remain relatively unexplored in this context. Tian et al. (2022) examined soil samples collected from the periphery of a glacier forefield in Svalbard, revealing distinct adaptations in recently exposed samples closely resembling subglacial microbial communities. These samples showcased an abundance of genes associated with coping mechanisms for high solar UV radiation, such as 15-cis-phytoene synthase (CrtB) and the excinuclease ABC system (UvrABC). These genes exhibited notably high Transcripts Per Million (TPM) values, indicating their significant prevalence and potential importance in the adaptation of subglacial microbes to the periglacial environment. In a related study, Feng et al. (2023) explored the functional genetic potential of the Damma glacier forefield, unveiling low genetic diversity yet a broad range of functional capabilities. Despite the lower organismal diversity, the functionalities observed were diverse, a phenomenon attributed by Feng et al. (2022) to selective pressure imposed by high levels of UV radiation, favouring the survival

and proliferation of organisms equipped with superior adaptive mechanisms. To improve our understanding, future research could integrate viability assessments, metabolic profiling, and genomic analyses, promising a more profound understanding of the adaptive strategies and limitations of subglacial microbial life exposed to light.

5.5 Competition Between Sulphate Reducing Bacteria and Methanogens in Glacial Sediments

Our study aimed to understand how glacial communities contribute to CH_4 production under elevated temperatures. Initially, we hypothesised that increased temperatures would enhance respiration, leaving the microcosms anoxic; this would then be followed by methanogenesis, leading to higher CH_4 concentrations. However, our GC analysis results did not align with this initial hypothesis. Instead, our GC analysis revealed that CH_4 concentrations remained close to atmospheric levels, indicating relatively low levels of methanogenic activity within the microcosms. Firstly, it is essential to acknowledge uncertainties about whether the headspace reached complete anoxia. Methanogenesis and denitrification (see Figure 24), being exclusively anaerobic processes, depends on the absence of oxygen (Tiedje et al., 1984). To ensure anoxic conditions, the experiment aimed to establish a small, sealed headspace combined with an active microbial community. Monitoring CO_2 concentrations offers valuable insights into this process since microorganisms in anoxic environments typically shift to anaerobic respiration, resulting in increased CO_2 production as a metabolic by-product. Thus, an expectation existed for CO_2 concentrations to rise in anoxic conditions. Based on the observed increase in CO_2 concentrations, accompanied by a gradual slowing in production rates toward the experiment's conclusion, it was inferred that anoxia had likely been achieved. Nevertheless, if anoxia had not been achieved it poses as a potential explanation for the low concentrations of CH_4 (and N_2O). Therefore, for increased confidence future investigations should consider conducting complimentary analyses such as dissolved oxygen measurements or microbial community analysis, as additional approaches can help identify anaerobic microbial populations and provide a more robust confirmation of anoxic conditions. However, under the assumption that microcosms did achieve anoxia, the low concentrations of CH_4 present within the microcosm headspace suggests that alternative metabolic pathways may have been predominantly occurring, overshadowing

methanogenesis. Methanogenesis, typically the final stage in organic matter decomposition, is primarily driven by methanogens, a group of fermentative bacteria that metabolise organic matter, generating CH_4. Glacial systems commonly feature two predominant methanogenic pathways: hydrogenotrophic and acetolactic, relying on hydrogen and acetate as substrates. The equations for these pathways are as follows-

$$4H^2 + H^+ + CH_4$$
$$Acetate \rightarrow HCO_3 + H^+ + CH_4$$

It's well-documented that sulphate reducing bacteria (SRB), which consume CH_4, can outcompete methanogens for hydrogen and acetate resources when sulphate is not limiting (Plugge et al., 2011). To delve deeper into this, future research could employ multiple approaches. A sulphate-reducing bacteria assay, for instance, can detect the presence of sulphate reducers by using specific substrates that SRB can utilise, resulting in the production of distinctive compounds or observable colour changes (Javed and Masgwani, 2020). Additionally, measuring sulphide concentrations could provide insights into sulphate reduction activity, and stable isotope analysis can help determine if sulphate has undergone microbial reduction (Londry and Des Marais, 2003). Combining these various methods could shed light on the potential role of sulphate reducers in modulating CH_4 dynamics in deglaciating ecosystems. If active sulphate reducers are responsible for reducing CH_4 levels in microcosms, it may have implications for periglacial environments. For instance, it suggests that CH_4 production may be delayed until conditions become more favourable for methanogens, and certain compounds (e.g., sulphate) are depleted by SRB. This consideration is crucial when designing predictive models for CH_4 release under climate change. Furthermore, active sulphate-reducing communities could alter the sulphate cycle, resulting in the accumulation of sulphate in periglacial soils. This accumulation could have biogeochemical impacts on microbial and plant communities, influencing nutrient cycling and ecosystems dynamics.

5.5.1 Methanogenesis in Oxic Conditions

The oxidation of CH_4 is strongly associated with anoxic conditions, however, the glacial system, its surrounding environment, and the sediments that inhabit them comprise both

aerobic and anaerobic zones (Buda et al., 2022). These regions are subject to frequent change, seasonally dictated by drainage systems and the presence/ absence of oxygenated meltwater (Bhatia, Sharp, and Foght, 2006). For example, during the summer months, higher redox potential has been recorded in the subglacial regions of high Arctic glaciers at times of peak discharge (Xing et al., 2022). Therefore, future work should not overlook the potential importance of using aerobic incubations to understand CH_4 pathways in glacial sediments. In marine environments, a process termed the marine methane paradox has been observed whereby CH_4 is microbially produced through an oxic pathway (Bartosiewicz et al., 2023). Here, the degradation of phosphonate esters in organic matter by marine bacteria saw the microbial production of CH_4 in the presence of oxygen (Repeta et al., 2006). Whilst this paradox has, so far, been explored in seawater, organophosphate esters are known to have been found in glacial meltwater (Sun et al., 2020). A 2016 study conducted by Repeta et al. (2006), found that this pathway was utilised in phosphate-limited environments, a finding echoed by Oliverio et al. (2020) who found carbon-phosphorus lyase occurred more in soils with lower phosphonate. The periglacial environment has potential to be nutrient poor (Zazovskaya et al., 2022), and thus there is the potential bacteria will utilise this pathway under oxic conditions resulting in CH_4 being produced. Whilst our results do not corroborate this theory, it is still an emerging area of research and thus future studies could investigate the potential for this pathway to be utilised by glacial communities.

5.6 The Importance of Nutrient Supply for Denitrification in Glacial Sediments

To explore the impact of Arctic warming on nitrogen cycling in glacial sediments, we measured nitrous oxide (N_2O) concentrations, a key product of nitrification and denitrification processes (see Figure 20), in the headspace of microcosms. We hypothesised that warmer temperatures would lead to higher N_2O concentrations, indicating enhanced denitrification (since our microcosms were expected to become anoxic). GC analysis did not indicate this, with samples maintaining N_2O concentrations consistent with atmospheric levels across all tested temperatures, suggesting that denitrification may not have played a dominant role in these samples. This result was unexpected based on research such as Sagawa et al. (2014) that detected abundant marker genes associated with both nitrification

and denitrification processes in cryoconite. Notably, Segawa et al. (2014) conducted their research on cryoconite from an Asian glacier in the High Mountain Asia (HMA) region. However, a study by Murakami et al. (2020) found significantly higher denitrification potential in Asian glaciers compared to polar glaciers in the Arctic and Antarctica. This discrepancy was attributed to environmental factors, including greater availability of substrates such as nitrate and dissolved organic carbon in glacial meltwater from HMA glaciers compared to polar regions. Taken together, these findings in the present study suggest that nitrate and nitrite, which are necessary for denitrification may have been present in limiting quantities in the tested samples, contributing to the observed low N_2O concentrations. Future research endeavours could employ a range of tests to probe this hypothesis. These approaches might encompass direct measurements of nitrate and nitrite concentrations within the samples or in the surrounding environment, such as glacial meltwater, utilising techniques like ion chromatography (Beaton et al., 2017). Alternatively, experimental manipulations could artificially elevate nitrate and nitrite concentrations, followed by measurements of any resulting changes in N_2O production. Additionally, delving into microbial community analysis, for instance, through Fluorescent In Situ Hybridization (FISH) could be used to identify denitrifying bacteria and assess their activity (Ryuda et al., 2011). By designing specific fluorescent probes that target the genetic material of denitrifying bacteria and incorporating them into the FISH protocol, you can identify the type and distribution of denitrifying bacteria present. The advantage of a protocol such as FISH is that when coupled with activity stains or substrates, FISH provides direct visuals on which denitrifying bacteria are actively participating in dentification processes. These multifaced investigations would provide valuable insights into the role of nitrate and nitrite availability in N_2O dynamics within cryoconite as well as provide knowledge on both taxonomy and function.

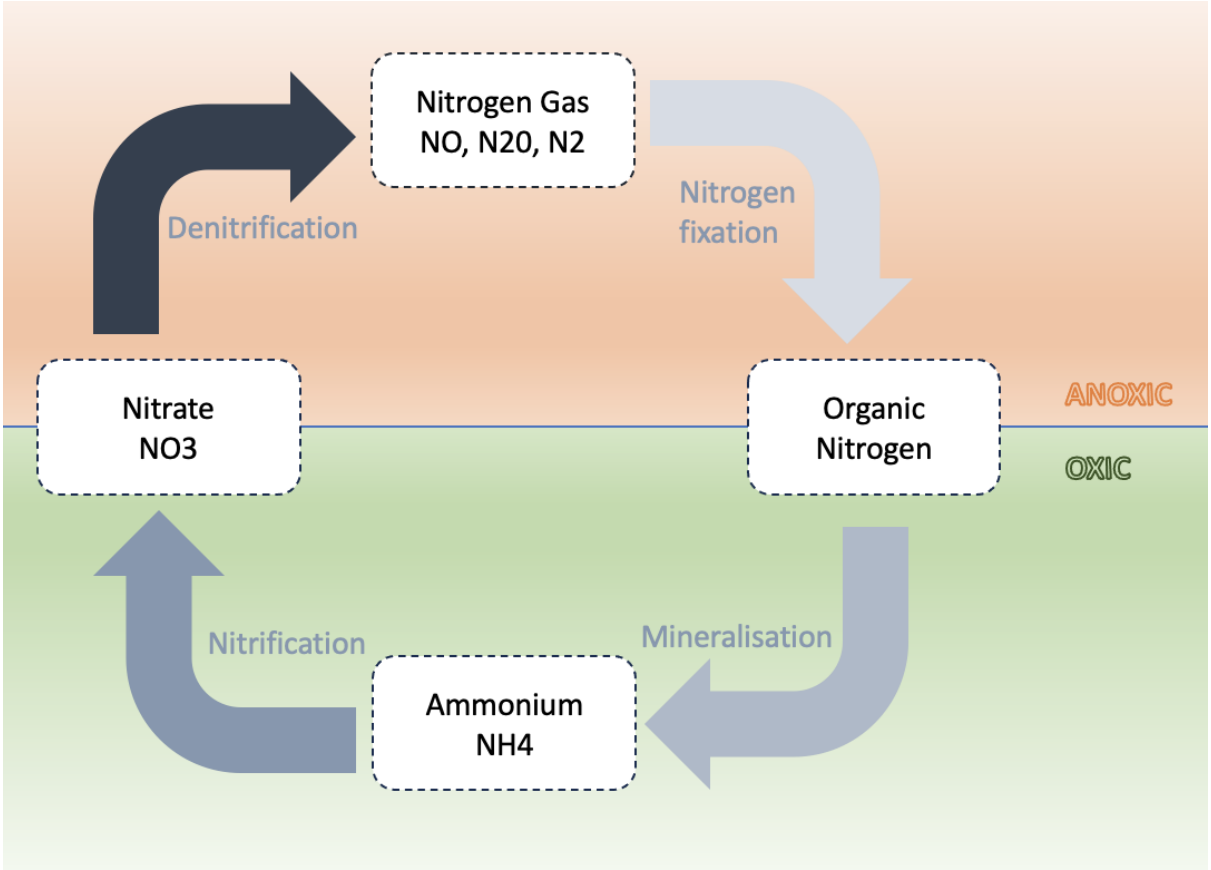

Figure 20: The Nitrogen Cycle. A depiction of processes associated with the nitrogen cycle highlighting those that occur under oxic (e.g., mineralisation and nitrification) and anoxic conditions (e.g., nitrogen fixing and denitrification). Adapted from Bicking et al. (2019)

