

CRYOCONITE HOLES ON ROOT GLACIER, ALASKA: AN EXPLORATION
OF DISSOLVED ORGANIC CARBON, BACTERIAL ABUNDANCE AND
PHYSICALITY

by

Stephanie Pierce

A Thesis

Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
June 2024

© 2024 by Stephanie Pierce. All rights reserved.

This Thesis for the Master of Environmental Studies Degree

by

Stephanie Pierce

has been approved for

The Evergreen State College

by

Erin Martin, Ph. D.

Member of Faculty

Date

Abstract

Cryoconite holes on Root Glacier, Alaska: An exploration of dissolved organic carbon and bacterial abundance

Stephanie Pierce

Cryoconite holes play a significant role in the biogeochemical cycling of glaciated ecosystems and the downstream rivers and water bodies being fed by their meltwater. This study examines the physicality, bacterial enumeration, respiration rates, and organic carbon compositions of cryoconite holes on Root Glacier in Alaska during June of 2023. Samples were collected from 15 cryoconite holes. Results of the study showed that dissolved organic carbon (DOC) concentrations in water samples taken on the Root glacier ranged from 0.077-5.83 mg C l⁻¹ during the beginning of the 2023 ablation season. Similar studies conducted worldwide have shown both analogous and disparate DOC concentrations in cryoconite hole water samples. This suggests that there can be significant differences in organic carbon content between glacier ecosystems. A negative correlation was also found between bacterial abundance and DOC within cryoconite holes, implying that as more bacteria is present within each hole, DOC concentrations may decrease. Respiration rates within selected combined cryoconite holes varied with an increase in respiration occurring within incubations containing sediment. This study highlights the importance of understanding the broader impacts of cryoconite holes, cryoconite sediment and glacial melt on aquatic ecosystems and water quality downstream.

Table of Contents

Chapter 1: Introduction	1
Chapter 2: Literature review	4
2.1 Description of study area	4
2.2 History of land	5
2.3 Glacial ecosystems	6
2.4 Cryoconite holes	6
2.5 Characteristic of cryoconite holes within landscapes	9
2.6 Cryoconite hole physicality	10
2.7 Dissolved organic matter in cryoconite holes	11
2.8 Diversity and life in cryoconite holes	13
2.9 Downstream impacts	15
2.10 Glacial inclusion into current hydrological models	17
2.11 Conclusion	19
Chapter 3: Methods	20
3.1 Site description	20
3.2 Preparation before departure	21
3.3 Field sampling	22
3.3.1 Standardization of sodium thiosulfate solution.....	22
3.3.2 Winkler titrations to determine oxygen concentration.....	23
3.3.3 Cryoconite hole structure	26
3.3.4 Dissolved organic carbon.....	26
3.3.5 Hole density: 5-m radius plots	27
3.3.6 Sediment weight.....	27
3.4 Microbially community analysis	27
3.4.1 Microbial microscopy	28
3.5 Statistics	28
Chapter 4: Results	29
4.1 Physicality	29
4.2 Five-meter radius plots	29
4.3 DOC	31

4.4 Bacterial abundance	31
4.5 Respiration rate.....	32
Chapter 5: Discussion	34
5.1 Field observations	35
5.2 Physicality	36
5.3 Bacterial abundance	37
5.4 DOC Concentration	39
5.5 Respiration rate in combined cryoconite holes	40
5.6 Five-meter radius plots	42
5.7 Limitations and future research	42
5.8 General recommendations	44
Chapter 6: Conclusion	45
Bibliography	Error! Bookmark not defined.

List of Figures

3.1: Map of Root Glacier, Alaska.....	21
3.2: Winkler Titrations.....	24
3.3: Five-Meter Radius Plots.....	27
4.1: Cryoconite Holes.....	30
4.2: Dried Sediment.....	31
4.3: Correlation of Bacterial Abundance by DOC.....	33
4.4: Respiration Rate vs. Treatment of Winkler Titrations.....	34
5.1: Meltwater Channel.....	36

List of Tables

Table 4.1: Respiration Rate: Winkler Titration Treatment.....	33
---	----

Acknowledgements

I would like to express my heartfelt gratitude towards all those who have contributed to the completion of this thesis. Firstly, I extend my deepest appreciation to my reader, Erin Martin for her invaluable guidance, experience and encouragement throughout the entirety of this project. I would next like to thank Jenna Nelson for all her knowledge and support, consistently going above and beyond to help facilitate research over 2000 miles away. I would also like to thank Mark Miller with the Wrangell St. Elias National Park and Preserve for his assistance with logistics and the permit process and Erin Larson with the University of Alaska Anchorage for use of her lab and all her assistance. Many thanks to Jon Erdman and the lovely people at the Wrangell Mountain Center who helped navigate the logistics of remote field research. I am grateful for funding from the MES thesis research fund and all the staff at the Evergreen State College who helped make this project possible.

Finally, I would like to thank all my friends and furry companions who have kept me grounded and positive. I am forever grateful for your love and support.

Chapter 1: Introduction

Glaciers consist of complex and understudied water systems and icy structures. Of these structures, cryoconite holes may be the least understood, receiving little study over the last couple of centuries. Cryoconite holes appear as vertical pools of water formed when sediment on the surface of glaciers, lake ice and sea ice, change the albedo of the snow, and temperature change creates melt. The word ‘cryoconite’ originates from the Greek words, “cryo”, which means ice and “conite” meaning dust (Fountain et al., 2004). Cryoconite holes have been found to cover 0.1-10% of glaciated surfaces (Cameron et al., 2011; Fountain et al., 2004). Often appearing small and lifeless, research has shown that each hole has the potential of harboring diverse life forms including communities of eukaryotic, bacterial and archaeal microorganisms (Musiliva et al., 2017; Cameron et al., 2012). As glacial mass continues to decline, the structures and systems that influence glacial inputs into downstream hydrological regimes drastically change (Samui et al., 2020; Cameron et al., 2012).

Ice sheets and glaciers are the largest freshwater ecosystems on Earth (Poneicka et al., 2020; Edwards et al., 2013), and continue experiencing rapid changes in melt patterns that have wide impacts on global sea levels, nutrient cycles, ecosystem dynamics, and human communities (Stocker et al., 2013). Ongoing and accelerating loss of these ice masses underscores the urgency of addressing climate change and its effects on these critical freshwater resources.

Along the Gulf of Alaska (GOA), where ice is abundant in areas close to tidal water, glacier retreat is especially serious (Hood and Scott, 2008). It is estimated that over the last 70 years, glaciers of Alaska and neighboring Canada have made the largest single glaciological contribution to sea level rising (Arendt et al., 2002). For example, in this region, the last ten years of the 20th century saw glacier ice depleting at a rate of approximately 90 km³/yr. This

provided a larger contribution to rising sea level than runoff from the Greenland ice sheet (Arendt et al, 2002). Studying glaciers like Root Glacier in Alaska is essential to evaluating the broader implications of glacial changes on downstream ecosystems. Monitoring glaciers provides baseline data on total mass balance, melt rate and seasonal variations. This data is crucial for detecting and understanding long-term trends in glacial dynamics and how they respond to climate change.

Over the years researchers have found that microbial assemblages within cryoconite holes are considerably diverse. Several eukaryotic and bacterial phyla, as well as viruses have been identified (Mueller et al., 2001, Sävström et al., 2002, Anesio et al., 2007). Ambrosini et al. (2017) assert that cryoconite holes are the most biologically potent environments on glaciers. Several studies have demonstrated that glacial runoff provides a significant source of highly bioavailable nutrients to downstream ecosystems (Smith et al., 2017; Cameron et al., 2012). Glacial runoff contains various nutrients, including nitrogen, phosphorus and trace metals, all of which are essential for the growth of microorganisms and plants (Stubbins et al., 2012). These nutrients support primary productivity by promoting the growth of phytoplankton, photosynthetic organisms and other aquatic plants, which form the base of the food web (Sävström et al., 2002; Hodson et al., 2010). Runoff also includes labile dissolved organic carbon (DOC), which plays a crucial role in influencing downstream marine heterotrophic and primary productivity at both local and regional scales (Cameron et al., 2011; Musilova et al., 2017). Labile DOC is easily utilized by bacteria and other microorganisms that rely on organic carbon for energy, stimulating microbial activity and heterotrophic productivity. This microbial activity plays a critical role in nutrient cycling and energy flow within aquatic ecosystems and can lead to higher rates of photosynthesis and carbon fixation. Enhanced microbial activity

fueled by this DOC in turn sustains higher trophic levels, including zooplankton and fish downstream, contributing to the overall richness of marine ecosystems.

The effects of glacial runoff are observed locally in the glaciers' immediate vicinity and regionally, as nutrients are transported downstream and dispersed over larger areas. This can influence the productivity of coastal and open ocean ecosystems, affecting fisheries, carbon cycling, and overall ecosystem health.

Despite their ecological importance, few studies have examined the contents found in cryoconite holes. By collecting and analyzing data on the physicality, bacterial enumeration, respiration rates, and organic carbon compositions in cryoconite holes on Root Glacier, this study aims to shed light on the significant role these microhabitats play in biogeochemical cycling and as nutrient sources for downstream ecosystems. This research is critical for understanding the ecological importance of glaciers in a changing climate. As such, the following work addresses these questions: What is the range of ecosystem metabolism rates found in cryoconite holes in the Root Glacier? Are DOC concentrations correlated w/ measures of metabolism? Does cryoconite sediment impact bacterial abundance? To address these questions, bacterial abundance was measured, respiration rates and primary production rates were assessed, and the abundance of cryoconite holes were enumerated. In measuring these parameters, even though bacterial abundance measurements were low, the relatively high DOC concentrations suggest that the Root Glacier is an important source of carbon to downstream ecosystems.

Chapter 2: Literature review

This literature review will investigate the role of cryoconite holes on glacial ecosystems and impacts on downstream systems. First, the chosen study area will be described, followed by an exploration of the land's history and glacial ecosystems. Cryoconite holes will then be defined, their characteristics within a landscape, physicality and dissolved organic matter within these holes will also be discussed. Afterwards cryoconite hole microbial diversity and abundance, and the impacts of these factors on downstream ecosystems will be explained. Finally, it is argued here that cryoconite hole discharge should be included in future hydrological models.

2.1 Description of study area

Root Glacier is located in south-central Alaska in the heart of the Wrangell-St. Elias National Park. The park itself is the largest area in the United States that is protected by the National Park Service, and contains the longest valley glacier in the world, several active volcanoes, and the 2nd tallest peak in North America. The Wrangell and St Elias Ranges (WRST) are a subpolar region that are the most heavily glaciated mountain ranges in Alaska, with changes in these glaciers being responsible for roughly half of the ice mass loss in Alaska (Armstrong et al., 2017).

This 24 km land terminating glacier lies about 500 km east of Anchorage, located in the eastern region of south-central Alaska. This 2-million-year-old glacier is around 90-150 meters thick, a thickness that changes each year through seasonal variation and climate change (Anderson et al., 2003). Root glacier is a major tributary to the Kennicott glacier (Mackeyett & Pecora, n.d). Meltwater from these glaciers flows into the Kennciott river which then flows into the Chitna River, the major tributary to the Copper River. Known as one of the world's most significant wild rivers, the Copper River flows through a vast wilderness for almost the entirety

of its length. Ranking as the tenth-largest river in North America, it boasts one of the highest silt loads globally. The accumulation of silt over time has formed the expansive Copper River delta at the river's mouth, which stands as the largest continuous wetland along the entire Pacific Coast (Anderson et al., 2003).

2.2 History of land

Within the lands of the Wrangell-St. Elias National Park and Preserve there are four indigenous groups with ties to the land (Holen, 2004). Of the four, the Ahtna and Upper Tanan Athabascans reside within the interior of the park where the Root Glacier is located. There is no solid evidence as to when humans first reached the Copper River Basin, but it is thought that as long as 8,000 years ago, caribou hunters began visiting the area. As the glaciers continued to retreat, eventually humans entered what we know today as the Wrangell Mountains. Some believe the area was originally settled by the Eyak people, but the Athna replaced them long ago.

