Unseen and Unknown: Microbial Community Diversity in a Rapidly Changing High Arctic Watershed on Northern Ellesmere Island, Canada

By

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ABSTRACT

Arctic watersheds are currently undergoing great alterations due to human induced climate change. Current models predict increases in High Arctic temperatures and precipitation of up to 8.3°C and 40%, respectively, by 2100, which will have profound impacts on the arctic hydrological cycle, including enhanced glacial melt and permafrost thaw. However, it remains uncertain how enhanced glacial melt and permafrost thaw will impact the structure of downstream resident freshwater microbial communities and ensuing microbially driven freshwater ecosystem services. Using the Lake Hazen watershed (Nunavut, Canada, 81 N; 71 W) as a sentinel system for change, we characterized microbial community composition, using 16S rRNA gene sequencing, over a complete annual hydrological cycle, in relation to measured physicochemical parameters (e.g., temperature, dissolved oxygen, nutrients, major ions) in three freshwater compartments within the Lake Hazen watershed: i) glacial rivers; ii) permafrost thaw streams and the waterbodies they drain into, including small lakes and wetland areas, and; iii) Lake Hazen into which i) and ii) drain. Our findings show that microbial communities throughout these freshwater compartments are highly interconnected and are often shaped by the availability of melt-sourced chemicals (such as carbonates and sulfate) as the melt season progresses over time. Within Lake Hazen in particular, microbial taxa were found to be generally stable over a spring and summer season, including members from phyla Chloroflexi and Elusimicrobia, indicating that microbial communities and the potential ecosystem services they provide therein may be somewhat resilient in the face of environmental change. Altogether, my research helps establish a baseline understanding of how microbial communities and the ecosystem services they provide in Arctic watersheds might be reacting in response to climate change.

PREFACE

My thesis is divided into three chapters, beginning with an introductory chapter (1), followed by a research chapter (2), written in manuscript format, and lastly, a concluding chapter (3). Chapter 1 introduces the overall context and relevancy of my M.Sc. research, the various field sites involved, and the overarching goals of the research undertaken. Chapter 2 is entitled "Microbial community diversity in a rapidly changing High Arctic watershed" and is co-authored by myself, Dr. Vincent St. Louis[1], Katja Engel[2], Kyra. A St. Pierre[1], Dr. Sherry Schiff [3], Dr. Marek Stibal[4] and Dr. Josh D. Neufeld[2], and will be submitted to the journal of FEMS Environmental Microbiology. Most microbiological samples were collected by myself, with additional help from Jessica Serbu, Kyra St. Pierre, Paul Dainard, Pieter Aukes, Dr. Sherry Schiff, Charles Talbot, Dr. Vincent St. Louis, Victoria Wisniewski, Stephany Varty and Graham Colby. I was responsible for data analysis, with significant help from Katja Engel, who performed 16S rRNA gene sequencing and preliminary data preparation, and Dr. Josh Neufeld and Dr. Sherry Schiff for feedback on data interpretation. Dr. Marek Stibal and the Cryosphere Ecology group were most helpful in the development of a conceptual model merging all of the microbial ecology and associated physicochemistry within the Lake Hazen watershed together. Chapter 3 contains a general overview of research findings, highlights of those findings, and suggestions for future research directions on this research topic in this, and other, Arctic watersheds.

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“Deep in the human unconscious is a pervasive need for a logical universe that makes sense. But the real universe is always one step beyond logic.”

-Frank Hebert,

Dune, 1965

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and pursuit of additional research directions and analyses that I definitely wouldn't have been able to complete on my own. Ashley, you're awesome.

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I would also like to thank all of the members of my supervisory committee, comprised of Dr. Yan Boucher and Dr. Maya Bhatia who reviewed my thesis.

And last of all, I would like to thank all of my friends and family for their stalwart support throughout my entire university career. To my parents, Rebeca and Jose, I can't thank you enough for the love, encouragement, humour and support you've lent me. I have learned so much from you; that sometimes nothing but hard work and a positive attitude (or just plain laughter) can take you anywhere, and truly, they have. You are both my inspiration, my motivators and my foundation. Although unorthodox, I would also like to acknowledge my budgie, Pippin, who was instrumental in maintaining my mental sanity throughout. To my excellent field mates, lab mates and friends: Kyra St. Pierre and Jessica Serbu, thank you for the good times in the field and for your excellent senses of humour. Kyra, your patience, help and invaluable insight into the Lake Hazen watershed was an absolute lifesaver for data amalgamation, interpretation and field site prep. To Igor Lehnherr, Charles Talbot (from whom I finally learned how to play Euchre), Paul Dainard, Pieter Aukes, Victoria Wisniewski, Stephany Varty, and Graham Colby, your good cheer and expertise, despite the sometimes adverse conditions, have made my field experiences all the more memorable. The field wouldn't have been the same without you guys. To Yuluan Wang, Michael Wong, Lee Campbell, Daniel Engelhardt, Sarah Shakil, Helena Magaldi, Patrick Neuberger, Alireza Saidi-Mehrabad, thanks for the laughs, and graduate student bonding over mutually confounding results, DNA extraction and microbial sequencing issues. You're all great. And, to Ansh Dubey, thanks for all of your caring support, understanding and sense of humour! You were one of my prime motivators in the writing of this here thesis.