5.7 Microbial Viability as a Predictor of Global GHG emissions

The study aimed to establish a link between viable cell percentages and GHG production, seeking to assess its potential as an indicator for terrestrial emissions across various warming scenarios. Such an indicator could significantly benefit predictive models that determine whether periglacial environments, when exposed to glacial organisms, act as GHG sources or sinks. However, despite employing linear regressions, this study did not reveal a clear relationship. This outcome likely stems from the complex nature of headspace gas concentrations, which reflect more than just gas production but encompass broader ecosystem fluxes. In incubation, microbial cells engage dynamically in assimilating and producing gases, complicating straightforward interpretations of gas concentrations. For instance, a significant population of viable methanotrophs might decrease headspace methane (CH_4) concentrations due to their methane-consuming activity. This could lead to a negative association between viable methanotrophs and CH_4 concentrations within the

headspace. Future research should prioritise employing a combination of direct field measurements and laboratory analyses of gas fluxes. This approach will deepen our comprehension of the intricate relationship between gas exchange processes and periglacial environments. For instance, Glanville et al.'s (2012) study merged field and laboratory analyses to elucidate the significance of temperature and water content in periglacial ecosystem dynamics. Field observations highlighted the dominance of temperature and nutrient availability in driving processes like carbon mineralisation. Conversely, laboratory investigations identified water content as the primary influence. Glanville et al. (2012) clarified this divergence by recognising water content as a limiting factor in the lab, indicating that temperature is likely to exert greater influence at the growing season's onset, while water content becomes more influential as the season progresses and water becomes a limiting factor. This highlights the complex link between microbial biogeochemical responses to climate-induced changes and emphasizes the need to integrate both field and laboratory analyses to unravel the intricate interplay among multiple variables.

5.8 Microcosm Gas Sampling Techniques: Avoiding the Bottle Effect

In this study, we aimed to investigate the potential accumulation of gases within the headspace of our microcosms, to provide valuable insights for the development of future research methodologies. When comparing the concentration of CO_2, N_2O, and CH_4 at different time points, we observed remarkably consistent results between the two different approaches. For instance, at time point T5, the concentration of CO_2 was 4000ppm when using approach one and two. This pattern was consistently observed for all three gases assessed, implying minimal differences in the recorded gas concentration between the two approaches. Our findings suggest that there was no substantial build-up of gases within the headspace of the microcosms, implying that the optimal sample to headspace ratio for glacial microcosms was successfully identified. While microcosms are a widely accepted technique in microbiological research, it is essential to acknowledge the potential influence of what is commonly referred to as the bottle effect. This phenomenon refers to a situation where observed outcomes are more a result of confinement within microcosm rather than the intended experimental manipulations. In this study, where we did not have direct control over the composition of the headspace, there was a potential risk of gases like CO_2 accumulating and potentially affecting microbial communities, pH levels, and the pressure within the microcosms. Some previous studies have addressed this concern by actively

managing the headspace composition. For example, as demonstrated by the work of researchers like Tang et al. (2013) anaerobic incubations were conducted with periodically flushing of the headspace using a N_2 to prevent gas accumulation which has potential to influence biogeochemical processes like carbon mineralisation. Similarly, in the study conducted by Knoblauch et al. (2013) aerobic microcosms were monitored to ensure that CO_2 levels remained below 3% throughout the experiment. If CO_2 levels exceeded this threshold, adjustments were made to maintain atmospheric air conditions. By shedding light on the considerations of headspace composition and related practises in microbiological research, our study contributes to the ongoing efforts to refine experimental methodologies.

5.9 Methodological Limitations

5.9.1 Heat Shock During Sample Thaw: Implications for Microbial Relocation

Live/Dead assays allowed for direct comparisons to be made between the percentage of viable microorganisms present in both cryoconite and subglacial samples. For example, when looking at T1 for Figure 19 noticeable discrepancy in the percentage of living microbes between both sample types can be noted. Cryoconite samples start with a much higher portion of the community living, ranging from 60-95%, whilst subglacial communities start with a higher mortality percentage (20-40% living). Because samples were not analysed at T0 the cause of this cannot be attributed with complete confidence. However, there are several possibilities which could have led to the low viability in the subglacial samples. For example, membrane damage could have occurred prior to sample collection. Unlike cryoconite which can be collected in situ, the inaccessibility of the subglacial environment means that subglacial samples are collected post-relocation or exposure from the retreating glacier. Here, samples were collected at the glacier forefield and from within an ice cave, where numerous unknown factors could have impacted viability. Alternatively, mortality could have occurred in the laboratory during the thawing process. Samples were left to thaw at room temperature (~21°C), which could have resulted in heat shock. A study conducted by Mondini et al. (2022) exposed subglacial samples to cycles of 25°C to test community response to heat stress, where heat shock was observed quickly after exposure. If this did occur in the present study, then it generates questions about how communities will respond to relocation in a warming world, suggesting that mortality could occur shortly

upon exiting the glacial system. Heat shock can be avoided in future work, however, by thawing samples at a controlled temperature. Because these factors, could explain the mortality in the subglacial samples at T1, it is hard to quantify the influence of the select temperature conditions. It is possible that subglacial communities had adverse responses to all three temperatures and saw a steep decline in viable cells over the first 7 days of incubation. Access to viability data at T0 would provide greater insight into community response to temperature.

5.9.2 Microcosm Set-up: Shading Effect on Photosynthesis

Light and dark microcosms are frequently deployed as a method to measure NEP and compare the rate of community respiration with primary production (Telling et al., 2012; Bagshaw, Tranter, and Wadham., 2016; McCormick, Phillips, and Ives, 2019). Whilst light microcosms reflect the flux between respiration and photosynthetic microorganisms, dark microcosms eliminate the process of photosynthesis and only measure the flux of chemotrophic reactions (Telling et al., 2010). It is the expectation, therefore, that dark microcosms will accumulate higher concentrations of CO_2 within the headspace, as CO_2 is not being fixed through primary producers. However, in this study, light microcosms measured slightly higher headspace concentrations of CO_2 than dark. This unexpected result could be attributed to several factors including potential shading effects that reduced the available light for photosynthetic organisms, subsequently reducing photosynthesis and increasing headspace CO_2 concentrations. To investigate this further, future work should focus on precise light measurements (e.g., using light sensors) and the effects of shading. For example, in their study investigating two cryoconite holes in Antarctica, Bagshaw et al (2016) employed Apogee quantum light sensors to quantify the PAR penetrating the ice lid and reaching the microbial communities. Sensors were calibrated to record the amount of PAR reaching cryoconite every 30 seconds for a month. This methodology illustrates the potential application of these sensors within incubators for continuous monitoring of microcosm light exposure. Additionally, investigating the composition of microbial communities', nutrient availability, and temperature variations in both light and dark microcosms could provide valuable insights into the mechanism underlying this unexpected result and help refine our experimental conditions for more accurate carbon cycling assessments.

5.9.3 Limitations of Long-term Sample Storage

Preparatory to analysis, samples were acclimatised over a seven-day period to each of the three temperatures. This was important because prior to thaw, samples had been stored at -18°C for a little over a decade. Originally, the plan was to collect fresh samples, but due to COVID-19 restrictions, fieldwork was postponed to a later point in the season. Unfortunately, this delay led to the sampling site being covered in snow, making it inaccessible. As an alternative, samples were obtained from collaborators, that had been frozen and transported to the laboratory years prior. The freezing of samples is a standard practice used to slow the rate of microbial activity and preserve samples as close to their natural state upon collection (Stibal and Tranter., 2007; Pautler et al., 2013; Rozwalak et al., 2022). However, there are limitations to this method which become prevalent when working with extremophiles. Metabolic activity has been measured at temperatures as low as -30^{o}C because glacial samples (especially subglacial microorganisms) have adapted to cope with these extreme temperatures (Margesin et al., 2019). The possibility thus exists that the community could have maintained some level of activity during freezing, which becomes more apparent over longer periods. Unless samples were analysed upon collection and again after thaw years later, it is hard to quantify the extent of change the community has potentially experienced during storage and therefore the sample may not accurately represent the in situ microbial composition of the glacier. This was a concern echoed by Stibal and Tranter (2007), where it was postulated that lower carbon uptake rates measured in ex-situ cryoconite compared to in-situ samples could be linked to the effects of freezing and transportation on community viability. However, as stated by Stibal and Tranter (2007)"this is unlikely due to the routine survival of microbes throughout the harsh conditions on Svalbard". The findings of this study have provided some interesting insights into the physiological capabilities of glacial communities to temperature and thus it would be beneficial for future work to use more recent samples which are less likely to have been affected by the issues mentioned above.

5.10 Understanding Abiotic Controls on Glacial Microbial Processes: Environmental Metabolomics

Environmental metabolomics is an analytical technique used to analyse how metabolites respond to an external stressor, and consequently the interactions of organisms with their environment (Lankadurai, Nagato, and Simpson., 2013). By studying the composition of metabolites in cryoconite, scientists can gain insight into the energy sources and metabolic pathways used by cryoconite microorganisms, and how this is affected by environmental changes, including those associated with climate change. Research conducted by Gokul et al. (2023) serves a key example of this where they conducted metabolomic analyses on cryoconite communities distributed across the Foxfonna ice cap in Svalbard. Their research highlights the profound impact of climate change on Arctic ecosystems, with specific focus on the pivotal roles played by temperature, nutrient availability, and light dynamics. One of the key findings of their study was the upregulation of cAMP and cGMP levels in cryoconite communities exposed to limited light conditions. This suggests that these organisms have evolved adaptive strategies to thrive in environments characterized by fluctuating light intensities. Elevated levels of cAMP and cGMP can influence photosynthesis in several ways, including the regulation of gene expression, modulation of enzyme activity, light perception, and responses to stressors. These molecular adjustments indicate that microorganisms have evolved mechanisms to maintain efficient carbon fixation through photosynthesis even under challenging light conditions. Moreover, Gokul et al. (2023) identified a preference among these microorganisms for utilising metabolites generated through energetically cost-effective pathways. For instance, the study revealed bias for recycling and scavenging nitrogen-containing metabolites rather than synthesizing them de novo. This adaptive strategy is particularly relevant in nitrogen-poor environments. Integrating the analysis of metabolites with the temperature conditions in the present study offers a potential avenue for assessing the physical and metabolic adaptations of microbial communities to rising temperatures.