Given that Root Glacier lies within Ahtna land, it's important to acknowledge their importance to this area. The name "Ahtna" is derived from the indigenous name for the Copper River, "Atna' tuu," which translates to "Copper River" (John et al., 1986). The Ahtna people's traditional territory spans the Copper River Basin, including areas around Root Glacier and the surrounding mountainous regions. The Ahtna people have worked to retain and protect their traditional lands through various legal and political efforts, including land claims and participation in the Alaska Native Claims Settlement Act (ANCSA) (Holen, 2004). Root Glacier's location within the historic lands of the Ahtna people highlights the importance of integrating indigenous knowledge and perspectives into the study and management of these environments. Understanding the cultural and historical context enriches scientific research and promotes a more inclusive and respectful approach to environmental stewardship.

2.3 Glacial ecosystems

Glaciers are complex structures about which much remains unknown. Meltwater running from these ice formations plays an important role in high alpine water catchments (Milner et al., 2017). Glacial surfaces and ice sheets contain a range of diverse prokaryotic and eukaryotic organisms (Anesio et al., 2009; Telling et al., 2010). Glacial runoff is an important source of labile organic matter, with some glaciated watersheds being responsible for 66% of the dissolved organic matter available in the watershed (Hood et al., 2009). Castello and Rogers (2005), suggest that between 10^{17} and 10^{21} microorganisms were released from glaciers globally every year. They likely are sourced from the remains of ancient peatlands and forests exported by glaciers, anthropogenic aerosols deposited on glaciers, and microbial communities found in cryoconite holes (Stubbins et al., 2012; Bagshaw et al., 2013).

2.4 Cryoconite holes

Cryoconite holes exist all over our world's glaciers and ice sheets, covering 0.1-10% of glacial surface area and often persisting for decades (Anesio et al., 2009, Tranter et al., 2004). Formed from the granular sediment known as cryoconite, these holes establish when dark sediment absorbs solar radiation and begins to melt the ice. Cryoconite sediments darken the ice sheets and snowpack and change the albedo of the snow surface (Gerdel and Drouet, 1960; Takeuchi et al., 2001; Takeuchi, 2002). The low albedo organic matter allows for absorption of solar radiation which heats the ice and forms mostly cylindrical depressions known as cryoconite holes (Cook et al., 2016). They are filled with water on the surface of glaciers and cover 4-6% of all glaciated surfaces around the world (Fountain et al., 2004). The formation and expansion of cryoconite holes enhance the melt rates of glaciers, the absorbed heat accelerates the melting process in these specific areas. This localized melting can contribute to the overall reduction in

glacial mass, affecting the mass balance of glaciers (Fountain et al., 2004; Kohshima et al., 1992). Many studies indicate that meltwater inputs from these formations are significant enough to warrant inclusion into models of downstream ecosystem processes due to the diversity of life forms, nutrients and organic carbon they contain (Bagshaw et al., 2013).

Cryoconite holes hold sediment, harbor diverse microbial communities, and trap upland plant matter, and in some regions, holes may contain organisms including tardigrades, nematodes, rotifers, copepods, protozoa and insect larvae (S awstr om et al., 2002; Anesio et al., 2009; Edwards et al., 2011; Cameron et al., 2012). Cryoconite holes are often referred to as the “icy hot spots” because of the densely diverse life held within them, with microbial processing enabling nutrient sequestration from the atmosphere (Tranter et al., 2004; Anesio et al., 2009; Edwards et al., 2013). These sediments can be composed of both organic and inorganic materials (Cook et al., 2016). Organic constituents can include microbes that are both living and dead, algal material, exudates and products of decomposition (Hodson et al., 2008). Organic matter, biota and geological and hydrological processes influence biogeochemistry, morphology and the formation of cryoconite holes (Edwards et al., 2011; Cook et al., 2016).

These icy apertures can greatly vary in size, ranging from 5-145 cm and 4-56 cm in depth (Cook et al., 2016). Some recorded holes are much larger with 5 m depths and 30 m diameters (Fountain et al., 2004). Once these sediments are deposited on the snow surface and within the cryoconite hole, they can exist there for many years (Tranter et al., 2004). During the winter, cryoconite holes undergo a phase of complete freezing due to the low temperatures. The microorganisms and organic matter within these holes become trapped in ice, effectively pausing their metabolic activities until the temperatures rise again (Anesio et al., 2009). In the summer, increased solar radiation penetrates the ice and heats the dark sediment at the bottom of the

cryoconite holes. This sediment absorbs more heat due to its darker color and higher thermal conductivity compared to the surrounding ice (Cook et al., 2016). As the sediment warms, it causes localized melting around and above it, leading to the reformation of the cryoconite hole (Tranter et al., 2004).

There are three types of cryoconite holes; open, submerged and closed. Within open holes, rates of photosynthesis can be significant, and exposure to the atmosphere can stimulate microbial activity (Hodson et al, 2008). This stimulation could lead to an increase in degradation within these systems (Sanyal et al., 2018). Closed holes are sealed off from the atmosphere by overhead ice, acting as an isolating lid. This partitioning greatly restricts gas exchange and can create extreme conditions as photoautotrophic processes continue (Poniecka et al., 2020, Hodson et al., 2008). Closed cryoconite holes that are entombed by a layer of ice eventually experience nutrient limitations within their melted niche. These limitations in combination with high levels of respiration due to the insulation of ice, become significant when recycling within the organic nutrient pool is mandated (Hodson et al., 2008).

Sanyal et al. (2018) showed higher microbial diversity was recorded in open and hydrologically connected cryoconite holes in comparison to those that were closed. The greater diversity observed in open and hydrologically connected cryoconite holes compared to closed holes may be attributed to the distinct conditions between these systems, which impose different environmental pressures on microbial communities within the holes. Open holes facilitate increased exchange of gases and the influx of microbes, nutrients, water and organic matter into the cryoconite hole (Hodson et al., 2008). The contrasting geochemical conditions within open and closed holes could impact microbial activity and community composition, resulting in distinct microbial communities in each type of system (Hodson et al., 2008; Pautler et al., 2013).

Sanyal et al. (2018) observed significant amounts of bacterial biomarkers associated with open cryoconite holes, indicating that exposure to the atmosphere may stimulate microbial activity, potentially leading to further degradation of organic matter in these systems.

2.5 Characteristic of cryoconite holes within landscapes

The biogeochemical characteristics of cryoconite holes are affected by the position of a glacier within the landscape. More biologically productive areas have higher concentrations of carbon and nutrients such as nitrogen and phosphorus (Bagshaw et al., 2013). Influenced by abiotic factors, location specific and spatially significant conditions include elevation, sediment load, temperature, cryoconite volume and debris cover. The position on the glacier can also affect the diversity and composition of cryoconite holes. Variables such as these create environmental conditions in glaciated spaces which greatly dictate variability in microbial community structure (Bagshaw et al., 2013; Hodson et al., 2008).

Environmental factors influencing the growth of photosynthetic microorganisms in glacier ecosystems include altitude, slope angle, nutrient concentration, mineral particle amounts or composition, distance from the glacier terminus, surrounding vegetation and surface hydrology (Cook et al., 2016; Uetake et al., 2016). Geographical position, encompassing both altitude and latitude, significantly affects the duration of snow cover. At lower altitudes and latitudes, winter snow melts more rapidly, and the summer snow line's altitude fluctuates annually. These environmental variations lead to differences in the abundance and species composition of algae (Takeuchi 2001), bacteria (Segawa et al. 2017), and fungi (Uetake et al. 2012).

Stibal et al. (2012) and Edwards et al. (2011) suggest that surface hydrology can explain variations in bacterial diversity between different glaciers, for example, varying slopes can impact the velocity of meltwater which could potentially wash away both nutrients and microorganisms needed for growth. Nutrient availability, particularly nitrogen and phosphorus, can limit growth in glacier ecosystems (Stibal et al. 2015; Telling et al. 2010). Additionally, the input of carbon, nitrogen, and phosphorus fosters bacterial growth in cryoconite granule layers (Sävström et al. 2002). The distance from the glacier terminus also affects microbial growth due to variations in the supply of allochthonous organic carbon from adjacent deglaciated areas (Stibal et al., 2012). In addition, cryoconite holes located near the ice margin typically contain less biogenic material and greater mineral diversity compared to those found in the glacial interior (Pautler et al., 2013).

Besides geography playing a role on a landscape level, it also impacts dependent variables such as wind speed and ability to move sediments over distances and terrain (Weisleitner et al., 2020). Impacts of landscape are important to consider while studying cryoconite holes. Further research and modeling are needed to interpret the intricate relationship between geography, morphology and microbial makeup within these holes and how these variations impact cryoconite hole physicality.

2.6 Cryoconite hole physicality

As the cryoconite holes deepen, their rate of growth decelerates due to the decrease in radiation energy which is caused by an increase in optical depth along the ray path to the sediment. A steady-state depth or equilibrium is achieved when the rate of downward melting matches the glacial surface ablation rate. (Wharton et al., 1985; Fountain et al., 2004; Banerjee et al., 2023). This occurs because the dark cryoconite sediment absorbs more solar radiation,

causing the cryoconite to be buried deeper into the ice and away from the surface, thereby reducing the solar energy absorbed. This negative feedback mechanism stabilizes the hole geometry, with both the bright ice surface and the darker cryoconite bottom melting at the same rate (Wharton et al., 1985).

The evolution of cryoconite holes is further complicated by various factors such as the cross-sectional geometry, the presence of meltwater, heat advection by meltwater, refrozen ice lids covering the holes (Fountain et al., 2008), energy partitioning between melting and sublimation at the glacier surface and heat production due to microbial activities (Fountain et al., 2004). While cylindrically shaped holes are found most often, complex shapes can also form. These differences in shape also reflect the relationship between ice type, hydrology, regional and local topography, aspect and sediment dynamics (Cook et al., 2016). Due to these complexities, a general understanding of the steady-state hole geometry is still lacking. It is unclear whether there is a consistent depth-radius or volume-diameter relationship for steady-state holes (Fountain et al., 2004; Wharton et al., 1985; Banerjee et al., 2023).

2.7 Dissolved organic matter in cryoconite holes

Within cryoconite holes, dissolved organic matter (DOM), defined as organic matter <0.7 μm in size, comes from a variety of sources (Feng et al., 2021). These include organic matter leached from sediments deposited at the hole site, autochthonous production within the hole, atmospheric deposition and allochthonous debris (Sanyal et al., 2018; Smith et al., 2018). DOM in cryoconite holes exhibits distinct characteristics depending on the source of the glaciers (Feng et al., 2021). In Antarctic glaciers, the DOM is predominantly autochthonous, originating primarily from microbial sources within the cryoconite holes themselves (Smith et al., 2018). This DOM is highly labile, due to its low carbon to nitrogen (C/N) ratios and low aromaticity

(Sanyal et al., 2018). The lack of higher plant inputs in these Antarctic environments leads to a simpler chemical composition of the organic matter, dominated by microbial contributions (Sanyal et al., 2018; Smith et al., 2018).

In contrast, DOM in cryoconite holes on mountain glaciers is more chemically heterogeneous (Smith et al., 2018; Feng et al., 2021; Guo et al., 2022). This heterogeneity is due to significant inputs of allochthonous material, such as mosses and vascular plants, from surrounding environments (Pautler et al., 2013; Smith et al., 2018; Sanyal et al., 2018). These inputs contribute to a more complex mixture of organic compounds in the DOM, including more aromatic structures and higher C/N ratios compared to Antarctic cryoconite holes. The presence of plant-derived material introduces a wider variety of organic molecules, which can include more recalcitrant compounds, thereby influencing the overall chemical nature of the DOM (Antony et al., 2017). These variations have significant implications for the microbial ecology and biogeochemical processes within the cryoconite holes and for the downstream environments influenced by the meltwater they produce.