To all: thank you, truly.

TABLE OF CONTENTS

LIST OF FIGURES:

LIST OF TABLES

1 INTRODUCTION

1.1 Climate change in the Arctic and its impacts on freshwater systems

Human induced climate change is occurring more rapidly in the Arctic than anywhere else on Earth, with climate models predicting increases in temperature and precipitation at these northernmost latitudes of up to 8.3ºC and 40%, respectively, by 2100 (RCP8.5 in CMIP5; Stocker et al., 2013). The arctic landscape, predominantly underlain by permafrost and covered with snow, glaciers, and ice caps, is especially prone to warming (Serreze, et al., 2009, Serreze, 2011). This is due to arctic amplification; a positive feedback loop whereby decreases in the reflectivity of the seascape and landscape due to the melt of snow and ice cover result in increases in ambient temperature. This ensuing warming then perpetuates the further melting of snow and ice on land and at sea in high northern latitudes (Serreze et al., 2011). This phenomenon thus serves to increase the sensitivity of an already fragile ecoregion to further warming and ensuing changes (Cronin et al., 2017). The gradual loss of these features will also profoundly affect the Arctic hydrological cycle (Dery et al., 2009), increasing glacial melt and permafrost thaw run off, with uncertain consequences for the ecological integrity of downstream freshwater systems (Hood et al., 2007; Rydberg et al., 2010; Zdanowicz et al., 2013). This is particularly important because in polar deserts, freshwater systems act as geochemical reactors (Emmerton et al., 2016) and biological focal points on the landscape, supporting highly specialized and fragile ecological webs of life that are found nowhere else on the planet (Vincent et al., 2011).

1.2 Freshwater microorganisms

In nutrient-poor oligotrophic or ultra-oligotrophic Arctic freshwater systems, microorganisms are extremely important in regulating nutrient and energy fluxes (Cotner and Biddanda, 2002), and

hence, overall water quality and ecosystem productivity, by forming the base of food webs and driving key biogeochemical cycles in ecosystems (Graham et al., 2016). With short generation times and sometimes flexible metabolisms, microorganisms can respond rapidly to variability in the physicochemistry of their environment (Logue et al., 2015). However, daily and seasonal fluctuations in precipitation, glacial melt, and permafrost thaw water inputs to Arctic freshwaters create highly unpredictable conditions for organisms living there. Given the climate changes already occurring in, and predicted for, the Arctic, understanding microbial community structure and functional capabilities as a system is paramount to predicting how overall freshwater productivity and quality will respond to rapidly changing climates.

1.3 The Lake Hazen watershed

We used the Lake Hazen watershed, located within Quttinirpaaq National Park on Northern Ellesmere Island (81N; 71W) to characterize freshwater microbial ecology and their relationships with the physicochemical environment of freshwater systems across a rapidly changing High Arctic watershed. As with much of the High Arctic, the Lake Hazen watershed is experiencing an acceleration of glacial melt and permafrost thaw, and subsequent associated changes in the stability and fluxes of several physicochemical parameters (Lehnherr et al. 2018). This watershed contains a highly diverse and interconnected freshwater system (Figure 1.1), at the heart of which is the world's largest lake by volume north of the Arctic Circle: Lake Hazen. Lake Hazen is approximately 75 km long, ~11 km across and has a maximum depth of ~267 m (Kock et al., 2012). Lake Hazen is drained by the Ruggles River out to Chandler Fjord on the northeastern coast of Ellesmere Island, which eventually feeds into the Nares Strait-North Water polynya circulation (Melling et al. 2001). In particular, my research project elucidated microbial community composition and physicochemical relationships within three main freshwater

compartments of the watershed: 1) glacial rivers, 2) permafrost thaw streams and the waterbodies they drain into, including small lakes and wetland areas, during the summer, all feeding into: 3) Lake Hazen. To the best of my knowledge, this is the first study to address microbial community diversity and its relationship with associated physicochemical parameters on a whole-watershed scale for an entire hydrological year in the High Arctic. This is important because freshwater microbial communities and their relationships with seasonal physicochemical relationships underlie many of the fragile biogeochemical relationships of the region, connecting the cryosphere with the terrestrial and freshwater environment as waters originating from these systems flow across the landscape. As the Lake Hazen watershed encompasses a variety of these systems interconnected together by a major lake, this site represents a unique sentinel site to assess, for the first time, the composition and relationship of microbial communities with various physicochemical conditions represented by three basic but interconnected freshwater compartments.