6.0 Conclusions

In summary, this study explored the intricate dynamics of glacial sediments when relocated from glaciers into the periglacial environment, specifically examining their metabolic activity and viability under elevated temperatures. The findings presented here have not only corroborated existing literature but have also showcased the remarkable adaptability of cold-adapted microorganisms. Even when subjected to temperatures akin to the current temperature extremes in Svalbard of 21^0C, both cryoconite and subglacial communities have demonstrated remarkable resilience, maintaining high viability levels of 80% and 70%, respectively. Furthermore, our research has highlighted the complexity of metabolic activities within these communities and showcased some of the challenges of studying these microcosmically, reflected by the low levels of CH_4 and N_2O having been detected. Our study is novel in its direct comparison of cryoconite and subglacial communities, elucidating the impact of abiotic treatments on both. Specifically, our research has revealed a potential connection between increased light exposure and fluctuations in the viability of subglacial communities. This suggests the potential for significant compositional changes within these communities following glacial expulsion. Additionally, we have identified a potential link between the presence of glacial life and increased CO_2 emissions in deglaciated soils, a phenomenon likely exacerbated by rising air and soil temperatures. Our study has conducted one of the first assessments of the temperature sensitivity of cryoconite, yielding a Q10 value of 0.935. Our findings indicate that cryoconite exhibits lower sensitivity to temperature changes compared to other Arctic ecosystems. In conclusion, our research underscores the imperative for additional studies, as glacial communities are poised to become key drivers in global biogeochemical cycles during the 21^{st} century's warming climate. As we move forward, understanding these communities' responses and impacts will be crucial in navigating the challenges of a changing world. This can be achieved through interdisciplinary research, and the development of techniques like BONCAT that could provide more in depth understanding of the changing geobiology of these systems.

References

Abakumov, E., Polyakov, V., Kushnov, I. and Tembotov, R., eds. 2021. *BIO Web of Conferences* [on- line] EDP Sciences.

Ambrosini, R., Musitelli, F., Navarra, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Mayer, C., Minora, U., Azzoni, R.S., Diolaiuti, G. and Smiraglia, C., 2017. Diversity and assembling processes of bacterial communities in cryoconite holes of a Karakoram glacier. *Microbial ecology*, *73*, pp.827-837.

Andrews, M.G., Jacobson, A.D., Osburn, M.R. and Flynn, T.M., 2018. Dissolved carbon dynamics in meltwaters from the Russell Glacier, Greenland Ice Sheet. *Journal of Geophysical Research: Biogeosciences*, *123*(9), pp.2922-2940.

Anesio, A.M. and Laybourn-Parry, J., 2012. Glaciers and ice sheets as a biome. *Trends in Ecology & Evolution,* [e-journal] 27 (4), pp.219-225. https://doi.org/10.1016/j.tree.2011.09.012. <https://www.sciencedirect.com/science/article/pii/S0169534711002722>.

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

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

Anesio, A.M., Lutz, S., Chrismas, N.A. and Benning, L.G., 2017. The microbiome of glaciers and ice sheets. *npj Biofilms and Microbiomes,* [e-journal] 3 (1), pp.1-11. Available through: google.

Anesio, A.M., Lutz, S., Chrismas, N.A. and Benning, L.G., 2017. The microbiome of glaciers and ice sheets. *npj Biofilms and Microbiomes*, *3*(1), p.10.

Ashun, E., Toor, U.A., Kim, H.S., Kim, K.R., Park, S.J., Hong, S. and Oh, S.E., 2022. A direct contact bioassay using sulfur-oxidizing bacteria (SOB) for toxicity assessment of contaminated field soils. *Chemosphere*, *286*, p.131599.

Bagshaw, E.A., Wadham, J.L., Tranter, M., Perkins, R., Morgan, A., Williamson, C.J., Fountain, A.G., Fitzsimons, S. and Dubnick, A., 2016. Response of Antarctic cryoconite microbial communities to light. *FEMS microbiology ecology,* [e-journal] 92 (6) Available through: google

Bartosiewicz, M., Venetz, J., Läubli, S., Sepúlveda Steiner, O., Bouffard, D., Zopfi, J. and Lehmann, M.F., 2023. Detritus-hosted methanogenesis sustains the methane paradox in an alpine lake. *Limnology and Oceanography*, *68*(1), pp.248-264.

Battin, T.J., Brittain, J.E., Barrand, N.E., Füreder, L., Cauvy-Fraunié, S., Gíslason, G.M., Jacobsen, D., Hannah, D.M. and Hodson, A.J., 2017. Glacier shrinkage driving global changes in downstream systems. *Proceedings of the National Academy of Sciences*, *114*(37), pp.9770-9778.

Bearzot, F., Colombo, N., Cremonese, E., Di Cella, U.M., Drigo, E., Caschetto, M., Basiricò, S., Crosta, G.B., Frattini, P., Freppaz, M. and Pogliotti, P., 2023. Hydrological, thermal and chemical influence of an intact rock glacier discharge on mountain stream water. *Science of The Total Environment*, p.162777.

Beaton, A.D., Wadham, J.L., Hawkings, J., Bagshaw, E.A., Lamarche-Gagnon, G., Mowlem, M.C. and Tranter, M., 2017. High-resolution in situ measurement of nitrate in runoff from the Greenland ice sheet. *Environmental science & technology*, *51*(21), pp.12518-12527.

Bhatia, M., Sharp, M. and Foght, J., 2006. Distinct bacterial communities exist beneath a high Arctic polythermal glacier. *Applied and Environmental Microbiology*, *72*(9), pp.5838-5845.

Bicking, S., Burkhard, B., Kruse, M. and Müller, F., 2019. Bayesian Belief Network-based assessment of nutrient regulating ecosystem services in Northern Germany. *PloS one*, *14*(4), p.e0216053.

Bischofberger, T., Cha, S.K., Schmitt, R., König, B. and Schmift-Lorenz, W., 1990. The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. *International Journal of Food Microbiology*, *11*(1), pp.51-71.

Blagodatskaya, E. and Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology and Biochemistry*, *67*, pp.192-211.

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

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

Bosson, J.B., Huss, M. and Osipova, E., 2019. Disappearing world heritage glaciers as a keystone of nature conservation in a changing climate. *Earth's Future*, *7*(4), pp.469-479.

Bosson, J.B., Huss, M., Cauvy-Fraunié, S., Clément, J.C., Costes, G., Fischer, M., Poulenard, J. and Arthaud, F., 2023. Glaciers and postglacial ecosystems: common goods to protect in the Anthropocene.

Bouwman, A.F., 1990. Land use related sources of greenhouse gases: Present emissions and possible future trends. *Land use policy*, *7*(2), pp.154-164.

Boyd, E.S., Hamilton, T.L., Havig, J.R., Skidmore, M.L. and Shock, E.L., 2014.

Chemolithotrophic primary production in a subglacial ecosystem. *Applied and environmental microbiology*, *80*(19), pp.6146-6153.

Boyd, E.S., Lange, R.K., Mitchell, A.C., Havig, J.R., Hamilton, T.L., Lafrenière, M.J., Shock, E.L., Peters, J.W. and Skidmore, M., 2011. Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial assemblages in a subglacial ecosystem. *Applied and environmental microbiology*, *77*(14), pp.4778-4787.

Boyd, E.S., Lange, R.K., Mitchell, A.C., Havig, J.R., Hamilton, T.L., Lafrenière, M.J., Shock, E.L., Peters, J.W. and Skidmore, M., 2011. Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial assemblages in a subglacial ecosystem. *Applied and environmental microbiology*, *77*(14), pp.4778-4787.

Boyd, E.S., Lange, R.K., Mitchell, A.C., Havig, J.R., Hamilton, T.L., Lafrenière, M.J., Shock, E.L., Peters, J.W. and Skidmore, M., 2011. Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial assemblages in a subglacial ecosystem. *Applied and environmental microbiology*, *77*(14), pp.4778-4787.

Bradley, J., 2021. Microbial dynamics in forefield soils following glacier retreat. In *Microbial Life In the Cryosphere and its Feedback on Global Change*. Walter de Gruyter GmbH & Co KG.

Bradley, J.A., Arndt, S., Šabacká, M., Benning, L.G., Barker, G.L., Blacker, J.J., Yallop, M.L., Wright, K.E., Bellas, C.M., Telling, J. and Tranter, M., 2016. Microbial dynamics in a High Arctic glacier forefield: a combined field, laboratory, and modelling approach. *Biogeosciences*, *13*(19), pp.5677-5696.

Brankatschk, R., Töwe, S., Kleineidam, K., Schloter, M. and Zeyer, J., 2011. Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. *The ISME journal*, *5*(6), pp.1025-1037.

Buda, J., Poniecka, E.A., Rozwalak, P., Ambrosini, R., Bagshaw, E.A., Franzetti, A., Klimaszyk, P., Nawrot, A., Pietryka, M., Richter, D. and Zawierucha, K., 2022. Is oxygenation related to the decomposition of organic matter in cryoconite holes?. *Ecosystems*, *25*(7), pp.1510-1521.

Cameron, K.A., Hodson, A.J. and Osborn, A.M., 2012. Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities. *Polar biology*, *35*, pp.1375-1393.

Cameron, K.A., Hodson, A.J. and 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), pp.254-267

Cameron, K.A., Müller, O., Stibal, M., Edwards, A. and Jacobsen, C.S., 2020. Glacial microbiota are hydrologically connected and temporally variable. *Environmental Microbiology*, *22*(8), pp.3172-3187.

capabilities. *MBio*, *11*(5), pp.10-1128.

Cebrián, J. and Duarte, C.M., 1995. Plant growth-rate dependence of detrital carbon storage in ecosystems. *Science*, *268*(5217), pp.1606-1608.

Chen, L., Zhao, B., Li, X., Cheng, Z. and Xia, Y., 2021. Isolating and characterizing translationally active fraction of anammox microbiota using bioorthogonal non-canonical amino acid tagging. *Chemical Engineering Journal*, *418*, p.129411.

Chen, Y., Liu, Y., Liu, K., Ji, M. and Li, Y., 2022. Snowstorm Enhanced the Deterministic Processes of the Microbial Community in Cryoconite at Laohugou Glacier, Tibetan Plateau. *Frontiers in Microbiology*, *12*, p.4357.

Cho, J.C., Lee, D.H., Cho, Y.C., Cho, J.C. and Kim, S.J., 1996. Direct extraction of DNA from soil for amplification of 16S rRNA gene sequences by polymerase chain reaction. *The Journal of Microbiology*, *34*(3), pp.229-235.

Cicerone, R.J. and Oremland, R.S., 1988. Biogeochemical aspects of atmospheric methane. *Global biogeochemical cycles*, *2*(4), pp.299-327.

Cook, J., 2016. 3.4. 11 Supraglacial Biogeochemistry. [e-journal] Available through: google

Darcy, J.L., Schmidt, S.K., Knelman, J.E., Cleveland, C.C., Castle, S.C. and Nemergut, D.R., 2018. Phosphorus, not nitrogen, limits plants and microbial primary producers following glacial retreat. *Science advances*, *4*(5), p.eaaq0942.

Davey, K.G., Campbell, C.K. and Warnock, D.W., 1996. Mycological techniques. *Journal of clinical pathology*, *49*(2), p.95.

Davis, J.W., Gonsior, S., Marty, G. and Ariano, J., 2005. The transformation of hexabromocyclododecane in aerobic and anaerobic soils and aquatic sediments. *Water Research*, *39*(6), pp.1075-1084.

Delaney, I. and Adhikari, S., 2020. Increased subglacial sediment discharge in a warming climate: Consideration of ice dynamics, glacial erosion, and fluvial sediment transport. *Geophysical Research Letters*, *47*(7), p.e2019GL085672.

Dieser, M., Broemsen, E.L., Cameron, K.A., King, G.M., Achberger, A., Choquette, K.,

Hagedorn, B., Sletten, R., Junge, K. and Christner, B.C., 2014. Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland Ice Sheet. *The ISME journal*, *8*(11), pp.2305-2316.