Glacial runoff and meltwater contribute microorganisms, nutrients and DOM to downstream ecosystems (Hood et al., 2009; Fellman et al., 2010). Microorganisms that are released from meltwaters can act as primary producers (Fellman et al., 2010; Musilova et al., 2017; Li et al., 2018), releasing mostly labile and low molecular weight matter which are important sources of DOM (Allan & Castillo, 2007). Recent data indicates that a significant portion of the organic matter derived from cryoconite ecosystems and snow is bio-labile and available for heterotrophic activity (Antony et al., 2017; Musilova et al., 2017; Feng et al., 2021). DOM is an important energy source, contributing to microbial populations and being foundational in the river food web, impacting both lower and higher consumers.

Glacial masses have historically followed predictive patterns of accumulation and recession, melting during warm seasons and usually regaining ice and snowpack during cold seasons (Seibert et al., 2018). Historically normative patterns have become altered by climate change, and repetitive annual cycles of glacial mass balances are increasingly unpredictable. Recognizing that glacial inputs into riverine and marine habitats are significant, changing melt patterns could indicate a significant change in the stability of supply of labile DOM and nutrients into coastal food webs. Hood et al. (2015) created a database of glacier dissolved organic carbon (DOC) concentrations on glaciers located on five continents around the world. This mass data collection included cryoconite holes, surface ice, englacial ice, basal ice and glacial meltwater. Across various regions and glacier types, this research revealed that DOC concentrations varied from 0.01-43.2 mgC L⁻¹, with an average of 0.97 mgC L⁻¹. Hood et al. (2015) found that the highest DOC concentrations were in cryoconite holes and basal ice. The concentrations found for cryoconite holes ranged from 0.71-1.42 mgC L⁻¹. The authors of this study suggest that these findings reflect the accumulation of organic matter into cryoconite holes. Another study conducted by Musilova et al. (2017) found DOC concentrations within cryoconite holes to be 0.15 ± 0.01 mgC l⁻¹ throughout the summer season on the Greenland ice sheet.

2.8 Diversity and life in cryoconite holes

Cryoconite holes exist all over our world's glaciers, providing essential habitats for a range of life in an otherwise hostile environment (Cook et al., 2016, Cook et al., 2018). They are inhabited by many organisms including bacteria, viruses, fungi, ciliates and algae (Bagshaw et al., 2013; Edwards et al., 2013). Cyanobacteria have been found to be dominant within cryoconite holes, being responsible for much of the carbon fixation that occurs. Their abundance, structure and role within the holes stimulates granule growth by entrapping additional organic

matter and minerals (Anesio et al., 2010; Cameron et al., 2012; Cook et al., 2016). Cryoconite holes also provide habitat to higher level heterotrophs including tardigrades, copepods, rotifers, midge larvae and ice worms (Anesio et al., 2009; Cook et al., 2016). These larger life forms survive by feeding off smaller organisms. On the melting ice, these ecological niches represent possibly the most active microbial habitat within glacial ecosystems (Hodson et al., 2008). Cryoconite holes host a diverse array of life and elevated rates of primary and secondary productivity as well as respiration. Some studies suggest that these rates of production are comparable to rates found in temperate soils (Anesio et al., 2009; Hodson et al., 2008; Musilova et al., 2017; Sanyal et al., 2018). In a study done by Anesio et al. (2009), it was found that the primary production and respiration in the cryoconite hole water was low yet comparable in scale to those observed in oligotrophic aquatic ecosystems. The microbial activity in these holes' sediment was comparable to that of soils in warmer climates. The study found that an average community respiration and primary production between the water column and the soil to be 0.95 and 7.35 $\mu\text{gCg}^{-1}\text{h}^{-1}$ respectively. This large amount of activity could potentially represent a globally substantial reservoir of carbon (Hodson et al., 2008).

Sanyal et al (2018) compared microbial communities in cryoconite holes on two geographically different glaciers in Antarctica and the Himalayas. Between both glaciers, 14 different genera of microorganisms were isolated and identified from cryoconite hole water samples. Mieczan et al. (2013) found and identified 16 phyla of ciliate.

When regarding glacially bound food web structures, habitats persisting in cryoconite holes make up some of the most important in the supraglacial ecosystem (Hodson et al., 2008). Bagshaw et al. (2013) conducted a study in Antarctica that found that despite melt water in cryoconite holes being mostly isolated from the surrounding hydrological regime, within them

nutrient recycling occurs from both autotrophic and heterotrophic organisms. Viral population within cryoconite holes also plays a role in the amount of recycling and exportation of carbon and nutrients that occurs through bacterial mortality (Cook et al., 2016). Viruses are important in cryoconite ecosystems (Hodson et al., 2008). Considered predators, these organisms control bacterial populations through viral lysis, infecting bacteria and causing apoptosis (Cook et al., 2016). S awstr om et al. (2007) found that virus-infected cells within the cryoconite community occurred at a frequency of 13%. This and other more recent studies have highlighted the crucial role that viruses play in how dissolved organic carbon is recycled and made labile in cryoconite holes. This role is important to understand because it affects the flux of resources to higher trophic levels along with our general comprehension of the cryoconite food web. While photoautotrophs are foundational in this food web, heterotrophic communities metabolize the organic matter from primary producers (Cook et al., 2016).

Anesio et al. (2010) found bacterial abundance within open cryoconite holes to be 7.7×10^6 cells ml^{-1} . Sanyal et al. (2018) found microbial cell numbers in the Antarctic samples ranged from 0.07×10^4 to 9.57×10^4 cells ml^{-1} . They also found microbial cell numbers in the Himalayan samples ranging from 4.83×10^4 to 2.75×10^5 cells ml^{-1} . This variation in bacterial findings in holes on both the same and different glaciers highlights the importance of understanding the complex community within the holes in order to allow for evaluation of total DOM to downstream ecosystems.

2.9 Downstream impacts

While more studies need to be conducted to understand the role cryoconite hole water plays on downstream ecosystems, current data indicates that there is a significant downstream impact from cryoconite hole activity. Current molecular and microbiological glacial studies only

show a fragment in time without comprehending environmental fluctuations and temporal shifts at a community level within the cryoconite holes (Cook et al., 2016). Glaciers comprise one of the largest freshwater reservoirs in the world. Annual influxes of water discharge from glaciers have been historically recorded on largely predictable levels. Climate change has introduced new variables into these complex and fragile systems, creating a widening gap in the literature which strives to understand the seasonal flux of glaciers (Bliss et al., 2014). These high fluxes in glacial meltwater play an important role in heterotrophic and primary production downstream (Musilova et al., 2017). Because glaciers are an integral source of dissolved organic matter (DOM) and dissolved organic carbon (DOC) on downstream ecosystems, accurate modeling of glacier melt is fundamental to understanding their current and future impacts on downstream riparian, riverine and oceanic habitat.

Cryoconite holes are perhaps one of the most understudied yet important structures of a glacier. Only 13-15% of glacier meltwater originates from these holes, yet studies have found up to 60% of biologically available DOM is dispersed downstream as these icy niches melt into glacial water systems (Fountain et al., 2004). Melting glaciers export runoff, nutrients and organisms into downstream ecosystems in unprecedented ways. Current studies suggest that riverine and coastal marine ecosystems rely on the nutrients provided by glacier melt. Often, watersheds with glacier contributors experience higher levels of key nutrients and bioavailable carbon and organic matter than their non-glaciated counterparts (Slemmons et al., 2013). In some cases, the current melt rate spurred by climate change exposes isolated cryoconite hole pockets that would otherwise persist for years. Glacial headwaters provide an essential source of nutrients and DOC for downstream ecosystems (Anesio et al., 2009; Bliss et al., 2014). Increases in glacial runoff are climatically driven and are expected to greatly impact the export of nutrients

on the downstream environment (Musilova et al., 2017). -Models working to understand fluxes in glacial melt and cryoconite DOC production are integral to understanding glacial ecosystems, and downstream hydrological systems.

2.10 Glacial inclusion into current hydrological models

Glaciers and ice sheets are the second largest water reservoir in the global hydrological cycle (Hood et al., 2008). Despite this, glaciers are not well represented in hydrological models (Seibert et al., 2018). Enhanced global warming and climate driven influxes in glacial runoff are thought to have a larger impact on the hydrological system than any other component (Hood et al., 2015). Due to the complexity of glaciers and computational limitations, glacial models do not accurately resolve changes in glaciated areas and simulated glacial mass balances associated with climate change (Seibert et al., 2018). Additionally, new studies suggest that the established release of DOC from mountain glaciers is 1.4 times higher than what is historically estimated (Sanyal et al., 2018).

Glacial runoff contains water from cryoconite holes. In studies conducted on the glaciers of Taylor Valley, Antarctica, up to 15% of observed runoff water is attributed to these holes (Fountain et al., 2004). Subsurface water systems that consist of cryoconite holes and interconnected passages provide much of the meltwater contributing to total glacial runoff. These passageways create a hydraulic resistance that in combination with melt production, controls the influx and discharge of water (Fountain et al., 2004). Traveling through ephemeral streams and seasonal lakes, these various hydrological connections allow for the release of dissolved nutrients and particulate matter stored in the water column of the cryoconite holes to be released

into downstream ecosystems (Bagshaw et al., 2013). This geographic hydraulic glacial drainage system determines the distribution of nutrients and water throughout the entire glacial ecosystem (Hodson et al., 2008).

Cryoconite holes contribute vastly to the annual hydrological cycle, in some cases 13-15% of all glacial runoff is from melt water within these holes (Fountain et al., 2008). This significant fraction of runoff connects to other downstream ecosystems (Bagshaw et al., 2013).

Within the holes are diverse habitats with a range of microbial life as well as other higher-level organisms. These organisms are responsible for significant rates of primary and secondary productivity (Hodson et al., 2008). The total amount of carbon, nitrogen and phosphorus stored within these holes is comparable to that of ephemeral streams, meaning cryoconite holes should be considered as a separate source when modeling systems (Bagshaw et al., 2013). The mobility of organisms within these momentarily isolated waters are washed into supraglacial streams and play a role in downstream biological processes (Fountain et al., 2004). Shifting hydrologic regimes show that in downstream ecosystems that are reliant on glacial input, there are observed changes in secondary production due to influx of protein-like fluorescence.

When considering models for hydrological regimes of high-mountain catchments over larger periods of time, shifts in glacial mass need to be considered, especially when glacial retreat continues to accelerate (Seibert et al., 2018). Arguably any system that maximizes the creation of organic matter and stores nutrients is important to landscape models. This is extremely important in scenarios where these nutrients and organic matter can be exported through the hydrological connection that interlinks ecosystems (Bagshaw et al., 2013). In

addition, the model should include several geological landscape features that impact not only glacial melting but microbial composition within cryoconite holes. When considering future glacial models, representing diversity within varying holes is essential for accuracy of the model, as explained earlier, open holes have a greater capacity for gas exchange, and flush of microbes and nutrients while closed systems have a restriction on these interactions and exchanges (Sanyal et al., 2018).

2.11 Conclusion

Glaciers and ice sheets, which make up the majority of the continental cryosphere, contain most of the Earth's freshwater. Cryoconite holes are crucial hydrological and biological systems in glacial environments, supporting diverse microbial communities and biogeochemical processes. In recent years, the study of biological processes in these holes has gained interest due to growing evidence that they can significantly influence the energy flows of glaciers and, consequently, the climate. Additionally, these environments are particularly sensitive to ongoing global warming.

Studying dissolved organic carbon, respiration rates, bacterial abundance and physical characteristics on the Root Glacier in Alaska represents a crucial advancement in comprehending the diversity of glaciers worldwide. Analyzing the interplay between these factors, including their influence on bacterial abundance and DOC levels, as well as the extent of glacier coverage by these features, provides essential insights into the broader ecosystem dynamics. These investigations are necessary to grasp the potential effects of these distinct ecosystems.