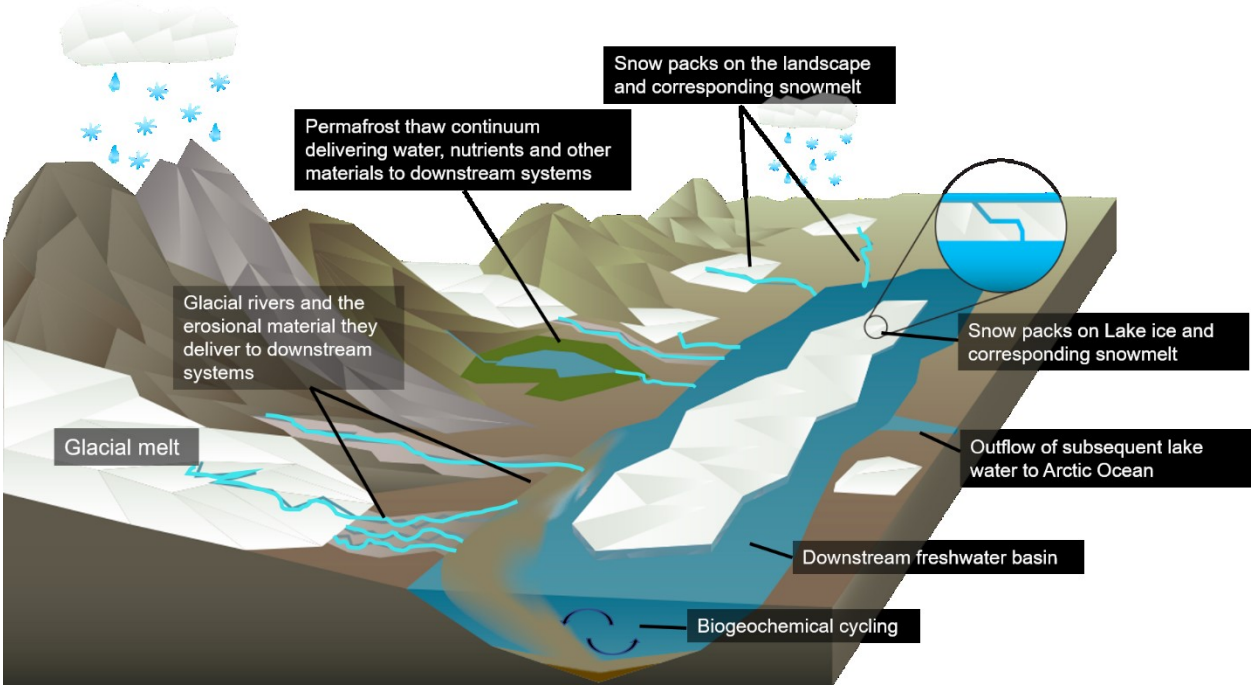


Figure 1.1 Schematic of the Lake Hazen watershed depicting the general landscape and the flow of water and potential nutrients and/or contaminants to downstream Lake Hazen from various freshwater compartments. These freshwater compartments comprise of snow, snowmelt, glacial melt, which is the primary source of freshwater to Lake Hazen, and a permafrost thaw continuum.

2 MICROBIAL COMMUNITY DIVERSITY IN A RAPIDLY CHANGING HIGH ARCTIC WATERSHED

2.1 INTRODUCTION

Human-induced climate change is profoundly altering Arctic watersheds (Prowse et al., (2015), Bring et al., (2016) & Wrona et al., (2016). Current climate models predict increases in temperature and precipitation in the High Arctic of up to 8.3°C and 40%, respectively, by 2100 (RCP 8.5 with CMIP5; Stocker et al., 2013). These changes will not only have overwhelming impacts on the Arctic hydrological cycle due to increased snowfall, glacial melt and permafrost thaw, but will also result in the release of previously archived nutrients and pollutants in ice and soils to downstream freshwater systems (Hood et al., 2007; Rydberg et al., 2010). These fluxes are important because distinct freshwater systems across these landscapes (lakes, wetlands, rivers) each have unique biogeochemical processes and levels of productivity (Emmerton et al., 2016; Vincent et al., 2013), intricately balanced food webs and energy flows (Wrona et al., 2016), and important hydrological linkages with the surrounding landscape (Newton et al., 2011). Collectively, freshwater systems are important sentinel systems for environmental change in the Arctic (Adrian et al., 2009). However, how the stability of microbial community composition is affected by physicochemical variability across High Arctic freshwaters due to seasonal glacial melt and permafrost thaw is largely unknown.

Freshwater microorganisms are critical mediators in the mineralization of organic matter and in the biogeochemical cycling of nutrients and contaminants (Barkay et al., 2011; Newton et al., 2014; Graham et al., 2016). As such, any environmental perturbation that may affect the structure and subsequent function of resident freshwater microbial communities will therefore have an impact on overall water quality and ecosystem services. Although several studies have

addressed the biogeochemistry of Arctic freshwaters (Pokrovsky et al., 2011; Dubnick et al., 2017) and the roles and fluctuations in microbial community composition throughout various Arctic landscapes (Schutte et al., 2010; McCann et al., 2016), including freshwater systems (Sheik et al., 2015; Comte et al., 2015; Hauptmann et al., 2016; Cameron et al., 2017), none have addressed temporal variations in High Arctic microbial community composition for freshwater compartments fed by both glacial melt and permafrost thaw on a watershed scale throughout an annual hydrological cycle.