Ding, Y., Mu, C., Wu, T., Hu, G., Zou, D., Wang, D., Li, W. and Wu, X., 2021. Increasing cryospheric hazards in a warming climate. *Earth-Science Reviews,* [e-journal] 213, pp.103500. Available through: google.

Dubnick, A., Wadham, J., Tranter, M., Sharp, M., Orwin, J., Barker, J., Bagshaw, E. and Fitzsimons, S., 2017. Trickle or treat: the dynamics of nutrient export from polar glaciers. *Hydrological Processes*, *31*(9), pp.1776-1789.

Edwards, A., Irvine-Fynn, T., Mitchell, A.C. and Rassner, S.M., 2014. A germ theory for glacial systems?. *Wiley Interdisciplinary Reviews: Water*, *1*(4), pp.331-340.

Elrys, A.S., Chen, Z., Wang, J., Uwiragiye, Y., Helmy, A.M., Desoky, E.S.M., Cheng, Y., Zhang, J.B., Cai, Z.C. and Müller, C., 2022. Global patterns of soil gross immobilization of ammonium and nitrate in terrestrial ecosystems. *Global Change Biology*, *28*(14), pp.4472-4488.

Elser, J.J., Wu, C., González, A.L., Shain, D.H., Smith, H.J., Sommaruga, R., Williamson, C.E., Brahney, J., Hotaling, S. and Vanderwall, J., 2020. Key rules of life and the fading cryosphere: Impacts in alpine lakes and streams. *Global Change Biology,* [e-journal] 26 (12), pp.6644-6656. Available through: google.

Esser, G., Kattge, J. and Sakalli, A., 2011. Feedback of carbon and nitrogen cycles enhances carbon sequestration in the terrestrial biosphere. *Global Change Biology*, *17*(2), pp.819-842.

Fanin, N., Mooshammer, M., Sauvadet, M., Meng, C., Alvarez, G., Bernard, L., Bertrand, I., Blagodatskaya, E., Bon, L., Fontaine, S. and Niu, S., 2022. Soil enzymes in response to climate warming: Mechanisms and feedbacks. *Functional ecology*, *36*(6), pp.1378-1395.

Førland, E.J., Benestad, R., Hanssen-Bauer, I., Haugen, J.E. and Skaugen, T.E., 2011. Temperature and precipitation development at Svalbard 1900–2100. *Advances in Meteorology*, *2011*.

Forster, J., 1887. Über einige eigenschaften leuchtender bakterien. *Centr.Bakteriol.Parasitenk.,* [e- journal] 2, pp.337-340. Available through: google.

Franzetti, A., Pittino, F., Gandolfi, I., Azzoni, R.S., Diolaiuti, G., Smiraglia, C., Pelfini, M., Compostella, C., Turchetti, B., Buzzini, P. and Ambrosini, R., 2020. Early ecological succession patterns of bacterial, fungal and plant communities along a chronosequence in a recently deglaciated area of the Italian Alps. *FEMS Microbiology Ecology*, *96*(10), p.fiaa165

Franzluebbers, A.J., Haney, R.L., Hons, F.M. and Zuberer, D.A., 1996. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. *Soil Science Society of America Journal*, *60*(4), pp.1133-1139.

Fraser, L.H. and Keddy, P., 1997. The role of experimental microcosms in ecological research. *Trends in ecology & evolution*, *12*(12), pp.478-481.

Garcia-Lopez, E. and Cid, C., 2017. The role of microbial ecology in glacier retreat. *Glaciers Evolution in a Changing World*.

García-Palacios, P., Crowther, T.W., Dacal, M., Hartley, I.P., Reinsch, S., Rinnan, R., Rousk, J., van den Hoogen, J., Ye, J.S. and Bradford, M.A., 2021. Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming. *Nature Reviews Earth & Environment*, *2*(7), pp.507-517.

Gokul, J.K., Mur, L.A., Hodson, A.J., Irvine-Fynn, T.D., Debbonaire, A.R., Takeuchi, N. and Edwards, A., 2023. Icescape-scale metabolomics reveals cyanobacterial and topographic control of the core metabolism of the cryoconite ecosystem of an Arctic ice cap. *Environmental Microbiology*.

Grzesiak, J., Zdanowski, M.K., Górniak, D., Świątecki, A., Aleksandrzak-Piekarczyk, T., Szatraj, K., Sasin-Kurowska, J. and Nieckarz, M., 2015. Microbial community changes along the ecology glacier ablation zone (King George Island, Antarctica). *Polar Biology*, *38*, pp.2069-2083.

Guo, J., Feng, H., Peng, C., Chen, H., Xu, X., Ma, X., Li, L., Kneeshaw, D., Ruan, H., Yang, H. and Wang, W., 2023. Global climate change increases terrestrial soil CH4 emissions. *Global Biogeochemical Cycles*, *37*(1), p.e2021GB007255.

Haeberli, W. and Whiteman, C., 2015. Snow and ice-related hazards, risks, and disasters: a general framework. [e-book] 2015. *Snow and Ice-Related Hazards, Risks and Disasters.* Elsevier. , pp.1-34. Available through: google.

Hallang, H., Hiemstra, J.F., Los, S.O., Matthews, J.A. and Froyd, C.A., 2020. Carbon dioxide emissions from periglacial patterned ground under changing permafrost conditions and shrub encroachment in an alpine landscape, Jotunheimen, Norway. *Permafrost and Periglacial Processes*, *31*(4), pp.524-537.

Hamilton, T.L., Peters, J.W., Skidmore, M.L. and Boyd, E.S., 2013. Molecular evidence for an active endogenous microbiome beneath glacial ice. *The ISME journal*, *7*(7), pp.1402-1412.

Han, G., Zhou, G., Xu, Z., Yang, Y., Liu, J. and Shi, K., 2007. Biotic and abiotic factors

controlling the spatial and temporal variation of soil respiration in an agricultural ecosystem. *Soil Biology and Biochemistry*, *39*(2), pp.418-425.

Hauber, E., Reiss, D., Ulrich, M., Preusker, F., Trauthan, F., Zanetti, M., Hiesinger, H., Jaumann, R., Johansson, L., Johnsson, A. and Olvmo, M., 2011. Periglacial landscapes on Svalbard: Terrestrial analogs for cold-climate landforms on Mars. *Geological Society of America Special Papers*, *483*, pp.177-201.

Hell, K., Edwards, A., Zarsky, J., Podmirseg, S.M., Girdwood, S., Pachebat, J.A., Insam, H. and Sattler, B., 2013. The dynamic bacterial communities of a melting High Arctic glacier snowpack. *The ISME Journal*, *7*(9), pp.1814-1826.

Hodson, A., Anesio, A.M., Ng, F., Watson, R., Quirk, J., Irvine-Fynn, T., Dye, A., Clark, C.,

McCloy, P., Kohler, J. and 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*(G4).

Hodson, A., Anesio, A.M., Ng, F., Watson, R., Quirk, J., Irvine-Fynn, T., Dye, A., Clark, C., McCloy, P., Kohler, J. and 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*(G4).

Hodson, A., Anesio, A.M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J. and Sattler, B., 2008. Glacial Ecosystems. [e-journal] 78 (1) <https://esajournals.onlinelibrary.wiley.com/doi/full/10.1890/07-0187.1?casa_token=vYR7SFSfcWoAAAAA%3AnyA1sl8ajIrX4ETHHaiQaip8o8Ol4aPP9_KBUyDrmlFSVO 3arY_Yg-BDy34Yc4wcofTBhmwpNE8QMA>.

Hodson, A., Bøggild, C., Hanna, E., Huybrechts, P., Langford, H., Cameron, K. and Houldsworth, A., 2010. The cryoconite ecosystem on the Greenland ice sheet. *Annals of Glaciology*, *51*(56), pp.123-129.

Hoover, R.B. and Pikuta, E.V., 2010. *Psychrophilic and psychrotolerant microbial extremophiles in polar environments* (No. M09-0601). CRS Press.

Hornberger, G. and Winter, T.C., 2009. Snow and Ice. [e-book] Likens, G.E., ed. 2009. *Encyclopedia of Inland Waters.* Oxford:Academic Press. , pp.773. Available through: <https://www.sciencedirect.com/science/article/pii/B9780123706263000132>.

Hotaling, S., Hood, E. and Hamilton, T.L., 2017. Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections and implications of a warming climate. *Environmental microbiology*, *19*(8), pp.2935-2948.

Hotaling, S., Hood, E. and Hamilton, T.L., 2017. Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections and implications of a warming climate. *Environmental microbiology*, *19*(8), pp.2935-2948.

Hu, Y., Jiang, H., Chen, Y., Wang, Z., Yan, Y., Sun, P. and Lu, X., 2021. Nitrogen addition altered the microbial functional potentials of carbon and nitrogen transformation in alpine steppe soils on the Tibetan Plateau. *Global Ecology and Conservation*, *32*, p.e01937.

Intergovernmental Panel on Climate Change (IPCC) 2021, 'Polar Regions', in Climate Change 2021: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, eds. Serdeczny, O., D. Oki, E. F. Lambin, D. S. Gutzler, E. L. Tompkins, A.H.M. Ramons, B. Osman-Elasha, H. Carr, Y. Dibabah, M. M. Ibrahim, and L. Zommers, Cambridge University Press.

Isaksen, K., Lutz, J., Sørensen, A.M., Godøy, Ø., Ferrighi, L., Eastwood, S. and Aaboe, S., 2022. Advances in operational permafrost monitoring on Svalbard and in Norway. *Environmental Research Letters*, *17*(9), p.095012.

Javed, B. and Mashwani, Z.U.R., 2020. Synergistic effects of physicochemical parameters on bio-fabrication of mint silver nanoparticles: structural evaluation and action against HCT116 colon cancer cells. *International journal of nanomedicine*, pp.3621-3637.

Jenkinson, D.S., 1990. An introduction to the global nitrogen cycle. *Soil use and management*, *6*(2), pp.56-61.

Jett, B.D., Hatter, K.L., Huycke, M.M. and Gilmore, M.S., 1997. Simplified agar plate method for quantifying viable bacteria. *Biotechniques*, *23*(4), pp.648-650.

Kaštovská, K., Elster, J., Stibal, M. and Šantrůčková, H., 2005. Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial ecology*, *50*, pp.396-407.

Kim, S., Lee, H., Hur, S.D., Sul, W.J. and Kim, O.S., 2022. Glaciers as microbial habitats: Current knowledge and implication. *Journal of Microbiology*, *60*(8), pp.767-779.

Knight, P., 2013. *Glaciers.* [e-book] Routledge. Available through: google.

Knoblauch, C., Beer, C., Sosnin, A., Wagner, D. and Pfeiffer, E.M., 2013. Predicting long-term carbon mineralization and trace gas production from thawing permafrost of N ortheast S iberia. *Global change biology*, *19*(4), pp.1160-1172.

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. and Kohler, T.J., 2019. Greenland melt drives continuous export of methane from the ice-sheet bed. *Nature*, *565*(7737), pp.73-77.

Leghari, S.J., Wahocho, N.A., Laghari, G.M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., Bhutto, T.A., Wahocho, S.A. and Lashari, A.A., 2016. Role of nitrogen for plant growth and development: A review. *Advances in Environmental Biology*, *10*(9), pp.209-219.

Li, L., Zheng, Z., Wang, W., Biederman, J.A., Xu, X., Ran, Q., Qian, R., Xu, C., Zhang, B., Wang, F. and Zhou, S., 2020. Terrestrial N2O emissions and related functional genes under climate change: A global meta-analysis. *Global Change Biology*, *26*(2), pp.931-943.

Lindbäck, K., 2015. *Hydrology and Bed Topography of the Greenland Ice Sheet: Last known surroundings* (Doctoral dissertation, Acta Universitatis Upsaliensis).