Chapter 3: Methods

3.1 Site description

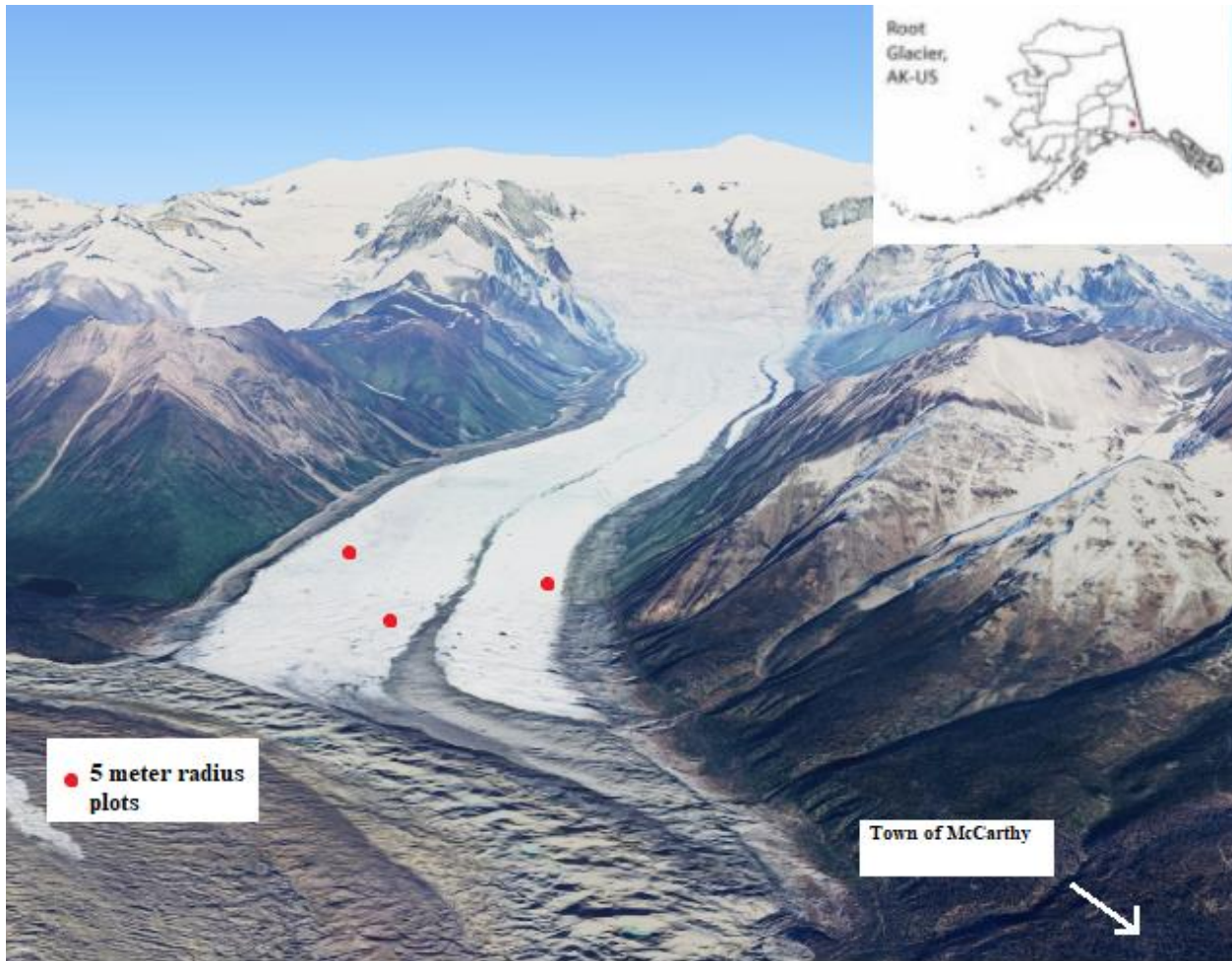
The study sites were located on the Root glacier, which lies in the heart of Wrangell St. Elias National Park (fig. 3.1). At a little over 300 miles southeast of Anchorage, this glacier is a popular hiking site due to its proximity to the unique tourist town of McCarthy Alaska. The site was reached by a fourteen-mile roundtrip hike from the town of McCarthy to the glacier. Named in 1899 for the U.S. Secretary of War Elihu Root, the Root glacier is an alpine glacier and a major tributary to the Kennicott glacier. It is approximately 24.14 km long, however as with all the glaciers in the park, its mass is decreasing at increasing rates each year. The cryoconite hole water for each of the holes utilized was crystal clear, with no coloration. The color of cryoconite hole water has varied in other studies, including some holes having a tea like coloration (Bagshaw et al., 2007).

Sampling began on Friday, May 26th, 2023, and was conducted for the next three consecutive days. The temperature outside was 6.11°C and the temperature of the water was 1.1°C. Wind speed at its max was recorded at 27 miles per hour on the glacier. There was a light drizzle that persisted throughout the day. This time of year was chosen for two reasons. One, May in Alaska is the beginning of the melt season when water flow would be at its potential highest (Arendt et al., 2002). The second is permit availability on the glacier, located in a National Park and Preserve, permits are only awarded during certain times of the year.

All holes sampled were found within 150 meters of the glacial terminus and at least 25 meters from the medial moraine. The coordinates for the start of the study are 61°30'41.8"N 142°54'33.5"W.

Figure 3.1

Map of Root Glacier, Alaska



Note. Root Glacier, Alaska Upper right shows where Root Glacier is located in respect to the state of Alaska. Location of five-meter radius plots are represented with red dots. (Google Earth 10.46.0.2 (2024) Root Glacier, Alaska, 61°48'7"N -142 °89'08"W, elevation 960m [online].

3.2 Preparation before departure

Sampling equipment used for DOC analysis was prepared at Evergreen State College before departure. Thirty GF/F filters were carbon cleaned by wrapping them in aluminum foil and combusting them in a muffle furnace at 450°C for 4 hours. Twenty 40-ml glass bottles were also wrapped in aluminum foil in groups of 7-8 and combusted at 500°C for 5 hours. The Teflon

lids were acid washed and rinsed with deionized (DI) water, dried and placed immediately into a Ziplock bag for travel. The filter tower was cleaned using carbon-clean soap, rinsed with DI water, and stored for travel.

Solution preparation for field sampling took place at the University of Anchorage Alaska, in the aquatic's lab in Beatrice McDonald Hall.

For the Winkler titrations, the following reagents were made:

2.5 M Manganese Sulfate (MgSO_4), Alkaline Iodide Solution, 0.025 M Sodium Thiosulfate Solution ($\text{Na}_2\text{S}_2\text{O}_3$), Potassium Iodate Standard Solution (KIO_3), 0.175M KI Solution

All solutions were transported in a closed tote and remained at ambient air temperature while being transported from the University of Anchorage Alaska to McCarthy Research Station.

3.3 Field sampling

Water from cryoconite holes were sampled for pH, temperature, respiration rate, microbial abundance, and Dissolved Organic Carbon (DOC) concentration.

3.3.1 Standardization of sodium thiosulfate solution

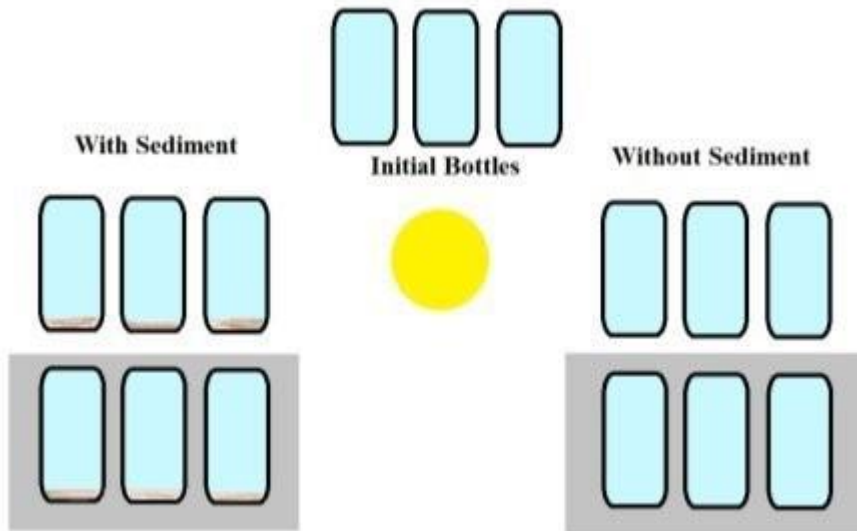
The sodium thiosulfate solution was standardized at the McCarthy Research Station, 25 ml of the started iodate solution was transferred to a 100-ml beaker and 5-ml of the 0.175M potassium iodide was added. Finally, 1-ml of 6M HCl (hydrochloric acid) was added. Next the solution was titrated with sodium thiosulfate using a Gilmont Burette. The solution was titrated until the ambertcolor had almost disappeared. Starch was then added as an indicator and the solution was titrated to completion. The volume of titrant added was recorded.

3.3.2 Winkler titrations to determine oxygen concentration

Water-column respiration rates were measured in 125-ml BOD (biological oxygen demand) bottles. Due to the small volume of water available in each hole, on average less than 50 ml per hole, water from 13 holes was collected into a plastic carboy using a 60 ml syringe, 20-60 ml was removed from each hole. Collection was done carefully as to reduce agitation as much as possible. Sediment from the bottom of each hole (not organic matter—that's different) was also collected from each of the thirteen holes and mixed thoroughly together in a separate 200-ml beaker. All the mixed sediment was carefully distributed into six BOD bottles, with a final sediment depth of 2-4 mm in each bottle. Sediment was added as a treatment to see if it impacted the rates of respiration within the holes. All 15 bottles were then filled with *in situ* supraglacial water from the carboy, once again using caution to prevent agitation. While caution was taken, an unavoidable small amount of agitation occurred. Bottles were filled, leaving no headroom, with water within a 5-minute span at 12:06 on Friday May 26th, 2023. Three of the 15 bottles were prepared to assess initial oxygen concentration (they contained only glacial water, no sediment) (fig. 3.2). Temperature was held constant (or experienced daily fluctuations) by doing the incubations *in situ*.

Figure 3.2

Winkler Titrations



Note. Fifteen BOD bottles used in Winkler titrations. Bottles outlined in grey represent dark bottles covered in aluminum foil, bottles next to the yellow sun represent the bottles being exposed to light. Three initially incubated bottles shown at the top.

Oxygen concentrations were measured in each individual bottle using Winkler titrations (Strickland & Parsons, 1972; American Public Health Association, 1975). This manual titration method was used to determine the dissolved oxygen content in water samples. Immediately after BOD bottles were filled completely, leaving no head space, 2 ml of manganese sulfate was added into the collection bottle. This was done by inserting a calibrated pipette just below the surface of the collected water so that no air was introduced. Care was also taken to make sure no bubbles were introduced during the pipetting process.

Next, 2 ml of alkali-iodide-azide reagent was added to the BOD bottles using the same technique outlined above. Finally, 2 ml of concentrated sulfuric acid was pipetted into the bottle just above the surface of the water sample. The bottle was then stoppered carefully making sure

no air was introduced and inverted several times until all precipitate was dissolved. Any bottles that had bubbles present after the inversion were discarded and new samples were taken. If a cloud of brownish-orange precipitate indicating the presence of oxygen was seen, the floc was allowed to settle to the bottom and the sample was mixed again.

All fifteen bottles were closed with a glass stopper, and three of the bottles containing sediments were designated as dark bottles (i.e., no photosynthesis occurring, only respiration) and were covered with aluminum foil. Similarly, three of the bottles with no sediment were designated as dark bottles and covered with aluminum foil. The light bottles, or bottles without aluminum foil, were used to aid in determining photosynthesis rates because photosynthesis and respiration were occurring in these bottles. Bottles were then placed into a Styrofoam cooler with permit ID displayed and placed into the glacial headwater and incubated for 24 +/- 2 hours. During this 24-hour period, the sun did not set until 2247 and rose the next day at 411.