The Lake Hazen watershed, located on northern Ellesmere Island, Nunavut, represents a uniquely interconnected and diverse freshwater system comprised of three basic freshwater compartments: 1) numerous glacially-sourced rivers, 2) permafrost thaw-driven sub-catchments, and 3) Lake Hazen, the High Arctic's largest lake by volume, which receives input from 1) and 2), at the heart of the watershed. The Lake Hazen watershed has experienced profound alterations due to climate change over recent decades, including a ten-fold increase in glacial melt since 2007 (Lehnherr et al., 2018), with yet unknown consequences for the freshwater microbial ecology in downstream systems.

Over the course of an annual hydrological cycle, we studied microbial community composition and corresponding physicochemical properties in each of these three freshwater compartments. We also explored the flow of microbial community members through the first two compartments and investigated how these compartments contributed to overall Lake Hazen microbiota. We here suggest that microbial communities within glacial rivers are structured by mass dispersal of microorganisms sourced from the erosion of river banks, and secondarily, from potential supraglacial routing. Once deposited into Lake Hazen, we predict that members of glacial river microbial communities likely undergo strong species selection due to long water

residence time and subsequent exposure to the lake physicochemical environment. To the best of our knowledge, the work presented here is one of the first watershed-scale studies linking freshwater microbial community composition to sourcing and physicochemical properties within and between various freshwater compartments. Hence, we here provide a baseline understanding of the composition and drivers of freshwater microbial community dynamics across a large High Arctic watershed.

2.2 MATERIALS AND METHODS

2.2.1 Site description

The Lake Hazen watershed is located in central Quttinirpaaq National Park, on Northern Ellesmere Island, Canada (81°N, 71°W). The watershed is dominated by ultra-oligotrophic Lake Hazen, a trench lake hydrologically fed by eleven glacial rivers (St. Pierre et al., under review) and, to a much lesser extent, by several permafrost thaw streams. The dominant glacial inflows into Lake Hazen are those from the Gilman and Henrietta Nesmith outlet glaciers (Figure 2.1), which collectively account for ~50% of the water inputs into Lake Hazen each year. Lake Hazen is solely drained by the Ruggles River, which flows into Chandler's Fjord and then Nares Strait between Ellesmere Island and Greenland. Lake Hazen is typically ice-covered most of the year but goes ice-free almost annually by late July/early August (Lehnherr et al., 2018).

Near the Parks Canada base camp at Lake Hazen exists what we have termed a "permafrost thaw continuum". This continuum consists of a permafrost thaw seep (Figure 2.1) in the nearby foothills that begins to flow mid-July downstream into Skeleton Lake (maximum depth of 4 m). Skeleton Lake then drains into a series of two small and shallow ponds to the east (Keatley et al., 2008), here referred to as "post ponds", which then flow into a wetland complex before flowing

through Skeleton Creek into Lake Hazen. Skeleton Lake and the two downstream systems are completely ice free from early July to late August. Table A1 summarizes the sites sampled, their locations and date accessed.

2.2.1a Sample sites

For microbial community and physicochemical analysis, we sampled four of the eleven glacial inflows into Lake Hazen, the permafrost thaw continuum, and Lake Hazen itself, including its outflow to the Ruggles River, between the summer season of July 2016 and spring 2017. Our sampling scheme encompassed melt/thaw dynamics over the course of a melt season to assess how glacial rivers and permafrost thaw contributed to the physicochemistry and microbial community structuring of Lake Hazen. Over the course of the spring season in May-June 2017, we then assessed how the microbial community and physicochemistry of Lake Hazen and the permafrost thaw lake, Skeleton lake, changed over the winter and spring months during absence of hydrological inputs and the sustained presence of ice and snow cover.

2.2.1b Sampling of glacial inflows, snow, and snowmelt

Over a seven-week period in July-August 2016 that encompassed the onset of initial glacial melt, the height of melt and the slowing of the melt season, we sampled Snowgoose and Blister glacial rivers weekly at their mouths to assess temporal changes in microbial community structure and physicochemical parameters. Despite the fact that these two glacial inputs into Lake Hazen were not the major glacial inputs into the lake, they were sampled frequently due to their ease of access from base camp. We also accessed the Henrietta Nesmith and Gilman glacial rivers, the two major inputs, by helicopter twice in 2016, collecting samples that coincided with the peak of glacial melt and the subsequent slowing flow, to assess whether there were significant microbial

community and physicochemical differences from the Snowgoose and Blister rivers. In May 2017, composite snowpack samples were obtained in triplicate along parallel transects spanning the frozen Lake Hazen surface and the nearby landscape parallel to Lake Hazen (Figure 2.1). Corresponding snowmelt samples were obtained in June 2017 at the very start of melt, from Blister Creek, the Abbé River, and Skeleton Creek.