Liu, J., Long, A., Deng, X., Yin, Z., Deng, M., An, Q., Gu, X., Li, S. and Liu, G., 2022. The Impact of Climate Change on Hydrological Processes of the Glacierized Watershed and Projections. *Remote Sensing*, *14*(6), p.1314.

Londry, K.L. and Des Marais, D.J., 2003. Stable carbon isotope fractionation by sulfate-reducing bacteria. *Applied and Environmental Microbiology*, *69*(5), pp.2942-2949.

López-Moreno, J.I., Boike, J., Sanchez-Lorenzo, A. and Pomeroy, J.W., 2016. Impact of climate warming on snow processes in Ny-Ålesund, a polar maritime site at Svalbard. *Global and Planetary Change*, *146*, pp.10-21.

Ma, H., Yan, W., Xiao, X., Shi, G., Li, Y., Sun, B., Dou, Y. and Zhang, Y., 2018. Ex situ culturing experiments revealed psychrophilic hydrogentrophic methanogenesis being the potential dominant methane-producing pathway in subglacial sediment in Larsemann Hills, Antarctic. *Frontiers in Microbiology*, *9*, p.237.

Ma, H., Yan, W., Xiao, X., Shi, G., Li, Y., Sun, B., Dou, Y. and Zhang, Y., 2018. Ex situ culturing

experiments revealed psychrophilic hydrogentrophic methanogenesis being the potential dominant methane-producing pathway in subglacial sediment in Larsemann Hills, Antarctic. *Frontiers in Microbiology*, *9*, p.237.

Machefert, S.E., Dise, N.B., Goulding, K.W.T. and Whitehead, P.G., 2002. Nitrous oxide

emission from a range of land uses across Europe. *Hydrology and Earth System Sciences*, *6*(3), pp.325-338.

Manohar, S.M., Shah, P. and Nair, A., 2021. Flow cytometry: Principles, applications and recent advances. *Bioanalysis*, *13*(3), pp.181-198.

Mapelli, F., Marasco, R., Rizzi, A., Baldi, F., Ventura, S., Daffonchio, D. and Borin, S., 2011. Bacterial communities involved in soil formation and plant establishment triggered by pyrite bioweathering on Arctic moraines. *Microbial ecology*, *61*, pp.438-447.

Margesin, R. and Collins, T., 2019. Microbial ecology of the cryosphere (glacial and permafrost habitats): current knowledge. *Applied Microbiology and Biotechnology,* [e-journal] 103 (6), pp.2537- 2549. 10.1007/s00253-019-09631-3. <https://doi.org/10.1007/s00253-019-09631-3>.

Margesin, R. and Collins, T., 2019. Microbial ecology of the cryosphere (glacial and permafrost habitats): current knowledge. *Applied microbiology and biotechnology*, *103*, pp.2537-2549.

Margesin, R. and Collins, T., 2019. Microbial ecology of the cryosphere (glacial and permafrost habitats): current knowledge. *Applied microbiology and biotechnology*, *103*, pp.2537-2549.

Margesin, R., Zacke, G. and Schinner, F., 2002. Characterization of heterotrophic microorganisms in alpine glacier cryoconite. *Arctic, Antarctic, and Alpine Research*, *34*(1), pp.88-93.

Martinez-Alonso, E., Pena-Perez, S., Serrano, S., Garcia-Lopez, E., Alcazar, A. and Cid, C., 2019. Taxonomic and functional characterization of a microbial community from a volcanic englacial ecosystem in Deception Island, Antarctica. *Scientific reports*, *9*(1), p.12158.

Matthews, J.A., 1999. Disturbance regimes and ecosystem response on recently-deglaciated substrates. *Ecosystems of the World*, pp.17-38.

McConnell, J.R., 2006. Role and importance of cryospheric processes in climate system. *Encyclopedia of Hydrological Sciences*.

McInerney, M.J., Bryant, M.P. and Pfennig, N., 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. *Archives of Microbiology*, *122*, pp.129-135.

McIntyre, N.F., 1984. Cryoconite hole thermodynamics. *Canadian Journal of Earth Sciences*, *21*(2), pp.152-156.

Meissner, K.J., Brook, E., Finkelstein, S.A. and Rae, J., 2021. Carbon cycle dynamics during episodes of rapid climate change. *Environmental Research Letters*, *16*(4), p.040201.

Michaud, L., Lo Giudice, A., Mysara, M., Monsieurs, P., Raffa, C., Leys, N., Amalfitano, S. and Van Houdt, R., 2014. Snow surface microbiome on the High Antarctic Plateau (DOME C). *PloS one*, *9*(8), p.e104505.

Miner, K.R., Turetsky, M.R., Malina, E., Bartsch, A., Tamminen, J., McGuire, A.D., Fix, A., Sweeney, C., Elder, C.D. and Miller, C.E., 2022. Permafrost carbon emissions in a changing Arctic. *Nature Reviews Earth & Environment*, *3*(1), pp.55-67.

Miteva, V.I., Sheridan, P.P. and Brenchley, J.E., 2004. Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. *Applied and Environmental Microbiology*, *70*(1), pp.202-213.

Mondini, A., Anwar, M.Z., Ellegaard-Jensen, L., Lavin, P., Jacobsen, C.S. and Purcarea, C., 2022. Heat Shock Response of the Active Microbiome From Perennial Cave Ice. *Frontiers in Microbiology*, *12*, p.4370.

Mondini, A., Anwar, M.Z., Ellegaard-Jensen, L., Lavin, P., Jacobsen, C.S. and Purcarea, C., 2022. Heat Shock Response of the Active Microbiome From Perennial Cave Ice. *Frontiers in Microbiology*, *12*, p.809076.

Monitoring, A., 2015. *AMAP Assessment 2015: Methane as an Arctic climate forcer*. Arctic Monitoring and Assessment Programme (AMAP).

Moore, T.R., Roulet, N.T. and Waddington, J.M., 1998. Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. *Climatic change*, *40*, pp.229-245.

Morgan-Kiss, R.M., Priscu, J.C., Pocock, T., Gudynaite-Savitch, L. and Huner, N.P., 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and molecular biology reviews*, *70*(1), pp.222-252.

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

Murakami, T., Takeuchi, N., Mori, H., Hirose, Y., Edwards, A., Irvine-Fynn, T., Li, Z., Ishii, S. and

Segawa, T., 2022. Metagenomics reveals global-scale contrasts in nitrogen cycling and cyanobacterial light-harvesting mechanisms in glacier cryoconite. *Microbiome*, *10*(1), pp.1-14.

Nakagawa, S. and Takai, K., 2008. Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance. *FEMS microbiology ecology*, *65*(1), pp.1-14.

Nemergut, D.R., Anderson, S.P., Cleveland, C.C., Martin, A.P., Miller, A.E., Seimon, A. and Schmidt, S.K., 2007. Microbial community succession in an unvegetated, recently deglaciated soil. *Microbial ecology*, *53*, pp.110-122.

Oliverio, A.M., Bissett, A., McGuire, K., Saltonstall, K., Turner, B.L. and Fierer, N., 2020. The role of phosphorus limitation in shaping soil bacterial communities and their metabolic

Paul, F., Frey, H. and Le Bris, R., 2011. A new glacier inventory for the European Alps from Landsat TM scenes of 2003: challenges and results. *Annals of Glaciology*, *52*(59), pp.144-152.

Pautler, B.G., Dubnick, A., Sharp, M.J., Simpson, A.J. and Simpson, M.J., 2013. Comparison of cryoconite organic matter composition from Arctic and Antarctic glaciers at the molecular-level. *Geochimica et Cosmochimica Acta*, *104*, pp.1-18.

Perkins, R.G., Bagshaw, E., Mol, L., Williamson, C.J., Fagan, D., Gamble, M. and Yallop, M.L., 2017. Photoacclimation by Arctic cryoconite phototrophs. *FEMS microbiology ecology*, *93*(5), p.fix018

Perkins, R.G., Bagshaw, E., Mol, L., Williamson, C.J., Fagan, D., Gamble, M. and Yallop, M.L., 2017. Photoacclimation by Arctic cryoconite phototrophs. *FEMS microbiology ecology*, *93*(5), p.fix018.

Plugge, C.M., Zhang, W., Scholten, J.C. and Stams, A.J., 2011. Metabolic flexibility of sulfate-reducing bacteria. *Frontiers in microbiology*, *2*, p.81.

Poniecka, E. and Bagshaw, E.A., 2021. 11 The cryoconite biome. [e-book] 2021. *Microbial Life in the Cryosphere and Its Feedback on Global Change.* De Gruyter. , pp.227-238. Available through: google.

Poniecka, E.A., Bagshaw, E.A., Sass, H., Segar, A., Webster, G., Williamson, C., Anesio, A.M. and Tranter, M., 2020. Physiological capabilities of cryoconite hole microorganisms. *Frontiers in Microbiology*, *11*, p.1783.

Previdi, M., Smith, K.L. and Polvani, L.M., 2021. Arctic amplification of climate change: a review of underlying mechanisms. *Environmental Research Letters*, *16*(9), p.093003.

Ren, Z., Martyniuk, N., Oleksy, I.A., Swain, A. and Hotaling, S., 2019. Ecological stoichiometry of the mountain cryosphere. *Frontiers in Ecology and Evolution*, *7*, p.360.

Repeta, D.J., Ferrón, S., Sosa, O.A., Johnson, C.G., Repeta, L.D., Acker, M., DeLong, E.F. and Karl, D.M., 2016. Marine methane paradox explained by bacterial degradation of dissolved organic matter. *Nature Geoscience*, *9*(12), pp.884-887.

Rick, W.Y. and Thomas, S.M., 2001. Microbial nitrogen cycles: physiology, genomics and applications. *Current Opinion in Microbiology*, *4*(3), pp.307-312.

Rime T, Hartmann M, Frey B. Potential sources of microbial colonizers in an initial soil ecosystem after retreat of an alpine glacier. The ISME journal. 2016 Jul;10(7):1625-41.

Rosentreter, J.A., Borges, A.V., Deemer, B.R., Holgerson, M.A., Liu, S., Song, C., Melack, J., Raymond, P.A., Duarte, C.M., Allen, G.H. and Olefeldt, D., 2021. Half of global methane emissions come from highly variable aquatic ecosystem sources. *Nature Geoscience*, *14*(4), pp.225-230.

Ruiz-Fernández, J., Oliva, M. and Hughes, P., 2019. Permafrost and periglacial processes in mid-and low-latitude mountain regions. *Permafrost and Periglacial Processes*, *30*(4), pp.245-248.

Sattin, S.R., Cleveland, C.C., Hood, E., Reed, S.C., King, A.J., Schmidt, S.K., Robeson, M.S., Ascarrunz, N. and Nemergut, D.R., 2009. Functional shifts in unvegetated, perhumid, recently-deglaciated soils do not correlate with shifts in soil bacterial community composition. *The Journal of Microbiology*, *47*, pp.673-681.

Schuur, E.A., McGuire, A.D., Schädel, C., Grosse, G., Harden, J.W., Hayes, D.J., Hugelius, G., Koven, C.D., Kuhry, P., Lawrence, D.M. and Natali, S.M., 2015. Climate change and the permafrost carbon feedback. *Nature*, *520*(7546), pp.171-179.

Seckbach, J., 2000. Extremophilies as models for extraterrestrial life. In *Bioastronomy 99* (Vol. 213).

Segawa, T., Ishii, S., Ohte, N., Akiyoshi, A., Yamada, A., Maruyama, F., Li, Z., Hongoh, Y. and Takeuchi, N., 2014. The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environmental microbiology*, *16*(10), pp.3250-3262.