On Saturday May 27th, 2024, after the incubation period of 24 +/- 2 hours was complete each sample was then titrated in a glass flask using 40.2 ml of the sample water and sodium thiosulfate. The bottles in the cooler were collected at 10:15 and brought to a flatter surface near the glacial terminus. The ambient temperature was 3.33°C and there was light occasional wind. Fifteen bottles were incubated, the initial DO concentrations were recorded in three BOD bottles containing only glacial water. Of the 6 bottles containing sediment, 3 were exposed to light for 24 hr, and 3 were exposed to darkness for 24 hrs. For the 6 bottles that did not contain sediment, 3 were exposed to light for 24 hr and 3 were exposed to darkness. After 24 +/- 2 hours, the DO concentrations were recorded. Respiration rates were calculated by using the amount of sodium thiosulfate needed to reach the end point, when the solution turned from purple to clear. The oxygen originally present in the water is stoichiometrically related to sodium thiosulfate. After

determining how many mmol of the thiosulfate was used, the conversion into mmol of O₂ was made. To achieve a concentration, the number of mmol of O₂ was then divided by the volume of the BOD bottle that was used, in this case, 125 ml. Respiration rate was determined by subtracting the O₂ concentration at the end of the 24 period by the O₂ concentration that was initially recorded and dividing that by the hours of incubation.

3.3.3 Cryoconite hole structure

Within the chosen plot, 15 cryoconite holes were located and recorded. The holes were chosen at random, at least 100 m from the glacial terminus and not within 25 m of the medial moraine. Width, both the major and minor axis, and depth of all holes in the plot were measured and GPS coordinates were noted for future georeferencing. Temperature and pH were also measured using a data logger.

3.3.4 Dissolved organic carbon

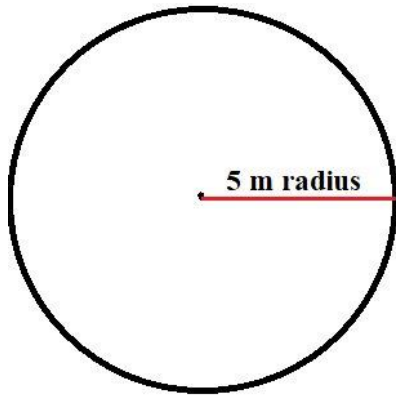
For dissolved organic carbon analysis, water samples were filtered through precombusted GF/F filters, and the filtrate was collected. These samples were taken from the same 15 cryoconite holes that were chosen for physicality data. The filtrate was collected into a 40 ml glass bottle (the bottle was pre-cleaned by combustion, and the lid was acid-washed) Care was taken to not fill the glass bottle more than $\frac{3}{4}$ of the way full. Each bottle was labeled with sample ID number and date and placed in a cooler for transport. They were stored in a freezer at the McCarthy Research Center and kept frozen during travel back to Washington State where they were shipped overnight to the University of New Hampshire to be evaluated for DOC concentration at the Water Quality Analysis Laboratory. The samples were analyzed using a Shimadzu TOC-V CSH with TNM-1 and Asl V autosampler.

3.3.5 Hole density: 5-m radius plots

Three 5-meter radius plots were created to count hole density (fig. 3.3). A stake was placed into a random spot on the glacier and a 5-meter-long string was used to create a circular plot (Takeuchi et al., 2001). Within this area, holes were counted and recorded.

Figure 3.3

5-meter Radius Plots



Note. Example of a five-meter radius plot created to count hole density, the red line represents the string used to measure.

3.3.6 Sediment weight

In the research laboratory at the Evergreen State College, sediment from each hole was dried and then weighed. Using pre-weighed petri dishes, the sediment was placed into a Quincy Lab Oven, model 30E and baked for 6 hours at 45 °C.

3.4 Microbially community analysis

Water collected from each of the 15 holes and were evaluated for microbial abundance using microscopy at the Advanced Microscopy Lab at the Evergreen State College. Using sterile 50- ml syringes, 20 ml was removed from each hole and placed into a 50-ml falcon tube. Immediately after, 20-ml of 4% paraformaldehyde was added to the same tube. The cover was tightly screwed on, the bottle was labeled and placed in a cooler for transport. 15 samples were

then kept in a freezer at the McCarthy Research station until departure. During travels back to the Evergreen State College, samples remained frozen until they were kept for storage in a freezer on campus.

3.4.1 Microbial microscopy

Frozen samples in the Falcon tubes (containing the cryoconite hole water and 4% paraformaldehyde) were removed from the freezer and placed into refrigerator at 4°C to melt in the dark 8 hours prior to filtration. Glassware, water and forceps were sterilized prior to filtration using an autoclave at 121 °C for a minimum of 30 minutes. Each sample was filtered through a grey Sterlitech 0.2 µm pore-size polycarbonate filter. These filters were then fixed onto permanent slides using SlowFade™ Gold Antifade Mountant with DAPI Product # S36942. After, the samples were stored in the dark for 24 hours before being evaluated for microbial abundance with epifluorescent microscopy. Images were recorded with a Hamamatsu ORCA-ER digital camera attached to an Olympus IX81 Microscope using an Olympus UPLANFL N 100X UIS2 oil lens. For each sample, 20 randomly selected areas were photographed using a DAPI fluorescent filter with an excitation wavelength of 403 nm and the filter set Emission and Beam splitter Quad 360/40 nm, 403/12 nm, 501/16 nm, 568/25 nm. Cells were counted with the count and measure plugin within the microscopy software cellSens Dimension.

3.5 Statistics

Statistics were conducted using JMP Pro 16 and Excel. For each of the measurements, mean and standard deviation was calculated using Excel. Each variable was assessed for normality using Shapiro-Wilk Test for Normality. All data was normally distributed so parametric statistics were utilized. The Pearson Correlation Coefficient was utilized to assess relationships between variables.

Chapter 4: Results

4.1 Physicality

Hole physicality varied across the 15 selected cryoconite holes and across the glacier. The randomly chosen holes ranged from 8.8 cm deep to 25.6 cm deep, all were open and filled almost completely with water. Cryoconite hole diameter varied greatly, ranging from 3 to 15 cm. The volume within each of the holes ranged from 87 cm³ to 3,553 cm³ (M= 927.51 cm³, SD= 852.06), that is a 40-fold difference in volume between the smallest and largest cryoconite hole measured. Volume was estimated using the radius from the average minor and major axis of each hole (to account for shape variety and irregularity) and then multiplied by depth. The depth of sediment within each hole ranged from 2– 4 mm thick. In comparison to the volume, this amount is quite small. There was no correlation between sediment weight and depth of the cryoconite holes ($r = -0.063$, $p = 0.8235$). The holes contained a mixture of fine-grained sediment with some coarse particles. The weight of sediment in each hole after being dried completely was 0.17 g to 3.23 g (M= 1.71, SD= 0.94). To get an idea of just how little sediment was in each hole, figure two shows the sediment once removed and dried in the laboratory. The pH in each hole varied with a range of 6.68-8.6 (M= 7.89, SD= 0.58). There was no algae or plant matter found in any of the study holes. Each hole had a unique shape, some were cylindrical and evenly round while others were more oblong and asymmetrical. Number 13 in figure 1. is a good example of how cryoconite holes are not strictly round in diameter. There was a large variation in pH measured in each hole with a range of 6.7-8.6 (M= 7.9, SD= 0.58).

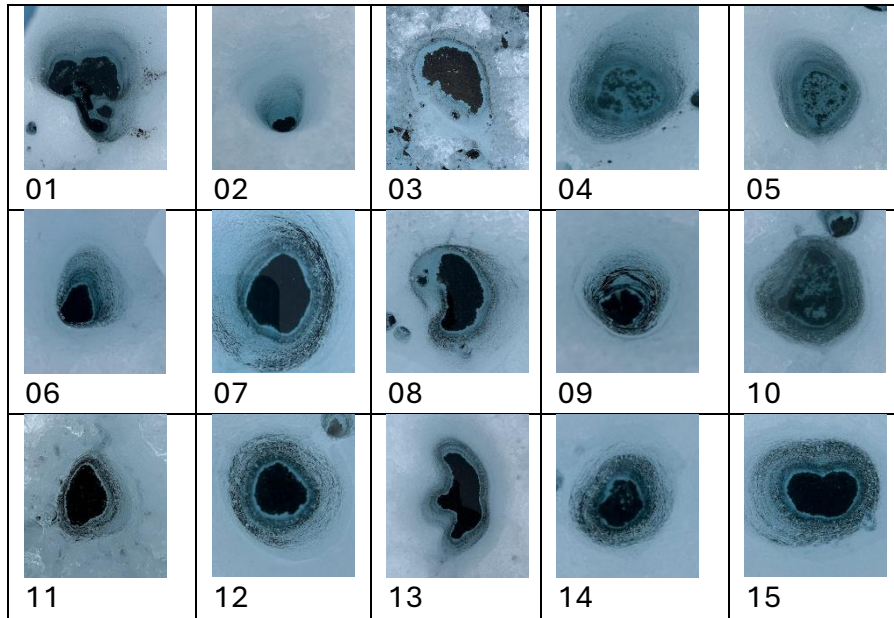
4.2 Five-meter radius plots

In the three 5 m radius plots, 442 cryoconite holes were counted in total, with a range from 106-184 in each (M=147.3, SD=39.2). Cryoconite holes varied greatly within these larger

plots. For the purpose of this study ‘extreme’ cryoconite holes and smaller clustered holes were counted individually.

Figure 4.1

Cryoconite Holes



Note. Pictures of the fifteen cryoconite holes randomly selected and sampled for DOC, bacterial abundance and physicality.

Figure 4.2

Dried Sediment



Note. Pictures of the dried sediment collected from the fifteen randomly selected cryoconite holes.

4.3 DOC

DOC varied considerably between the 15 holes in which it was examined. The range of DOC concentration found in the studied 15 holes was 0.08- 5.83 mg C L⁻¹ (M= 2.94, SD= 2.25).

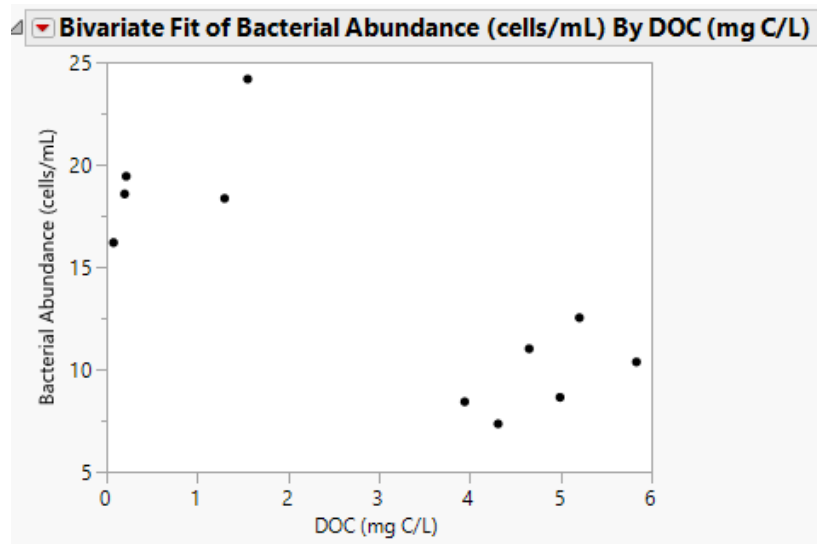
4.4 Bacterial abundance

Bacteria was found in the water column of each of the 15 holes. The bacterial abundance found in the holes ranged from 0.073 x 10³ and 0.24 x 10³ cells/ml (M= 0.15 x 10³ , SD= 0.057 x 10³). There was no correlation between bacterial abundance and the weight of sediment ($r= 0.31$,

p= 0.26). There was no correlation between bacterial abundance and the pH of the water in the holes ($r= -0.34$, $p= 0.22$). There was a negative correlation found between the DOC concentration and the bacterial abundance ($r= -0.79$, $p= 0.0035$)(fig. 4.3).

Figure 4.3

Correlation of Bacterial Abundance by DOC



Note. Correlation of bacterial abundance by dissolved organic carbon.