2.2.1c Sampling of the Skeleton permafrost thaw continuum

Over the same seven-week period between July and August 2016, we also sampled five sites within the permafrost thaw continuum on a weekly basis (Figure 2.1). Similar to glacial melt sampling, our sampling campaign in the permafrost thaw continuum coincided with the onset of permafrost thaw water flow, and the height of thaw. To this end, we obtained five seep water samples once permafrost had begun thawing, and two wetland and creek samples once enough thaw water was present to allow discharge of the permafrost thaw continuum to Lake Hazen. During May 2017, we also obtained water column samples from Skeleton Lake while it was completely ice covered to assess seasonal differences in microbial community composition and associated physicochemical parameters. Water samples here were taken at depths of 1.5 m (below the ice) and 4 m (bottom waters).

2.2.1d Sampling of Lake Hazen

To assess the contribution of glacial and permafrost thaw inflows to the microbial community and physicochemical makeup of Lake Hazen shoreline waters, we sampled near the Lake Hazen shoreline weekly in 2016, over the same period that we sampled the nearby Snowgoose and Blister rivers and the permafrost continuum. We also obtained samples from 1 m, 15 m, and 250 m depths at the deepest spot in Lake Hazen on August 9, 2016. Due to inclement weather,

more depths could not be safely sampled at that time. To determine how the microbial community changed over the winter and spring months within the Lake Hazen water column, we sampled in late May 2017 at the same site as our summer water column samples. Here, we sampled depths immediately below the surface of the ice, at 5 m, 10 m, 15 m, 25 m, 50 m, 100 m, 200 m, 235 m, 240 m, 245 m, and 250 m. Additionally, samples were obtained from the Ruggles River just as it exited Lake Hazen during both summer seasons (July 11 and August 2, 2016) and spring (June 3, 2017). These were combined into the Lake Hazen summer and spring samples, respectively, given the physicochemical and microbiological similarity between this outflow and the Lake itself.

2.2.2 Sampling for microbial and physicochemical analysis

Snow samples for microbial analyses were collected in duplicate using a clean stainless-steel snow corer, immediately placed into sterile 5 L Whirl-Pak Giant-Size sample bags and maintained at -20°C until further processing. Snow samples for water chemistry analyses were collected the same way, except that they were stored in large freezer bags. For microbial community analyses in flowing and standing waters, 600 mL of water were collected into Whirl-Pak Thio-Bag bagsBags™ specialized for microbial water sampling, in triplicate, with the thiosulfate tablet aseptically removed. Bags were rinsed three times with site water prior to being filled. Samples were returned to the Lake Hazen/Quttinirpaaq Field Laboratory clean room for further processing within a few hours of collection.

Microorganisms and associated sediment particles from water samples (including melted snow) were isolated onto a 0.2 µm filter membrane inserted into a glass vacuum filter holder and attached to a Büchner flask and filter pump (25 psi). Filters were immediately preserved in 1 mL

of RNA*later* (Fisher Scientific) in sterile Eppendorf tubes, and kept frozen at -20°C until subsequent DNA extraction could be completed.

At each water sampling site, we used an EXO2 multi-parametric sonde (YSI incorporated, Yellow Springs, OH, USA) to measure turbidity, dissolved oxygen concentration, pH, temperature, and electrical conductivity. Additional water samples were collected into pre-cleaned HDPE bottles for analyses of general chemical parameters including particulate nitrogen (PN), nitrate and nitrite (NO_3^- and NO_2^-), alkalinity, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved solids (TDS), major ions, and trace metals such as dissolved iron (Fe). Upon return to the base camp, these samples were immediately processed in the Field Laboratory clean room using established clean chemistry protocols. Processed samples were subsequently maintained in the dark at ~5°C or frozen until they were analyzed at the Canadian Association of Laboratory Accreditation-certified Biogeochemical Analytical Service Laboratory (BASL, University of Alberta, AB) using standard analytical protocols.

2.2.3 Processing of samples for microbial analyses

Microbial DNA was extracted from duplicate filters from all sampled sites using the DNeasy PowerSoil kit (Qiagen). The extraction procedure followed that recommended by the manufacturer, except for three modifications: 1) prior to DNA extraction, excess RNA*later* was removed from the filters via centrifugation at 10,000 *g* for 10 s, because RNA*later* may impede the efficiency of DNA extraction from environmental samples (McCarthy et al., 2015); 2) filters were unwrapped and cut with flame-sterilized tweezers and scissors prior to being placed inside extraction tubes to maximize the filter surface area exposed to sterile beads during a bead beating step, and 3) prior to mechanical lysis using bead beating, the prepared samples were chemically lysed by incubation at 70 °C for 10 min in the provided lysis solution to maximize total DNA

yield. The amount of isolated DNA from each sample was determined using a Qubit fluorometer and ranged between 1-100 ng, at concentrations of 0.046 to 14.22 ng/µl to serve as a template for PCR.