Segawa, T., Ishii, S., Ohte, N., Akiyoshi, A., Yamada, A., Maruyama, F., Li, Z., Hongoh, Y. and Takeuchi, N., 2014. The nitrogen cycle in cryoconites: naturally occurring nitrification-denitrification granules on a glacier. *Environmental microbiology*, *16*(10), pp.3250-3262.

Segawa, T., Takeuchi, N., Mori, H., Rathnayake, R.M., Li, Z., Akiyoshi, A., Satoh, H. and Ishii, S., 2020. Redox stratification within cryoconite granules influences the nitrogen cycle on glaciers. *FEMS microbiology ecology*, *96*(11), p.fiaa199.

Selvin, R., Reguera, B., Bravo, I. and Yentsch, C.M., 1989. Use of fluorescein diacetate (FDA) as a single-cell probe of metabolic activity in dinoflagellate cultures. *Biological oceanography*, *6*(5-6), pp.505-511.

Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L. and Luna-Guido, M., 2014. Methanogenesis and methanotrophy in soil: a review. *Pedosphere*, *24*(3), pp.291-307.

Serreze, M.C. and Barry, R.G., 2011. Processes and impacts of Arctic amplification: A research synthesis. *Global and planetary change*, *77*(1-2), pp.85-96.

Shahgedanova, M., 2021. Climate change and melting glaciers. In *The Impacts of Climate Change* (pp. 53-84). Elsevier.

Shand, C.A., Williams, B.L. and Coutts, G., 2008. Determination of N-species in soil extracts using microplate techniques. *Talanta*, *74*(4) Wehr, J.D. and Sheath, R.G., 2015. Habitats of freshwater algae. In *Freshwater Algae of North America* (pp. 13-74). Academic Press., pp.648-654.

Singh, B.K., Bardgett, R.D., Smith, P. and Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, *8*(11), pp.779-790.

Singh, P. and Singh, S.M., 2012. Characterization of yeast and filamentous fungi isolated from cryoconite holes of Svalbard, Arctic. *Polar Biology*, *35*, pp.575-583.

Slater, T., Lawrence, I.R., Otosaka, I.N., Shepherd, A., Gourmelen, N., Jakob, L., Tepes, P., Gilbert, L. and Nienow, P., 2021. Earth's ice imbalance. *The Cryosphere*, *15*(1), pp.233-246.

Smittenberg, R.H., Gierga, M., Göransson, H., Christl, I., Farinotti, D. and Bernasconi, S.M., 2012. Climate-sensitive ecosystem carbon dynamics along the soil chronosequence of the D amma glacier forefield, S witzerland. *Global Change Biology*, *18*(6), pp.1941-1955.

Stevens, I.T., Irvine-Fynn, T.D., Edwards, A., Mitchell, A.C., Cook, J.M., Porter, P.R., Holt, T.O.,

Huss, M., Fettweis, X., Moorman, B.J. and Sattler, B., 2022. Spatially consistent microbial biomass and future cellular carbon release from melting Northern Hemisphere glacier surfaces. *Communications Earth & Environment*, *3*(1), p.275.

Stibal, M., Wadham, J.L., Lis, G.P., Telling, J., Pancost, R.D., Dubnick, A., Sharp, M.J., Lawson, E.C., Butler, C.E., Hasan, F. and Tranter, M., 2012. Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting organic carbon sources. *Global Change Biology*, *18*(11), pp.3332-3345.

Strauss, S.L., Garcia-Pichel, F. and Day, T.A., 2012. Soil microbial carbon and nitrogen transformations at a glacial foreland on Anvers Island, Antarctic Peninsula. *Polar Biology*, *35*, pp.1459-1471.

Su, B., Xiao, C.D., Chen, D.L., Qin, D.H. and Ding, Y.J., 2019. Cryosphere Services and Human Well-Being. Sustainability, 11 (16).

Sun, S., Ma, B., Wang, G. and Tan, X., 2023. Linking microbial biogeochemical cycling genes to the rhizosphere of pioneering plants in a glacier foreland. *Science of The Total Environment*, *872*, p.161944.

Sun, Y., De Silva, A.O., St Pierre, K.A., Muir, D.C., Spencer, C., Lehnherr, I. and MacInnis, J.J., 2020. Glacial melt inputs of organophosphate ester flame retardants to the largest high Arctic lake. *Environmental Science & Technology*, *54*(5), pp.2734-2743.

Takeuchi, N., Nishiyama, H. and Li, Z., 2010. Structure and formation process of cryoconite granules on Ürümqi glacier No. 1, Tien Shan, China. *Annals of Glaciology*, *51*(56), pp.9-14.

Tandong, Y., Keqin, D., Baiqing, X., Ninglian, W., Jianchen, P., Shichang, K., Xiang, Q. and Thompson, L.G., 2002. Temperature and methane changes over the past 1000 years recorded in Dasuopu glacier (central Himalaya) ice core. *Annals of Glaciology*, *35*, pp.379-383.

Tang, G., Zheng, J., Xu, X., Yang, Z., Graham, D.E., Gu, B., Painter, S.L. and Thornton, P.E., 2016. Biogeochemical modeling of CO 2 and CH 4 production in anoxic Arctic soil microcosms. *Biogeosciences*, *13*(17), pp.5021-5041.

Töwe, S., Albert, A., Kleineidam, K., Brankatschk, R., Dümig, A., Welzl, G., Munch, J.C., Zeyer, J. and Schloter, M., 2010. Abundance of microbes involved in nitrogen transformation in the rhizosphere of Leucanthemopsis alpina (L.) Heywood grown in soils from different sites of the Damma glacier forefield. *Microbial ecology*, *60*, pp.762-770.

Tribelli, P.M. and López, N.I., 2018. Reporting key features in cold-adapted bacteria. *Life*, *8*(1), p.8.

Tscherko, D., Hammesfahr, U., Zeltner, G., Kandeler, E. and Böcker, R., 2005. Plant succession and rhizosphere microbial communities in a recently deglaciated alpine terrain. *Basic and applied ecology*, *6*(4), pp.367-383.

Turchetti, B., Buzzini, P., Goretti, M., Branda, E., Diolaiuti, G., D'Agata, C., Smiraglia, C. and Vaughan-Martini, A., 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS microbiology ecology*, *63*(1), pp.73-83.

Turchetti, B., Buzzini, P., Goretti, M., Branda, E., Diolaiuti, G., D'Agata, C., Smiraglia, C. and Vaughan-Martini, A., 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS microbiology ecology*, *63*(1), pp.73-83.

Vaughan, D.G., J.C. Comiso, I. Allison, J. Carrasco, G. Kaser, R. Kwok, P. Mote, T. Murray, F. Paul, J. Ren, E. Rignot, O. Solomina, K. Steffen and T. Zhang, 2013. *Observations: Cryosphere.* Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.

Vincent, W.F., Mueller, D.R. and Bonilla, S., 2004. Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology*, *48*(2), pp.103-112.

Vitousek, P.M., 1994. Beyond global warming: ecology and global change. *Ecology*, *75*(7), pp.1861-1876.

Wadham, J.L., Tranter, M., Tulaczyk, S. and Sharp, M., 2008. Subglacial methanogenesis: a potential climatic amplifier?. *Global Biogeochemical Cycles*, *22*(2).

Walworth, J.L., Woolard, C.R. and Harris, K.C., 2003. Nutrient amendments for contaminated peri-glacial soils: use of cod bone meal as a controlled release nutrient source. *Cold regions science and technology*, *37*(2), pp.81-88.

Wang, F., Liang, X., Ding, F., Ren, L., Liang, M., An, T., Li, S., Wang, J. and Liu, L., 2022. The active functional microbes contribute differently to soil nitrification and denitrification potential under long-term fertilizer regimes in North-East China. *Frontiers in microbiology*, *13*, p.1021080.

Wang, X., Liu, S. and Zhang, J., 2019. A new look at roles of the cryosphere in sustainable development. *Advances in Climate Change Research,* [e-journal] 10 (2), pp.124-131. https://doi.org/10.1016/j.accre.2019.06.005. <https://www.sciencedirect.com/science/article/pii/S1674927818301084>.

Wang, Y., Ma, A., Liu, G., Ma, J., Wei, J., Zhou, H., Brandt, K.K. and Zhuang, G., 2020. Potential feedback mediated by soil microbiome response to warming in a glacier forefield. *Global change biology*, *26*(2), pp.697-708.

Wehr, J.D. and Sheath, R.G., 2015. Habitats of freshwater algae. In *Freshwater Algae of North America* (pp. 13-74). Academic Press.

Weijma, J., Gubbels, F., Hulshoff Pol, L.W., Stams, A.J.M., Lens, P. and Lettinga, G., 2002. Competition for H2 between sulfate reducers, methanogens and homoacetogens in a gas-lift reactor. *Water Science and Technology*, *45*(10), pp.75-80.

Welte, C. and Deppenmeier, U., 2014. Bioenergetics and anaerobic respiratory chains of aceticlastic methanogens. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1837*(7), pp.1130-1147.

Williamson, C.J., Cameron, K.A., Cook, J.M., Zarsky, J.D., Stibal, M. and Edwards, A., 2019. Glacier algae: A dark past and a darker future. *Frontiers in microbiology*, *10*, p.524.

Winton, M., 2006. Amplified Arctic climate change: What does surface albedo feedback have to do with it?. *Geophysical Research Letters*, *33*(3).

Xing, T., Liu, P., Ji, M., Deng, Y., Liu, K., Wang, W. and Liu, Y., 2022. Sink or source: alternative roles of glacier foreland meadow soils in methane emission is regulated by glacier melting on the Tibetan plateau. *Frontiers in Microbiology*, *13*, p.862242.

Yang, Z., Wullschleger, S.D., Liang, L., Graham, D.E. and Gu, B., 2016. Effects of warming on the degradation and production of low-molecular-weight labile organic carbon in an Arctic tundra soil. *Soil Biology and Biochemistry*, *95*, pp.202-211.

Yarzábal, L.A., Salazar, L.M.B. and Batista-García, R.A., 2021. Climate change, melting cryosphere and frozen pathogens: Should we worry...? *Environmental Sustainability,* [e-journal] , pp.1-13. Available through: google.

Yiqi, L. and Zhou, X., 2010. *Soil respiration and the environment*. Elsevier.

Zarsky, J.D., Stibal, M., Hodson, A., Sattler, B., Schostag, M., Hansen, L.H., Jacobsen, C.S. and Psenner, R., 2013. Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea. *Environmental Research Letters*, *8*(3), p.035044.

Zawierucha, K., Porazinska, D.L., Ficetola, G.F., Ambrosini, R., Baccolo, G., Buda, J., Ceballos, J.L., Devetter, M., Dial, R. and Franzetti, A., 2021. A hole in the nematosphere: tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing. *Journal of zoology,* [e- journal] 313 (1), pp.18-36. Available through: google.

Zazovskaya, E.P., Mergelov, N.S., Shishkov, V.A., Dolgikh, A.V., Dobryansky, A.S., Lebedeva, M.P.,

Zazovskaya, E.P., Mergelov, N.S., Shishkov, V.A., Dolgikh, A.V., Dobryansky, A.S., Lebedeva, M.P., Turchinskaya, S.M. and Goryachkin, S.V., 2022. Cryoconites as factors of soil development in conditions of rapid retreat of the Aldegonda Glacier, Western Svalbard. *Eurasian Soil Science*, *55*(3), pp.299-312.

Zdanowski, M.K., Bogdanowicz, A., Gawor, J., Gromadka, R., Wolicka, D. and Grzesiak, J., 2017. Enrichment of cryoconite hole anaerobes: implications for the subglacial microbiome. *Microbial ecology*, *73*, pp.532-538.