4.5 Respiration rate

Respiration rate was measured from 13 combined cryoconite holes. For the bottles kept in the light without sediment the mean respiration rate was $1.2 \text{ mg O}_2 \text{ L}^{-1} \text{ hr}^{-1}$ (SD= 0.0004), for the bottles kept in the light with sediment the mean respiration rate was $16.3 \text{ mg O}_2 \text{ L}^{-1} \text{ hr}^{-1}$ (SD= 0.002). For bottles kept in the dark without sediment the mean respiration rate was $1.8 \text{ mg O}_2 \text{ L}^{-1} \text{ hr}^{-1}$ (SD= 0.0006), for bottles kept in the dark with sediment the mean respiration rate was $17.1 \text{ mg O}_2 \text{ L}^{-1} \text{ hr}^{-1}$ (SD= 0.002) (tab. 4.1). Bottles with sediment had much higher respiration rates than those without, in both light and dark treatments (fig. 4.5). There is slight difference between the dark versus the light treatment in that the darker bottles have slightly higher respiration rates.

Table 4.1

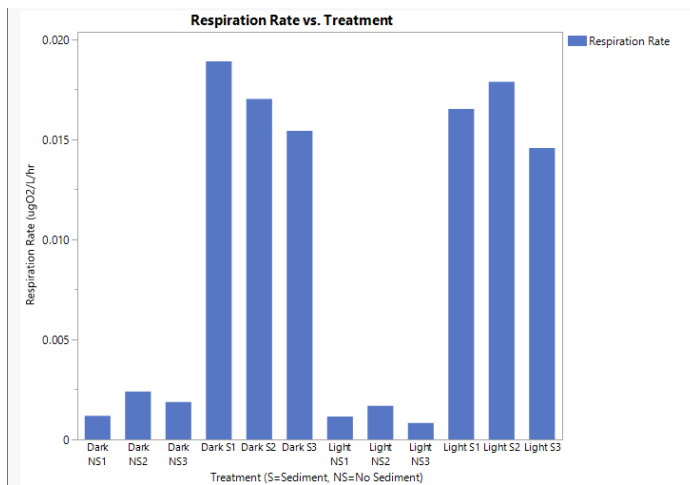
Respiration Rate: Winkler Titration Treatment

Treatment	Mean Respiration ($\mu\text{g O}_2/\text{L/hr}$)	Standard Deviation
Light w/ Sediment	16.3	0.002
Dark w/ Sediment	17.1	0.002
Light w/out Sediment	1.2	0.0004
Dark w/out Sediment	1.8	0.0006

Note. Results of respiration rates in each treatment measured in oxygen consumed per hour.

Figure 4.5

Respiration Rate vs. Treatment of Winkler Titrations



Note. Bar graph depicting respiration rate differences in each of the four treatments. Measured in oxygen consumed per hour.

Chapter 5: Discussion

Glacial runoff is a crucial component of the hydrological cycle, and its changes in runoff are directly impacted by climate change. Increased ambient temperatures, fluctuations in precipitation patterns, and other climate-related factors can lead to higher runoff from glaciers. Temporal changes in glacial runoff not only reflect seasonal and climate variations in surface ablation and meltwater production but also involve melt balance, refreezing processes, and alterations in the storage of meltwater in alpine glacial environments (Li et al., 2018).

Glacial environments, including their hydraulic systems, play a vital role in the distribution of water and nutrients from microbial communities predominantly found on glaciers to downstream ecosystems (Smith et al., 2017). Cryoconite holes, which can be present on up to 13% of the surface area of the world's glaciers and ice sheets, are often understudied (Sanyal et al., 2013, Fountain et al., 2004). Despite their small size, these holes host thriving ecosystems that contribute to the availability of labile material flowing into glacial headwaters and eventually to downstream ecosystems.

Within each of these holes, ecosystems thrive, contributing to the availability of labile material flowing into glacial headwaters and to downstream ecosystems. Thus cryoconite holes have an important role in the potential for transformation, storage and export of nutrients (Anesio et al., 2009; Anesio et al., 2010; Edwards et al., 2010; Murakami et al., 2020), with significant effects on downstream ecosystems. Understanding the dynamics of cryoconite holes and their role in nutrient cycling in glacial environments is essential for comprehending the broader impact of glacial melt and runoff on aquatic ecosystems and water quality downstream.

5.1 Field observations

May 26th - 28th 2023

In late May 2023, some surface water was observed on flat surface of the glacier, liquid water was observed in the form of small stream in surface meltwater channels (fig. 5.1). These channels were opened to the atmosphere and contained large volumes of sediment at the base. This sediment catalyzed the creation of what is known as ‘extreme’ cryoconite holes, holes that can be tens of meters across and up to 5 meters deep (Fountain et al., 2004). Samples were not collected in these extreme holes due to the inability to confirm that they were not connected below the surface to each other or to other glacial waterways. For this study's purpose, only cryoconite holes that have not yet melted into any subglacial streams or into each other were measured and sampled.

Figure 5.1

Meltwater Channel



Note. Picture of a melt water channel on the Root Glacier.

5.2 Physicality

Fountain et al., 2004, suggests that the initial size of the sediment deposit may determine diameter of the hole. In addition, they postulate that the hole's depth may be influenced by the sediment's thickness; if the sediment on the bottom of the hole is thick, the solar energy absorbed might be greater and increase its diameter. In this study, this was not observed. There was no correlation between the depth of the hole and the weight of the sediment and the cryoconite holes observed here varied by depth and diameter.

The fifteen randomly selected holes ranged from 8.8 cm deep to 25.6 cm deep, all were open and filled almost completely with water. Cryoconite hole diameter ranged from 3 cm to 15 cm. While some found that deeper cryoconite hole trended towards a greater diameter (Weisleitner et al., 2020), other studies (Fountain et al., 2004) found no relation between the two. There was no significant correlation between depth and diameter on the holes evaluated on Root Glacier. One possibility is that the holes studied on Root Glacier had not yet reached their equilibrium, which given the sampling time of May would make sense. Studies suggest that these holes will reach depths reported as 30 cm-50 cm once they have completely melted out and their depth and diameter become positively correlated (Gribon, 1979, Weisleitner et al., 2020). These depths are often reached at the end of the melt season (Fountain et al., 2008, Banerjee et al., 2023). The ice surrounding the holes and study area was still partially snow covered, which can limit the solar heating of the ice surrounding the study area significantly (Hoffman et al., 2008).

The physical characteristics of cryoconite holes can vary greatly across a glacier, even within a small area. The size, shape, depth, volume, sediment composition, sediment weight, and pH of each hole can differ significantly. This variability may be influenced by factors such as temperature, sunlight exposure, water flow, and sediment sources. Understanding the physicality

of cryoconite holes is important for studying their role in glacier ecosystems and their potential impacts on glacier melting and water quality. Further research is needed to explore the factors driving the variability in cryoconite holes and their implications for glacier ecosystems.

The variation in pH observed in the cryoconite holes on the Root Glacier could be influenced by the surrounding terrestrial area. The presence of nearby forests and other terrestrial ecosystems could introduce organic matter, nutrients, and other substances that influence the chemical composition of the cryoconite holes. For example, organic acids from decomposing plant matter could lower the pH in some areas, while mineral weathering processes may contribute to higher pH levels in other areas. Additionally, factors such as water flow patterns, sunlight exposure, and microbial activity within the cryoconite holes can also play a role in shaping the pH of the water within them. Pittino et al. (2023) found a variation in the pH in cryoconite holes sampled to be between 8.57 and 10.47. A study done by Ambrosini et al. (2017) found bacterial community structure variance depending on the pH of the water in the cryoconite holes. While this study did not find any correlation between pH and other variables, understanding the sources and processes that contribute to the variability in pH within cryoconite holes could provide valuable insights into the overall ecosystem dynamics of glacier systems and their interactions with the surrounding environment.

5.3 Bacterial abundance

The range of bacterial cell numbers within cryoconite holes reported in the literature is wide. Cell numbers reported worldwide vary with technique, some being measured in cells/ gram while others measuring cell/ml. For this study, cell abundance was calculated in cells/ml and the

findings are one order of magnitude different from other studies using the same technique (Sanyal et al., 2018). The bacterial abundance found in the holes ranged from 0.0073×10^5 and 0.024×10^4 cells/ml ($M= 0.015 \times 10^4$, $SD= 0.0057 \times 10^4$). Sanyal et al. (2018) found a range from $0.07- 9.57 \times 10^4$ cells/ml. While another study using a similar technique found bacterial abundances from selected cryoconite holes to be $1.00- 4.50 \times 10^4$ cells/ml (S awstr om et al., 2002). The microbial abundance within the holes are often based the community that exists within the space and time samples are collected (Edwards et al., 2013), so the wide range of total abundance reported is understandable.

There was no statistical significance between the volume of water and bacterial abundance. This is consistent with other studies conducted (Weisleitner et al., 2020; Hodson et al., 2013). Weight of the soil and pH of the water also showed no correlation with bacterial abundance. There was a correlation with amount of DOC concentration and bacterial abundance in each hole (Fig. 4.3). The greater the abundance of bacteria, the smaller the DOC concentration. This negative correlation showed an R-value of -0.79. This finding reflects other studies where increase in microbial abundance saw a decline in DOC values. Sanyal et al. (2018) saw a net decline in DOC and an increase in cell abundance during their incubation of heterotrophic microbes from cryoconite hole water removed from three geographically different sites in Antarctica. In all but two of the samples, where microbial abundance remained relatively unchanged, microbial abundance increased by 3 to 11 times in the live samples after incubation. This increase in microbial abundance, concurrent with the decline in DOC, could indicate heterotrophic microbial activity and the utilization of DOC in cryoconite hole water.

5.4 DOC Concentration

The DOC concentrations were highly cluster driven. With concentrations being either very low or closer to 4-5, there is no data to represent concentrations in the 2-3 range. When looking at bacterial abundance, while there is a negative correlation, without middle variables, only part of the picture may be visible. Compared to a study conducted by Musilova et al., in 2017, the DOC concentrations in the cryoconite holes on Root Glacier were larger than those found in the study which took place on the Greenland ice sheet. This study found that the DOC in cryoconite hole water, unlike other supraglacial surfaces, remained fairly constant at $0.15 \pm 0.01 \text{ mgCl}^{-1}$ over the 2012 ablation season. Hood et al. (2015) also found levels of DOC concentrations that were less than those found on Root Glacier. They reported concentrations ranging from $0.71\text{-}1.42 \text{ mgC L}^{-1}$. Another study conducted by Samui et al., 2020 found DOC concentrations in cryoconite holes to measure $9\text{-}55 \mu\text{g C L}^{-1}$ depending on the time of the season. Their study found that as the season progressed towards summer, the DOC concentrations increased. The study also suggested that degradation of DOC within the holes was an outcome of potential heterotrophic activity which could explain the negative correlation between the bacterial abundance and the DOC concentrations. The concentration of DOC in cryoconite holes could be influenced by various factors, including organic-rich carbon sediment sources, differences in sediment sources, such as glacial till versus terrestrial input, leading to variations in organic carbon content.

Comparing the DOC concentrations in cryoconite holes on the Root Glacier to those on the Greenland ice sheet and in other studies, it is evident that there can be significant differences in organic carbon content between glacier ecosystems. The stability of DOC concentrations over an ablation season on the Greenland ice sheet contrasts with the increase in DOC concentrations

observed by Samui et al. (2020) on the Root Glacier as the season progresses towards summer. This seasonal variation may be linked to changing environmental conditions and microbial activity within the cryoconite holes.

The negative correlation between bacterial abundance and DOC concentrations suggests a potential relationship between heterotrophic activity and carbon degradation within the cryoconite holes. The findings of various studies highlight the complex interactions between organic carbon sources, microbial communities, and environmental factors in glacier ecosystems. Further research is needed to explore the specific mechanisms driving variations in DOC concentrations, bacterial abundance, and microbial activity in cryoconite holes and their implications for ecosystems in glacier environments.