2.2.4 Sequencing and computational analyses

The 16S ribosomal RNA genes (16S rRNA genes) were amplified from randomized samples using universal prokaryotic primers 515F-Y (Parada et al., 2016) and 926R (Quince et al., 2011). Each primer also contained a six base index sequence for sample multiplexing as well as Illumina flow cell binding and sequencing sites (Bartram et al., 2011). The PCR mix (25 µl total volume) contained 1X ThermoPol Buffer buffer, 0.2 µM forward primer, 0.2 µM reverse primer, 200 µM dNTPs, 15 µg bovine serum albumin (BSA), 0.625 U Hot Start *Taq* Microbial DNA polymerase (New England Biolabs), and 1 µl of template. Each PCR was prepared in triplicate. The PCR was performed as follows: 95°C for 3 min, 35 cycles of 95°C for 30 sec, 50°C for 30 sec, 68°C for 1 min, and a final extension of 68°C for 7 min. No-template controls (NTCs), field blanks, and extraction kit controls were processed along with samples and included for sequencing even if no PCR fragment was detected. Pooled 16S rRNA gene amplicons were excised from an agarose gel and purified using Wizard SV Gel and PCR Clean-Up System (Promega, WI, USA). A 4.5 pM library containing 15% PhiX Control v3 (Illumina Canada Inc, NB, Canada) was sequenced on a MiSeq (Illumina Inc, CA, USA) using a 2 × 250 cycle MiSeq Reagent Kit v2 (Illumina Canada Inc, Canada). The MiSeq reads were demultiplexed using Illumina MiSeq Reporter software version 2.5.0.5. Each read pair was assembled using the paired-end assembler for Illumina sequences (PANDAseq, Masella et al., 2012) with a quality threshold of 0.9. and assembled reads were analyzed using QIIME (Caporaso et al., 2010) managed by automated exploration of microbial diversity (AXIOME) version 1.5 pipeline

(Lynch et al., 2013). Sequences were clustered with UPARSE algorithm from USEARCH version 7.0.1090 (Edgar, 2013) and aligned with Python Nearest Alignment Space Termination tool (PyNAST version 1.2.2; Caporaso et al., 2010). All representative sequences were classified using the Ribosomal Database Project (RDP) (Wang et al., 2007) with a stringent confidence threshold of 0.8 and the Greengenes reference database (McDonald et al., 2012). For some analyses, reads were rarefied to the lowest read count of 10,109. The resulting OTU dataset was used for assessing alpha diversity, defined as diversity within a particular site, and indicator species analyses, using functions within Vegan (Oksanen et al., 2018), phyloseq (McMurdie and Holmes, 2014), and the labdsv packages (Roberts, 2016) in R (R core team, 2015). Here, the definition of indicator species (i.e., OTUs) were those microorganisms found only at certain sites or seasonal conditions with high fidelity and relative abundance (Dufrene and Legendre, 1997). In practice, indicator species were OTUs with an indicator value of ≥ 0.7, and an associated p-value less than or equal to 0.05. The Shannon index was chosen as the primary measure of alpha diversity in this study because it considers the effect of rare species in the dataset while incorporating both evenness and richness into its interpretations (Hill et al., 2006). Venn diagrams to visually assess the number of unique and shared OTUs between glacial rivers, Lake Hazen during the spring, and Lake Hazen during the summer were produced using the VennDiagram package in R (Chen and Boutros, 2011).

Beta diversity, or the diversity between sites, of the different freshwater compartments sampled throughout the watershed was demonstrated using NMDS plots of OTU data converted to a Bray-Curtis dissimilarity matrix, plotted in SigmaPlot (Systat Software, San Jose, CA). The significance of observed site clusters within the ordination were calculated using global

ADONIS (permutational multivariate analysis of variance), pairwise PERMANOVA tests (using *p* values corrected for multiple comparisons), and Betadisper functions in Vegan.

Principal component analyses (PCAs) of specific physicochemical data (e.g., those that were at or above the detection limit or had values for all sites involved in the study) were analyzed using the FactoMineR and factoextra packages (Le et al., 2008; Kassambara and Mundt, 2017) and then visualized in SigmaPlot (Systat Software, San Jose, CA). Environmental data were centered and standardized prior to the implementation of these analyses. Correlations of overall and indicator microbial community composition with physicochemical data for each freshwater compartment in the watershed were conducted in R using BIOENV functions within the Vegan package. Euclidean distances were calculated for environmental variables, whereas OTU data were converted to a Bray-Curtis dissimilarity matrix. Db-RDAs were conducted using Canoco 5 (Šmilauer and Lepš, 2014) to assess the extent to which BIOENV-identified physicochemical characteristics could explain microbial community variability within the three basic freshwater compartments of the Lake Hazen watershed. Relative abundances of the top ten taxonomic members of microbial communities found throughout freshwater compartments within the watershed were visualized using PhyloSeq (McMurdie & Holmes, 2014).