Zemp, M., Hoelzle, M. and Haeberli, W., 2009. Six decades of glacier mass-balance observations: a review of the worldwide monitoring network. *Annals of Glaciology,* [e-journal] 50 (50), pp.101-111. Available through: google.

Zhang, Y., Kang, S., Wei, D., Luo, X., Wang, Z. and Gao, T., 2021. Sink or source? Methane and carbon dioxide emissions from cryoconite holes, subglacial sediments, and proglacial river runoff during intensive glacier melting on the Tibetan Plateau. *Fundamental Research*, *1*(3), pp.232-239.

Zhu, X., Deng, Y., Hernández, M., Fang, J., Xing, P. and Liu, Y., 2023. Distinct responses of soil methanotrophy in hummocks and hollows to simulated glacier meltwater and temperature rise in Tibetan glacier foreland. *Science of The Total Environment*, *862*, p.160888.

Zuo, Y., Zhang, H., Li, J., Yao, X., Chen, X., Zeng, H. and Wang, W., 2021. The effect of soil depth on temperature sensitivity of extracellular enzyme activity decreased with elevation: Evidence from mountain grassland belts. *Science of the Total Environ*

Appendix

Initial Study Goal-

Originally, this study aimed to explore the intricate relationship between surface and subsurface glacier ecosystems in response to a warming climate. The acceleration of glacial melt, a consequence of global warming, has strengthened hydrological channels such as moulins and crevasses, enabling a greater transfer of organisms from the surface to the subglacial environment (Hodson et al.,2008). This phenomenon has been identified as a potential factor contributing to increased GHG emissions observed from retreating glaciers (Du et al., 2022). To investigate this, we devised a microcosm incubation experiment wherein cryoconite was to be exposed to various conditions characteristic of the subglacial environment, including oxygen-depleted, dark, and high-pressure conditions. To achieve this, an anaerobic chamber was to be set-up to create anoxic incubations, and samples were to be covered in aluminium foil and subjected to 1.5mpa (220 psi) at 1.5ºC. To analyse the response of cryoconite, live/dead staining would be employed in combination with BONCAT to evaluate changes in community viability and metabolic activity, respectively. BONCAT is a technique used to selectively label and track actively metabolizing microbial cells within a complex community. Given that BONCAT represents a relatively recent technique in polar sample applications, we sought to enhance current laboratory methodologies for studying metabolic activity in sediments by collaborating with Dr James Bradley (Queen Mary University, London) and building upon his preliminary work.

Project Setbacks-

Unfortunately, the project faced significant setbacks due to COVID-19 implications and unforeseen circumstances. Initial travel delays, a consequence of COVID-19 restrictions, led to the rescheduling of fieldwork until later in the melt season. This delay resulted in the inability to collect samples due to the onset of winter weather conditions, preventing access to the sampling site. As an alternative, we sought to find collaborators who could supply alternative material, which further added to delays. Upon successfully obtaining samples, a major setback occurred with the unplugging of the freezer storing them. The freezer

remained unplugged for an unknown period (likely weeks), leading to the warming of all samples, with consequences for the viability and validity of these samples for these investigations. This incident therefore required a reassessment of the project's design and timelines. When the revised project commenced, an irreparable breakage in the gas chromatography machine was discovered, requiring us to source an alternative machine from an external institute. Although eventually sourced in Bangor, this process involved collecting appropriate vials from Wales and transporting the samples to the university. Additionally, during preliminary research on microscopy techniques, issues arose with the microscope. A suitable replacement was obtained from another department. However, due to other students also facing setbacks due to COVID, the booking system became extremely limited, with sampling slots available weeks apart. Consequently, the project's scope and goals were adapted, as detailed above. Due to constraints in sample availability and limited laboratory time, BONCAT, 16S rRNA analysis, and anabolic chamber analyses were removed, as outlined in the appendix. Microcosm numbers additionally had to be limited on account of reduced sample availability.

Lost gas concentration data-

An error message on the gas chromatography machine reading- “Auto Themo Time out” suggesting a malfunction potentially linked to the overheating of the lab/ equipment overnight. This consequently led to CO_2 concentrations for cryoconite in the light at T5 being lost.

CH4 Concentrations (ppm) - Gas Chromatography										
Sample Type/ Conditions	**Time Point**	**R1**	**R2**	**R3**	**R4**	**Average**	**Lowest**	**Highest**	**Lowest D**	**Highest D**
Cryoconite 21^{O}C Light	T1	1.99	1.92	2.01	1.93	1.9625	1.92	2.01	0.0425	0.0475
	T2	1.91	1.96	1.87	1.98	1.93	1.87	1.98	0.06	0.05
	T3	2.03	2.03	2.2	2.06	2.08	2.03	2.2	0.05	0.12
	T5	3.29		0.9	1.82	2.00333333	0.9	3.29	1.10333333	1.28666667
Cryoconite 21^{O}C Dark	T1	2	1.93	1.92	1.88	1.9325	1.88	2	0.0525	0.0675
	T2	2	2.12	1.94	1.92	1.995	1.92	2.12	0.075	0.125
	T3	2.08	2.1	2.14	1.94	2.065	1.94	2.14	0.125	0.075
	T5	2.03	1.99	2.12	2.02	2.04	1.99	2.12	0.05	0.08
Cryoconite 7^{O}C Light	T1	1.92	1.96	1.87	2.03	1.945	1.87	2.03	0.075	0.085
	T2	1.99	2.01	1.94	2.02	1.99	1.94	2.02	0.05	0.03
	T3	2.17	1.92	2.12	1.91	2.03	1.91	2.17	0.12	0.14
	T4	2.1	2.06	2.11	2.01	2.07	2.01	2.11	0.06	0.04
Cryoconite 7^{O}C Dark	T1	1.96	2.02	1.98	1.88	1.96	1.88	2.02	0.08	0.06
	T2	2.05	2.12	2.13	2.04	2.085	2.04	2.13	0.045	0.045
	T3	2.01	2.07	2.1	1.96	2.035	1.96	2.1	0.075	0.065
	T5	2.11	1.99	2.06	2.06	2.055	1.99	2.11	0.065	0.055
Cryoconite 1^{O}C Dark	T1	1.96	2.02	2.02	1.95	1.9875	1.95	2.02	0.0375	0.0325
	T2	1.91	1.97	2.02	2.08	1.995	1.91	2.08	0.085	0.085
	T3	2.04	2.13	2.09	2.03	2.0725	2.03	2.13	0.0425	0.0575
	T5	2.11	2.05	2.06	2.15	2.0925	2.05	2.15	0.0425	0.0575
Cryoconite 1^{O}C Light	T1	1.85	1.8	1.98	1.99	1.905	1.8	1.99	0.105	0.085
	T2	2.03	2.11	1.97	2.09	2.05	1.97	2.11	0.08	0.06
	T3	2	1.94	1.98	2.04	1.99	1.94	2.04	0.05	0.05

	T5	2.05	2.03	2.08	1.95	2.0275	1.95	2.08	0.0775	0.0525
Subglacial $21^{O}C$ Light	T1	2.05	1.98	1.99	2.01	2.0075	1.98	2.05	0.0275	0.0425
	T2	2.01	1.97	2.02	2	2	1.97	2.02	0.03	0.02
	T3	2.06	2.06	2.14	2.02	2.07	2.02	2.14	0.05	0.07
	T5	1.92	1.97	1.88	1.81	1.895	1.81	1.97	0.085	0.075
Subglacial $7^{O}C$ Dark	T1	1.79	1.86	1.9	1.68	1.8075	1.68	1.9	0.1275	0.0925
	T2	1.91	1.9	1.85	1.88	1.885	1.85	1.91	0.035	0.025
	T3	1.83	1.9	2.01	1.9	1.91	1.83	2.01	0.08	0.1
	T5	1.85	1.88	1.83	1.85	1.8525	1.83	1.88	0.0225	0.0275
Subglacial $7^{O}C$ Light	T1	1.87	1.88	1.77	1.98	1.875	1.77	1.98	0.105	0.105
	T2	1.98	1.85	1.86	1.94	1.9075	1.85	1.98	0.0575	0.0725
	T3	1.93	1.93	1.9	1.96	1.93	1.9	1.96	0.03	0.03
	T5	1.92	1.9	1.99	1.91	1.93	1.9	1.99	0.03	0.06
Subglacial $21^{O}C$ Dark	T1	2.02	2.01	1.91	1.9	1.96	1.9	2.02	0.06	0.06
	T2	1.95	2.04	1.88	1.89	1.94	1.88	2.04	0.06	0.1
	T3	1.94	2.14	1.97	2.07	2.03	1.94	2.14	0.09	0.11
	T5	1.98	1.86	1.87	1.93	1.91	1.86	1.98	0.05	0.07
Subglacial $1^{O}C$ Dark	T1	1.82	1.88	1.93	1.79	1.855	1.79	1.93	0.065	0.075
	T2	1.59	1.93	2.01	1.9	1.8575	1.59	2.01	0.2675	0.1525
	T3	2.01	1.99	1.97	2.03	2	1.97	2.03	0.03	0.03
	T5	1.86	1.95	2.1	1.72	1.9075	1.72	2.1	0.1875	0.1925

CO2 Concentrations (ppm) - Gas Chromatography										
Sample Type/ Conditions	**Time Point**	**R1**	**R2**	**R3**	**R4**	**Average**	**Lowest**	**Highest**	**Lowest D**	**Highest D**
Cryoconite 21^{O}C Light	T1	249.33	255.14	249.65	247.92	250.51	247.92	255.14	2.59	4.63
	T2	2585.39	2686.89	2285.68	2517.22	2518.795	2285.68	2686.89	233.115	168.095
	T3	3183.32	2619.38	3490.91	3227.34	3130.2375	2619.38	3490.91	510.8575	360.6725
	T5	N/A	N/A	N/A	N/A	N/A	N.A	N/A	N/A	N/A
Cryoconite 21^{O}C Dark	T1	235.46	224.72	244.35	244.23	237.19	224.72	244.35	12.47	7.16
	T2	2862.08	2772.83	343.02	2614.5	2148.1075	343.02	2862.08	1805.0875	713.9725
	T3	3434.36	3521.62	3238.95	2979.7	3293.6575	2979.7	3521.62	313.9575	227.9625
	T5	4340.69	673.1	4313.37	3185.33	3128.1225	673.1	4340.69	2455.0225	1212.5675
Cryoconite 7^{O}C Light	T1	246.04	244.62	227.44	224.5	235.65	224.5	246.04	11.15	10.39
	T2	2626.19	2892.81	2471.34	2821.97	2703.0775	2471.34	2892.81	231.7375	189.7325
	T3	4049.74	3443.06	4223	3927.97	3910.9425	3443.06	4223	467.8825	312.0575
	T5	5788.47	2767.68	4492.16	5189.8	4559.5275	2767.68	5788.47	1791.8475	1228.9425
Cryoconite 7^{O}C Dark	T1	254.45	258.44	256.12	244.08	253.2725	244.08	258.44	9.1925	5.1675
	T2	1842.19	1951.45	1890.91	1972.77	1914.33	1842.19	1972.77	72.14	58.44
	T3	2763.73	2662	2501.16	1863.75	2447.66	1863.75	2763.73	583.91	316.07
	T5	2687.37	3243.92	438.8	2952.2	2330.5725	438.8	3243.92	1891.7725	913.3475
Cryoconite 1^{O}C Dark	T1	264.31	265.15	254.17	257.84	260.3675	254.17	265.15	6.1975	4.7825
	T2	1900.47	1996.43	1890.69	1931.75	1929.835	1890.69	1996.43	39.145	66.595
	T3	2371.55	2722.91	2459.55	2617.11	2542.78	2371.55	2722.91	171.23	180.13
	T5	2933.36	2922.85	3209.1	3006.35	3017.915	2922.85	3209.1	95.065	191.185
Cryoconite 1^{O}C Light	T1	273.15	257.11	253.36	270.15	263.4425	253.36	273.15	10.0825	9.7075
	T2	2672.94	2702.82	2933.5	2861.16	2792.605	2672.94	2933.5	119.665	140.895
	T3	3574.03	4115.74	4384.98	2423.64	3624.5975	2423.64	4384.98	1200.9575	760.3825