5.5 Respiration rate in combined cryoconite holes

The results of this study suggest that sediment in cryoconite holes significantly impacts respiration rates, with incubations containing sediment showing higher rates compared to those without sediment. This difference indicates that the sediment provides a substrate for bacterial growth and activity, leading to increased respiration rates. Several studies show that most of the biomass and biological activity is found in the sediment within the cryoconite holes (S awstr om et al., 2002; Cook et al., 2010; Edwards et al., 2010; Telling et al., 2012; Samui et al., 2020). In High Arctic cryoconite holes, a larger than 10-fold difference was found in the photosynthetic rates of the water containing sediment in comparison to only the glacial water from the holes (S awstr om et al., 2002). Samui et al. (2020) found primary production rates measured in cryoconite hole water to be an average of $0.29 \pm 0.12 \mu\text{C L}^{-1} \text{ day}^{-1}$ while in the sediment they found the rates to be on average $644 \pm 436 \mu\text{C L}^{-1} \text{ day}^{-1}$. In a study done on Svalbard Valley

Glaciers in the Arctic, Telling et al. (2012) found a significant increase in respiration rate as sediment depth was increased. They propose that this is likely due to an increase in both the number of microbial cells and the amount of labile organic matter.

Slightly higher respiration rates were found in dark versus light treatment but there were not enough samples to run statistical analysis to determine underlying patterns and trends. The lack of large variations between light and dark bottles with sediment could support the idea that photosynthesis, and therefore autotrophic activity, may not be a dominant process in these cryoconite holes. It could also suggest that the light bottles had photosynthesis and respiration occurring simultaneously, meaning there could have been more significant photosynthesis occurring, but it was not measured because the rate of respiration diminished the increase in observed oxygen. The study by Cook et al. (2010) also highlights the dominance of autotrophs on sediment surfaces, suggesting that the net heterotrophy observed in their study may be due to the reduced photosynthetic area caused by thick cryoconite layers shielding grains from photosynthetically active radiation. The destabilization of the arrangement of the sediment when combining sediment from multiple cryoconite holes could have impacted photosynthesis.

Photosynthesis occurring in the bottles exposed to light may have produced oxygen during the incubation period, this could have made the decrease in oxygen observed in the dark bottles appear more drastic. This study was conducted during a period in Alaska where the sun is present for almost 18 hours, ideally the bottles exposed to the light would have had less time in the sun. This could have impacted oxygen consumption from respiration and the production of oxygen from photosynthesis.

5.6 Five-meter radius plots

To calculate the percentage of the Root Glacier covered by cryoconite holes based on the density of holes found within the three 5m radius plots, the total count of 442 cryoconite holes can be used for the three plots with the assumption that these plots are representative of the overall distribution across the glacier.

Given that each plot had a range of 106-184 cryoconite holes, we can estimate the minimum and maximum number of holes across the glacier using the lowest and highest values. To estimate the percentage of the Root Glacier covered by cryoconite holes, we can calculate the range based on these total minimum and maximum numbers. To determine the total number of possible holes on the glacier, we would need to know the size of the entire glacier area and the average density of cryoconite holes per unit area.

Takeuchi et al. (2001) looks at the variability in hole distribution across the ablation zone and further emphasizes the importance of considering the spatial distribution and potential variability when extrapolating findings from sampled areas to the entire glacier. Careful interpretation and analysis of these statistics are crucial for accurately representing the extent of cryoconite hole coverage on the Root Glacier.

5.7 Limitations and future research

This study had several limitations. The first to consider is logistics and location. Carrying all the equipment many miles on mountainous and glacial terrain limited the number of samples that could be collected, decreasing sample size. Additionally, there was no 60 ml BOD available, meaning taking respiration rate for one individual hole was not possible. Instead, multiple holes

had to be combined to fit into larger 125 ml bottles. While this allowed for the finding that respiration rate was greater in the bottles containing sediment, it was not possible to evaluate respiration rate in the individual holes where bacterial abundance, DOC and physicality data was collected. This data would have added greatly to the larger picture and the microbial activity occurring in each hole. There was no way to not have some amount of aeration when combining the water from the 13 holes. In addition, the light bottles were exposed to the sun for a long period of time due to time of year and location. It would have been ideal for the light bottles to only be in the sun during the most intense times of sunlight. These factors could have potentially impacted the results.

The 5-meter plots were collected close to the terminus of the glacier. To have a more accurate understanding of total density of cryoconite holes on the glacier, plots would have to be made not only in the ablation zone but also on the medial moraine. In a study conducted by Weislietner et al., 2020, it was found that holes selected within the glacial terminus were on average 3.75 times deeper than those measured on the medial moraine. An accurate model would include holes from different topographic regions of the glacier.

Another impact on physicality, hole depth, diameter and volume more specifically could be seasonality. All the samples collected on Root Glacier were collected over a two-day period during the beginning of melt season. The size and volume of each hole changes seasonally and over time. Studies have looked at cryoconite holes over time and found that their size does not reach finite until often the end of the melt season, and sometimes over multiple years (Fountain et al., 2004). Having permanent plots and measuring these holes over time could provide a better understanding of the variation in isolation and recharge within each area (Bagshaw et al., 2007). This would add to the larger picture of the glacial hydraulic system.

Four of the vials to be tested for DOC were broken during transit. With such a small sample size to begin with, losing 27% of the samples could have impacted the overall results of the DOC measurements and how they pertained to bacterial abundance. The vials that broke all had a bacterial count that was in the middle of the dataset collected. It would have been valuable to know how their DOC concentration impacted the statistics.

On the surface of the glacier there were several surface meltwater channels and extreme cryoconite holes. These holes and water systems contained large amounts of sediment at the base. While these extreme holes and larger bodies of water were not looked at in this study, they could be an important source of material to be transported downstream. Their size and sediment content warrants inclusion into future studies and research.

5.8 General recommendations

Although it is difficult to link direct cause and effect between bacterial abundance and DOC concentration, more analyses should be explored. This should include seasonal variation in data collection with dedicated melt season and winter season plots. This would allow for an understanding of seasonal change and longterm, multi-year data for calculating the total water input from cryoconite holes that can persist on glacial surfaces for many years. In addition to multiple season data collection and longterm plots, larger sample size is recommended for more accurate analysis of the chosen area. Finally, hydrological models should include glacial runoff and output of cryoconite holes which could be responsible for over 10% of glacial meltwater.

Chapter 6: Conclusion

The findings from this study on cryoconite holes yield valuable insights into the complex dynamics and ecological significance of glacial ecosystems. This study revealed substantial variability in physical characteristics, bacterial abundance, dissolved organic carbon (DOC) concentrations and respiration rates among the cryoconite holes. Notably, the negative correlation observed between bacterial abundance and DOC concentrations suggests a potential relationship between heterotrophic activity and carbon degradation within these holes. Further, the high respiration rates in incubations containing sediment suggest that the sediment is an important source of organic matter contributing to ecosystem metabolism.

The study faced certain limitations, including logistical constraints, limited sample size and the impact of seasonality on hole characteristics. These limitations highlight the need for continued research. Long-term monitoring is necessary to better understand the seasonal and temporal changes within cryoconite holes, ideally this would be done using permanent plots. Furthermore, addressing the spatial variability of cryoconite holes and their distribution across the glacier would enhance the understanding of their role in the overall glacial ecosystem.

The study has implications for the integration of cryoconite hole and glacial runoff outputs into hydrological models, recognizing their substantial contribution to glacial meltwater. Recommendations for further research include exploring the processes driving variations in DOC concentrations, bacterial abundance, and microbial activity in cryoconite holes. Future research and inclusion into current hydrologic models are essential. As our world's glaciers continue to diminish, we need to be prepared for extreme shifts in the glacially fed hydrological systems.

References

- Allan, J. D., & Castillo, M. M. (2007). An introduction to fluvial ecosystems. *Stream Ecology: Structure and function of running waters*, 1-12.
- Ambrosini, R., Musitelli, F., Navarra, F., Tagliaferri, I., Gandolfi, I., Bestetti, G., Mayer, C., Minora, U., Azzoni, R. S., Diolaiuti, G., Smiraglia, C., & Franzetti, A. (2017). Diversity and Assembling Processes of Bacterial Communities in Cryoconite Holes of a Karakoram Glacier. *Microbial Ecology*, 73(4), 827–837. <https://doi.org/10.1007/s00248-016-0914-6>
- Anderson, S. P., Longacre, S. A., & Kraal, E. R. (2003). Patterns of water chemistry and discharge in the glacier-fed Kennicott River, Alaska: evidence for subglacial water storage cycles. *Chemical Geology*, 202(3-4), 297-312.
- Anesio, A. M., Mindl, B., Laybourn-Parry, J., Hodson, A. J., & Sattler, B. (2007). Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). *Journal of Geophysical Research: Biogeosciences*, 112(4). <https://doi.org/10.1029/2006JG000350>
- Anesio, A. M., Hodson, A. J., Fritz, A., Psenner, R., & Sattler, B. (2009). High microbial activity on glaciers: Importance to the global carbon cycle. *Global Change Biology*, 15(4). <https://doi.org/10.1111/j.1365-2486.2008.01758.x>
- Anesio, A. M., Sattler, B., Foreman, C., Telling, J., Hodson, A., Tranter, M., & Psenner, R. (2010). Carbon fluxes through bacterial communities on glacier surfaces. *Annals of Glaciology*, 51(56). <https://doi.org/10.3189/172756411795932092>

- Antony, R., Willoughby, A. S., Grannas, A. M., Catanzano, V., Sleighter, R. L., Thamban, M., Hatcher, P. G., & Nair, S. (2017). Molecular Insights on Dissolved Organic Matter Transformation by Supraglacial Microbial Communities. *Environmental Science and Technology*, 51(8), 4328–4337. <https://doi.org/10.1021/acs.est.6b05780>
- Arendt, A. A., Echelmeyer, K. A., Harrison, W. D., Lingle, C. S., & Valentine, V. B. (2002). Rapid wastage of Alaska glaciers and their contribution to rising sea level. *Science*, 297(5580), 382-386.
- Armstrong, W. H., Anderson, R. S., & Fahnestock, M. A. (2017). Spatial Patterns of Summer Speedup on South Central Alaska Glaciers. *Geophysical Research Letters*, 44(18), 9379–9388. <https://doi.org/10.1002/2017GL074370>
- Bagshaw, E. A., Tranter, M., Fountain, A. G., Welch, K. A., Basagic, H., & Lyons, W. B. (2007). Biogeochemical evolution of cryoconite holes on Canada Glacier, Taylor Valley, Antarctica. *Journal of Geophysical Research: Biogeosciences*, 112(4). <https://doi.org/10.1029/2007JG000442>
- Bagshaw, E. A., Tranter, M., Fountain, A. G., Welch, K., Basagic, H. J., & Lyons, W. B. (2013). Do cryoconite holes have the potential to be significant sources of C, N, and P to downstream depauperate ecosystems of Taylor Valley, Antarctica? *Arctic, Antarctic, and Alpine Research*, 45(4), 440–454. <https://doi.org/10.1657/1938-4246-45.4.440>

- Banerjee, A., Sarangi, C., Rashid, I., Vijay, S., Najar, N. A., & Chandel, A. S. (2023). A scaling relation for cryoconite holes. *Geophysical Research Letters*, 50(22), e2023GL104942.
- Bliss, A., Hock, R., & Radić, V. (2014). Global response of glacier runoff to twenty-first century climate change. *Journal of Geophysical Research: Earth Surface*, 119(4), 717-730.
- Cameron, K. A., Hodson, A. J., & Osborn, A. M. (2012). Carbon and nitrogen biogeochemical cycling potentials of supraglacial cryoconite communities. *Polar Biology*, 35(9), 1375–1393. <https://doi.org/10.1007/s00300-012-1178-3>
- Castello, J., & Rogers, S. (n.d.). *Life in Ancient Ice* .
- Cook, J., Edwards, A., Takeuchi, N., & Irvine-Fynn, T. (2016). Cryoconite: The dark biological secret of the cryosphere. *Progress in Physical Geography*, 40(1).
<https://doi.org/10.1177/0309133315616574>
- Cook, J. M., Sweet, M., Cavalli, O., Taggart, A., & Edwards, A. (2018). Topographic shading influences cryoconite morphodynamics and carbon exchange. *Arctic, antarctic, and alpine research*, 50(1), S100014.
- Edwards, A., Anesio, A. M., Rassner, S. M., Sattler, B., Hubbard, B., Perkins, W. T., Young, M., & Griffith, G. W. (2011). Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *ISME Journal*, 5(1), 150–160. <https://doi.org/10.1038/ismej.2010.100>

- Edwards, A., Pachebat, J. A., Swain, M., Hegarty, M., Hodson, A. J., Irvine-Fynn, T. D. L., Rassner, S. M. E., & Sattler, B. (2013). A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environmental Research Letters*, 8(3). <https://doi.org/10.1088/1748-9326/8/3/035003>
- Fellman, J. B., Spencer, R. G., Hernes, P. J., Edwards, R. T., D'Amore, D. V., & Hood, E. (2010). The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems. *Marine Chemistry*, 121(1-4), 112-122.
- Feng, L., An, Y., Xu, J., Kellerman, A. M., Chacón Patiño, M. L., & Spencer, R. G. M. (2021). Molecular Insights into Glacial Cryoconite Dissolved Organic Matter Evolution under Dark Conditions during the Ablation Season on the Tibetan Plateau. *ACS Earth and Space Chemistry*, 5(4). <https://doi.org/10.1021/acsearthspacechem.0c00361>
- Fountain, A. G., Tranter, M., Nylén, T. H., Lewis, K. J., & Mueller, D. R. (2004). Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica.
- Fountain, A. G., Nylén, T. H., Tranter, M., & Bagshaw, E. (2008). Temporal variations in physical and chemical features of cryoconite holes on Canada Glacier, McMurdo Dry

Valleys, Antarctica. *Journal of Geophysical Research: Biogeosciences*, 113(1).