2.3 RESULTS AND DISCUSSION

2.3.1 Physicochemical characteristics of three basic freshwater compartments within the Lake Hazen watershed

The measured physicochemical parameters of the three investigated compartments of the Lake Hazen watershed (i.e., glacial rivers, the permafrost continuum, and Lake Hazen) are shown in Table 2.1 and were visually assessed using PCA biplots (Figure 2.2). Here, three distinct

physicochemical groupings are evident, each demonstrating unique environmental conditions, based on 95% confidence intervals: 1) the permafrost thaw continuum, 2) glacial rivers and Lake Hazen during the summer, and 3) Lake Hazen during the spring (Figure 2.2A). Further, the physicochemical distinctiveness of the sub-compartments within each of the three basic watershed compartments were also explored (Figure 2.2B & 2.2C). Based on the arrangement of the samples in two-dimensional space, eight distinct site groupings were identified based on their physicochemical properties: 1) glacial rivers, the Lake Hazen summer depths and the Lake Hazen shore, 2) the Lake Hazen water column in the spring, 3) the permafrost thaw seep, 4) Skeleton Lake in the summer, 5) the two ponds just downstream, 6) the wetland, 7) Skeleton Creek, and 8) Skeleton Lake in the spring. Whereas we obtained snow and snowmelt samples, reflecting two other freshwater compartments within the Lake Hazen watershed, the numbers of samples for each of these compartments were low and we were not able to collect similar physicochemical properties (e.g., turbidity, dissolved oxygen, dissolved inorganic carbon (DIC), and so we did not include these samples in analyses here, but their physicochemical properties are described in Table 2.1.

In the biplot consisting of glacial rivers and Lake Hazen, parameters such as calcium (Ca^{2+}), sulfate (SO_4^{2-}), and nitrate content (NO_2^- &NO_3^-), primarily derived from the weathering of bedrock as glacial rivers flow downstream, contributed most to the first component axis (Table A2) whereas DIC, particulate nitrogen (PN) and temperature mostly contributed most to the second component axis (Table A3). Glacial inflows to Lake Hazen varied spatially and temporally in their physicochemical parameters (Figure 2.2B). Overall, the physicochemical parameters (Table 2.1) describing the Lake Hazen shoreline waters and glacial rivers were similar (Figure 2.2B). This signifies that the physicochemistry of waters along the Lake Hazen

shoreline were heavily influenced by glacial river inputs, especially during peak melt. Samples collected from the Lake Hazen water column during the summer also grouped with glacial river inflow samples (Figure 2.2B), probably because dense and turbid glacial river water rapidly permeated the depths of the lake via turbidity currents. With the formation of a ~2 m thick ice cover on the lake over the winter and spring months, the water column exhibited reverse temperature stratification and in the absence of glacial melt and permafrost thaw inputs to the lake, dissolved oxygen declined with depth, reflecting heterotrophic processes, resulting in a distinct environment within the Lake Hazen spring water-column (Figure 2.2B).

A separate ordination biplot analysis was completed for sites within the permafrost thaw continuum (Table 2.1). In contrast to what was observed for glacially derived freshwaters in the watershed, these biplots revealed entirely distinct environmental profiles corresponding to each sub-compartment making up the permafrost thaw continuum (Figure 2.2C). Sodium (Na^+), potassium (K^+), and Ca^{2+}, as well as sampling date, loaded highest on the first axis, whereas DIC, dissolved organic carbon (DOC), and total dissolved solids (TDS) concentrations loaded highest on the second axis (Table A4). Within the continuum, the seep is physicochemically distinct because it is likely a main originator of nutrients such as phosphates and nitrates, while being poor in DIC and DOC, relative to downstream freshwater compartments (Table 2.1). The lack of physicochemical similarity between Skeleton Lake in the summer and its neighboring downstream ponds and the downstream wetland and creek, despite their direct hydrological link signifies unique biogeochemical processes occurring within each compartment. In the winter and spring, the physicochemistry of Skeleton Lake becomes distinct from that in summer (Figure 2.2C), presumably driven by the complete lack of hydrological inputs from the seep, combined with ice cover restricting atmospheric exchange and high rates of biological oxygen consumption

in the water column and sediments. Na^+ and K^+ in particular may primarily originate from groundwater seepage and/or from thaw waters bringing in these major ions left over from snow sublimation. The relatively low concentrations of these ions in Skeleton Lake during the spring (Table 2.1) may thus reflect the absence of groundwater seepage or permafrost thaw.