	T5	4123.82	3786.53	3795.82	3739.32	3861.3725	3739.32	4123.82	122.0525	262.4475
Subglacial $7^{O}C$ Light	T1	210.52	277.85	255	271.14	253.6275	210.52	277.85	43.1075	24.2225
	T2	294.26	290.47	280.73	254.66	280.03	254.66	294.26	25.37	14.23
	T3	297.42	297.77	314.78	252.19	290.54	252.19	314.78	38.35	24.24
	T5	269.67	211.64	300.56	305.32	271.7975	211.64	305.32	60.1575	33.5225
Subglacial $7^{O}C$ Dark	T1	269.46	249.68	275.62	279.21	268.4925	249.68	279.21	18.8125	10.7175
	T2	214.15	232.9	248.41	256.91	238.0925	214.15	256.91	23.9425	18.8175
	T3	346.39	275.03	336.61	317.86	318.9725	275.03	346.39	43.9425	27.4175
	T5	295.31	299	296.37	245.8	284.12	245.8	299	38.32	14.88
Subglacial $21^{O}C$ Light	T1	249.86	237.86	255.7	236.1	244.88	236.1	255.7	8.78	10.82
	T2	247.97	248.13	240.97	252.85	247.48	240.97	252.85	6.51	5.37
	T3	302.64	305.47	331.43	177.43	279.2425	177.43	331.43	101.8125	52.1875
	T5	330.15	313.99	315.31	301.6	315.2625	301.6	330.15	13.6625	14.8875
Subglacial $21^{O}C$ Dark	T1	278.46	280.14	210.05	221.42	247.5175	210.05	280.14	37.4675	32.6225
	T2	283.06	282.12	191.56	236.3	248.26	191.56	283.06	56.7	34.8
	T3	305.88	307.27	293.53	308.36	303.76	293.53	308.36	10.23	4.6
	T5	285.1	270.82	286.43	266.81	277.29	266.81	286.43	10.48	9.14
Subglacial $1^{O}C$ Dark	T1	240.56	270.85	280.65	266.03	264.5225	240.56	280.65	23.9625	16.1275
	T2	214.65	256.42	276.42	292.42	259.9775	214.65	292.42	45.3275	32.4425
	T3	322.49	315.42	328.31	329.81	324.0075	315.42	329.81	8.5875	5.8025
	T5	333.25	299.88	314.77	286.19	308.5225	286.19	333.25	22.3325	24.7275

N2O Concentrations (ppm) – Gas Chromatography										
Sample Type/ Conditions	**Time Point**	**R1**	**R2**	**R3**	**R4**	**Average**	**Lowest**	**Highest**	**Lowest D**	**Highest D**
Cryoconite 21^{O}C Light	T1	0.3466	0.34	0.36	0.3564	0.3496	0.3403	0.3564	0.0093	0.0068
	T2	0.2661	0.30	0.29	0.3055	0.2888	0.2661	0.3055	0.0227	0.0167
	T3	0.2879	0.31	0.29	0.2955	0.2959	0.2879	0.3079	0.0080	0.0121
	T5	0.3886		0.05	0.2292	0.2218	0.0477	0.3886	0.1741	0.1668
Cryoconite 21^{O}C Dark	T1	0.3029	0.29	0.30	0.2886	0.2967	0.2886	0.3029	0.0081	0.0062
	T2	0.2356	0.25	0.32	0.2468	0.2653	0.2356	0.3247	0.0297	0.0594
	T3	0.1807	0.21	0.25	0.2724	0.2270	0.1807	0.2724	0.0463	0.0454
	T5	0.2296	0.33	0.20	0.2622	0.2560	0.2027	0.3296	0.0533	0.0736
Cryoconite 7^{O}C Light	T1	0.2915	0.27	0.29	0.282	0.2814	0.2661	0.2915	0.0153	0.0102
	T2	0.2789	0.27	0.27	0.2861	0.2747	0.2668	0.2861	0.0079	0.0114
	T3	0.2796	0.26	0.25	0.2622	0.2628	0.2510	0.2796	0.0118	0.0168
	T5	0.2325	0.29	0.27	0.2486	0.2592	0.2325	0.2876	0.0267	0.0284
Cryoconite 7^{O}C Dark	T1	0.3159	0.3274	0.3135	0.314	0.3177	0.3135	0.3274	0.0042	0.0097
	T2	0.2907	0.3046	0.2966	0.2976	0.297375	0.2907	0.3046	0.0067	0.0072
	T3	0.2808	0.292	0.3004	0.2986	0.29295	0.2808	0.3004	0.0122	0.0075
	T5	0.2981	0.279	0.3494	0.2948	0.305325	0.2790	0.3494	0.0263	0.0441
Cryoconite 1^{O}C Dark	T1	0.3402	0.3447	0.3083	0.3507	0.335975	0.3083	0.3507	0.0277	0.0147
	T2	0.3262	0.3314	0.3379	0.332	0.331875	0.3262	0.3379	0.0057	0.0060
	T3	0.3463	0.3365	0.3462	0.3413	0.342575	0.3365	0.3463	0.0061	0.0037
	T5	0.3165	0.3096	0.3082	0.3507	0.32125	0.3082	0.3507	0.0131	0.0295
Cryoconite 1^{O}C Light	T1	0.2631	0.2824	0.2757	0.2812	0.2756	0.2631	0.2824	0.0125	0.0068
	T2	0.2563	0.2846	0.2635	0.266	0.2676	0.2563	0.2846	0.0113	0.0170
	T3	0.2883	0.2886	0.2938	0.2966	0.291825	0.2883	0.2966	0.0035	0.0048

	T5	0.2937	0.3059	0.2954	0.2914	0.2966	0.2914	0.3059	0.0052	0.0093
Subglacial $7^{O}C$ Light	T1	0.3117	0.3183	0.3188	0.3311	0.319975	0.3117	0.3311	0.0083	0.0111
	T2	0.3159	0.3065	0.3154	0.3253	0.315775	0.3065	0.3253	0.0093	0.0095
	T3	0.3388	0.3246	0.3453	0.298	0.326675	0.2980	0.3453	0.0287	0.0186
	T5	0.3144	0.3047	0.3147	0.3046	0.3096	0.3046	0.3147	0.0050	0.0051
Subglacial $7^{O}C$ Dark	T1	0.3215	0.3301	0.318	0.3283	0.324475	0.3180	0.3301	0.0065	0.0056
	T2	0.3126	0.3195	0.3239	0.3406	0.32415	0.3126	0.3406	0.0116	0.0165
	T3	0.3286	0.3458	0.3418	0.3394	0.3389	0.3286	0.3458	0.0103	0.0069
	T5	0.3031	0.3276	0.3328	0.3236	0.321775	0.3031	0.3328	0.0187	0.0110
Subglacial $21^{O}C$ Light	T1	0.3051	0.3054	0.307	0.3142	0.307925	0.3051	0.3142	0.0028	0.0063
	T2	0.3111	0.3084	0.3046	0.312	0.309025	0.3046	0.3120	0.0044	0.0030
	T3	0.3143	0.3182	0.3372	0.3115	0.3203	0.3115	0.3372	0.0088	0.0169
	T5	0.2988	0.2924	0.3158	0.2862	0.2983	0.2862	0.3158	0.0121	0.0175
Subglacial $21^{O}C$ Dark	T1	0.3337	0.3008	0.3093	0.2825	0.306575	0.2825	0.3337	0.0241	0.0271
	T2	0.3124	0.3087	0.2953	0.2806	0.29925	0.2806	0.3124	0.0187	0.0132
	T3	0.3302	0.3197	0.3333	0.3121	0.323825	0.3121	0.3333	0.0117	0.0095
	T5	0.3372	0.2718	0.3006	0.2914	0.30025	0.2718	0.3372	0.0285	0.0370
Subglacial $1^{O}C$ Dark	T1	0.2859	0.3165	0.3229	0.2753	0.30015	0.2753	0.3229	0.0249	0.0228
	T2	0.2496	0.3108	0.3009	0.3163	0.2944	0.2496	0.3163	0.0448	0.0219
	T3	0.3342	0.3166	0.3323	0.3449	0.332	0.3166	0.3449	0.0154	0.0129
	T5	0.3379	0.3296	0.337	0.3212	0.331425	0.3212	0.3379	0.0102	0.0065

Percentage Living (%)									
Sample Type/ Conditions	**Time Point**	**R1**	**R2**	**R3**	**Average**	**Lowest N**	**Highest N**	**Lowest D**	**Highest D**
Cryoconite 21^{O}C Light	T1	68	65	66	66	65	68	1	2
	T2	77	95	86	86	77	95	9	9
	T3	71	43	57	57	43	71	14	14
	T5	94	76	85	85	76	94	9	9
Cryoconite 21^{O}C Dark	T1	55	67	61	61	55	67	6	6
	T2	87	86	88	87	86	88	1	1
	T3	95	63	79	79	63	95	16	16
	T5	64	67	66	65	64	67	1	2
Cryoconite 7^{O}C Light	T1	87	80	80	82	80	87	2	5
	T2	80	80	83	81	80	83	1	2
	T3	71	92	93	85	71	93	14	8
	T5	81	75	78	78	75	81	3	3
Cryoconite 7^{O}C Dark	T1	70	76	84	76	70	84	6	8
	T2	85	90	87	87	85	90	2	3
	T3	75	72	94	80	72	94	8	14
	T5	76	75	74	75	74	76	1	1
Cryoconite 1^{O}C Dark	T1	95	96	92	94	92	96	2	2
	T2	61	96	76	77	61	96	16	19
	T3	58	82	70	70	58	70	12	0
	T5	63	78	70	70	63	78	7	8
Cryoconite 1^{O}C Light	T1	91	80	88	86	80	91	6	5
	T2	92	74	87	84	74	92	10	8
	T3	89	78	83	83	78	89	5	6

	T5	72	95	82	83	72	95	11	12
Subglacial 21^{O}C Light	T1	20	19	21	20	19	21	1	1
	T2	8	18	29	18	8	29	10	11
	T3	17	7	28	17	7	28	10	11
	T5	18	10	14	14	10	18	4	4
Subglacial 21^{O}C Dark	T1	51	12	28	30	12	51	18	21
	T2	16	9	32	19	9	32	10	13
	T3	14	13	15	14	13	15	1	1
	T5	86	77	81	81	77	86	4	5
Subglacial 7^{O}C Light	T1	51	50	52	51	50	52	1	1
	T2	19	12	15	15	12	19	3	4
	T3	82	80	81	81	80	82	1	1
	T5	52	3	27	27	3	52	24	25
Subglacial 7^{O}C Dark	T1	12	43	27	27	12	43	15	16
	T2	32	43	37	37	32	43	5	6
	T3	8	5	6	6	5	8	1	2
	T5	39	38	40	39	38	40	1	1
Subglacial 1^{O}C Dark	T1	47	41	44	44	41	47	3	3
	T2	17	18	16	17	16	18	1	1
	T3	47	5	26	26	5	47	21	21
	T5	82	70	76	76	70	82	6	6