<https://doi.org/10.1029/2007JG000430>

Gerdel, R. W., & Drouet, F. (1960). The cryoconite of the Thule area, Greenland. *Transactions of the American Microscopical Society*, 79(3), 256-272.

Guo, B., Liu, Y., Liu, K., Shi, Q., He, C., Cai, R., & Jiao, N. (2022). Different dissolved organic matter composition between central and southern glaciers on the Tibetan Plateau. *Ecological Indicators*, 139, 108888.

Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., ... & Sattler, B. (2008). Glacial ecosystems. *Ecological monographs*, 78(1), 41-67.

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

Holen, D. L. (2004). The Atna and the political ecology of the Copper River Fishery, Alaska. *Arctic Anthropology*, 41(1), 58-70.

Hood, E., & Scott, D. (2008). Riverine organic matter and nutrients in southeast Alaska affected by glacial coverage. *Nature Geoscience*, 1(9), 583–587. <https://doi.org/10.1038/ngeo280>

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

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

John, K., Kari, J. M., & Center, A. N. L. (1986). *Headwaters People’s Country*. Alaska Native Language Center, University of Alaska.
<https://books.google.com/books?id=hO3WAAAAMAAJ>

Kohshima, S., Seko, K., & Yoshimura, Y. (1992). Biotic Acceleration of Glacier Melting in Yala Glacier 9 Langtang Region, Nepal Himalaya (Issue 218). IAHS Publ.

Li, X., Ding, Y., Xu, J., He, X., Han, T., Kang, S., Wu, Q., Mika, S., Yu, Z., & Li, Q. (2018). Importance of Mountain Glaciers as a Source of Dissolved Organic Carbon. *Journal of Geophysical Research: Earth Surface*, 123(9). <https://doi.org/10.1029/2017JF004333>

Mackevett, E. M., & Pecora, W. T. (n.d.). *Stratigraphy and General Geology of jke McCarthy C-5 Quadrangle; Alaska Descriptions of the rocks of a quadrangle famous for its copper*

mines UNITED STATES GOVERNMENT PRINTING OFFICE, WASHINGTON : 1971
UNITED STATES DEPARTMENT OF THE INTERIOR GEOLOGICAL SURVEY.

Mieczan, T., Górniak, D., Świątecki, A., Zdanowski, M., Tarkowska, M., Kukuryk, –, & Adamczuk, M. (2013). *Vertical microzonation of ciliates in cryoconite holes in Ecology Glacier, King George Island.* <https://doi.org/10.2478/popore-2013-0008>

Milner, A. M., Khamis, K., Battin, T. J., Brittain, J. E., Barrand, N. E., Füreder, L., Cauvy-Frauní, S., Ar Gíslason, M. ´, Jacobsen, D., Hannah, D. M., Hodson, A. J., Hood, E., Lencioni, V., Olafsson, S. ´, Robinson, C. T., Tranter, M., & Brown, L. E. (2017). Glacier shrinkage driving global changes in downstream systems. <https://doi.org/10.1073/pnas.1619807114/-/DCSupplemental>

Mueller, D., Vincent, W., Pollard, W., & Fritsen, C. (2001). Glacial cryoconite ecosystems: A bipolar comparison of algal communities and habitats. 173–197.

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

Standard Methods for the Examination of Water and Waste Water, 14th ed., 1975. American Public Health Association, Washington, D.C.

- Murakami, T., Takeuchi, N., Mori, H., Hirose, Y., Edwards, A., Irvine-Fynn, T., Li, Z., Ishii, S., & Segawa, T. (2022). Metagenomics reveals global-scale contrasts in nitrogen cycling and cyanobacterial light-harvesting mechanisms in glacier cryoconite. *Microbiome*, *10*(1). <https://doi.org/10.1186/s40168-022-01238-7>
- Pautler, B. G., Dubnick, A., Sharp, M. J., Simpson, A. J., & Simpson, M. J. (2013). Comparison of cryoconite organic matter composition from Arctic and Antarctic glaciers at the molecular-level. *Geochimica et Cosmochimica Acta*, *104*, 1-18.
- Pittino, F., Ambrosini, R., Seeger, M., Azzoni, R. S., Diolaiuti, G., Alviz Gazitua, P., & Franzetti, A. (2023). Geographical variability of bacterial communities of cryoconite holes of Andean glaciers. *Scientific Reports*, *13*(1). <https://doi.org/10.1038/s41598-022-24373-5>
- Poniecka, E. A., Bagshaw, E. A., Sass, H., Segar, A., Webster, G., Williamson, C., Anesio, A. M., & Tranter, M. (2020). Physiological Capabilities of Cryoconite Hole Microorganisms. *Frontiers in Microbiology*, *11*. <https://doi.org/10.3389/fmicb.2020.01783>
- Samui, G., Antony, R., & Thamban, M. (2020). Fate of Dissolved Organic Carbon in Antarctic Surface Environments During Summer. *Journal of Geophysical Research: Biogeosciences*, *125*(12). <https://doi.org/10.1029/2020JG005958>

Sanyal, A., Antony, R., Samui, G., & Thamban, M. (2018). Microbial communities and their potential for degradation of dissolved organic carbon in cryoconite hole environments of Himalaya and Antarctica. *Microbiological Research*, 208, 32–42.

<https://doi.org/10.1016/j.micres.2018.01.004>

Såwström, C., Mumford, P., Marshall, W., Hodson, A., & Laybourn-Parry, J. (2002). The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79°N). *Polar Biology*, 25(8), 591–596. [https://doi.org/10.1007/s00300-002-](https://doi.org/10.1007/s00300-002-0388-5)

[0388-5](https://doi.org/10.1007/s00300-002-0388-5)

Segawa, T., Yonezawa, T., Edwards, A., Akiyoshi, A., Tanaka, S., Uetake, J., Irvine-Fynn, T., Fukui, K., Li, Z., & Takeuchi, N. (2017). Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers. *Journal of Biogeography*, 44(12), 2849–2861.

<https://doi.org/10.1111/jbi.13089>

Seibert, J., Vis, M., Kohn, I., Weiler, M., & Stahl, K. (2018). Technical note: Representing glacier geometry changes in a semi-distributed hydrological model. *Hydrology and Earth System Sciences*, 22(4), 2211–2224. <https://doi.org/10.5194/hess-22-2211-2018>

Slemmons, K. E., Saros, J. E., & Simon, K. (2013). The influence of glacial meltwater on alpine aquatic ecosystems: a review. *Environmental Science: Processes & Impacts*, 15(10), 1794-1806.

- Smith, H. J., Foster, R. A., McKnight, D. M., Lisle, J. T., Littmann, S., Kuypers, M. M. M., & Foreman, C. M. (2017). Microbial formation of labile organic carbon in Antarctic glacial environments. *Nature Geoscience*, 10(5), 356–359. <https://doi.org/10.1038/ngeo2925>
- Smith, H. J., Dieser, M., McKnight, D. M., SanClements, M. D., & Foreman, C. M. (2018). Relationship between dissolved organic matter quality and microbial community composition across polar glacial environments. *FEMS Microbiology Ecology*, 94(7), fiy090.
- Stocker, B. D., Roth, R., Joos, F., Spahni, R., Steinacher, M., Zaehle, S., ... & Prentice, I. C. (2013). Multiple greenhouse-gas feedbacks from the land biosphere under future climate change scenarios. *Nature Climate Change*, 3(7), 666-672.
- Stibal, M., Šabacká, M., & Žárský, J. (2012). Biological processes on glacier and ice sheet surfaces. *Nature Geoscience*, 5(11), 771-774.
- Stibal, M., Schostag, M., Cameron, K. A., Hansen, L. H., Chandler, D. M., Wadham, J. L., & Jacobsen, C. S. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environmental Microbiology Reports*, 7(2), 293–300. <https://doi.org/10.1111/1758-2229.12246>
- Strickland, J.D.H., T. R. Parsons, 1972. *A Practical Handbook of Sea Water Analysis*, 2nd Ed. Fish. Res. Board. Canada, Ottawa, Canada.

Stubbins, A., Hood, E., Raymond, P. A., Aiken, G. R., Sleighter, R. L., Hernes, P. J., Butman, D., Hatcher, P. G., Striegl, R. G., Schuster, P., Abdulla, H. A. N., Vermilyea, A. W., Scott, D. T., & Spencer, R. G. M. (2012). Anthropogenic aerosols as a source of ancient dissolved organic matter in glaciers. *Nature Geoscience*, 5(3), 198–201.
<https://doi.org/10.1038/ngeo1403>

Telling, J., Anesio, A. M., Hawkings, J., Tranter, M., Wadham, J. L., Hodson, A. J., Irvine-Fynn, T., & Yallop, M. L. (2010). Measuring rates of gross photosynthesis and net community production in cryoconite holes: A comparison of field methods. *Annals of Glaciology*, 51(56). <https://doi.org/10.3189/172756411795932056>

Telling, J., Anesio, A. M., Tranter, M., Stibal, M., Hawkings, J., Irvine-Fynn, T., Hodson, A., Butler, C., Yallop, M., & Wadham, J. (2012). Controls on the autochthonous production and respiration of organic matter in cryoconite holes on high Arctic glaciers. *Journal of Geophysical Research: Biogeosciences*, 117(1). <https://doi.org/10.1029/2011JG001828>

Takeuchi, N. (2002). Optical characteristics of cryoconite (surface dust) on glaciers: the relationship between light absorbency and the property of organic matter contained in the cryoconite. *Annals of Glaciology*, 34, 409-414.

- Takeuchi, N., Kohshima, S., & Seko, K. (2001). Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. *Arctic, Antarctic, and Alpine Research*, 33(2), 115-122.
- Tranter, M., Fountain, A. G., Fritsen, C. H., Berry Lyons, W., Priscu, J. C., Statham, P. J., & Welch, K. A. (2004). Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice. *Hydrological Processes*, 18(2), 379-387.
- Uetake, J., Tanaka, S., Segawa, T., Takeuchi, N., Nagatsuka, N., Motoyama, H., & Aoki, T. (2016). Microbial community variation in cryoconite granules on Qaanaaq Glacier, NW Greenland. *FEMS Microbiology Ecology*, 92(9). <https://doi.org/10.1093/femsec/fiw127>
- Weisleitner, K., Perras, A. K., Unterberger, S. H., Moissl-Eichinger, C., Andersen, D. T., & Sattler, B. (2020). Cryoconite Hole Location in East-Antarctic Untersee Oasis Shapes Physical and Biological Diversity. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.01165>
- Wharton Jr, R. A., McKay, C. P., Simmons Jr, G. M., & Parker, B. C. (1985). Cryoconite holes on glaciers. *Bioscience*, 499-503.