2.3.2 Microbial communities of the Lake Hazen watershed

We identified five significantly distinct microbial communities associated with snow, snowmelt, glacial rivers, Lake Hazen, and the permafrost thaw continuum, as demonstrated by the five discrete 95% confidence intervals shown in Figure 2.3A. Discrete Lake Hazen microbial communities occurred according to season, rather than location (e.g., lake water column, mouth of the Ruggles River, shoreline), where summer communities were similar to one another regardless of sample location. Lake Hazen spring microbial communities were significantly different from summer samples ($p < 0.05$, non-parametric PERMANOVA); however, water column microbial communities were highly similar to each other, irrespective of depth (Figure 2.3B). Glacial rivers and the Lake Hazen shoreline microbial communities displayed relatively strong inter-sample interconnectivity (Figure A1), but there were distinct community-level differences between the microbiomes of glacial rivers and the Lake Hazen water column (Figure 2.3B), suggesting that differences in the abundances of specific taxa are what drives the observed community-level variation (Sheik et al., 2015).

Lake Hazen and the various glacial rivers flowing into it were comprised of microbial communities dominated by taxonomic members related to phyla Proteobacteria, Bacteroidetes, and Actinobacteria (Figure 2.4B), like other studies of glacially-sourced freshwaters (Sheik et al., 2015; Hauptmann et al., 2016).

Microbial taxa in the glacial rivers varied neither temporally nor between the different rivers (Figure 2.4B), similar to results from a glacial system in Southern Alaska (Sheik et al., 2015). To further explore microbial community composition, we undertook an indicator species analysis to identify taxa uniquely affiliated with snow, snowmelt, and river samples (Figure 2.5). Glacial river indicator taxa included members from the phyla Bacteroidetes, Verrucomicrobia, and Proteobacteria, all of which are common polar soil microorganisms (Ganzert, et al., 2014). Communities associated with snow and snowmelt contained the largest proportion of unique OTUs assigned to these sites (Figure 2.5), reflecting a unique composition of microbes sourced from the surrounding terrestrial environment and from aeolian deposition (Harding et al., 2011).

Waters along the Lake Hazen shoreline contained distinct and abundant microbial populations, with proportionally higher levels of Elusimicrobia and Chloroflexi (Figure 2.4B) compared to glacial rivers. The proportion of these taxa increased over the winter in the spring Lake Hazen water column samples, reflecting a shift in the microbiome of Lake Hazen in the absence of glacial melt and permafrost thaw inputs. This stable springtime Lake Hazen microbial community assemblage likely reflects a balance between microorganisms sourced from glacial runoff and permafrost thaw streams the previous summer and those that are selected against in the water column once those inputs cease. Indeed, our indicator species analysis suggests that spring Lake Hazen, regardless of depth, were associated with distinct microbial community indicators (Figure 2.5). These indicator microorganisms were dominated by taxa associated with Chloroflexi, Elusimicrobia, and Proteobacteria (Figure 2.5).

We also found discrete microbial communities belonging to Skeleton Lake during spring and summer, respectively (Figure 2.3C). In contrast, the microbial communities belonging to the seep, post ponds, wetland, and creek shared statistically similar ($p > 0.05$, non-parametric

PERMANOVA) community compositions (Figure 2.3C). Communities from the seep, post ponds, creek, and wetland were taxonomically similar despite the physical distance between sites dominated by microorganisms from phyla Proteobacteria, Bacteroidetes and Verrucomicrobia (Figure 2.4C). There were no indicator species among the sub-compartments of the permafrost thaw continuum (Figure 2.5), further suggesting a high degree of taxonomic interconnectivity with the rest of the freshwaters (the three basic freshwater compartments including snow and snowmelt) within the Lake Hazen watershed. Both hydrologically and microbiologically, the creek draining the continuum into Lake Hazen contributes insignificantly to the overall Lake Hazen microbiota.

The microbiome of Skeleton Lake in the summer contained distinct taxonomic assemblages not observed in Skeleton Lake during spring, or in other downstream compartments of the continuum (Figure 2.4D). Like Lake Hazen, this smaller permafrost thaw-fed lake contained unique lake microbial assemblages, including lineages putatively related to *Deinococcus-Thermus* that are also commonly found in other polar freshwaters (Moller et al., 2013), as well as various photosynthetic microorganisms that do not appear to be exported downstream, or are unable to survive in these downstream sub-compartments (Figure 2.4D).

2.3.3 Correlational analysis between microbial communities and respective physicochemical parameters

Although ordinations are powerful tools for visualizing differences in physicochemistry and microbial community structures among sites, neither informs how each is influenced by the other. Hence, we used BIOENV analysis to first determine the best set of physicochemical parameters that maximally correlated with the microbial community structure from each of the

freshwater compartments we sampled. Secondly, we used db-RDAs to determine the extent to which these parameters explained microbial community variation.

Microbial community composition was significantly correlated ($p < 0.05$) to specific physicochemical characteristics of the sites. Glacial river microbial community structure was best correlated to oxygen, major ion concentrations, and temperature (Table 2.2). These select physicochemical parameters explained 37.1% of the total community variation (Table 2.2). These components are implicated in influencing microbial community structure within glacial rivers, and variability in these parameters may reflect river discharge and the subsequent weathering of surrounding sediments, rather than specific constraints on microbial metabolic potential. Weathering across the proglacial zone increases the loads of erosional material, SO_4^{2-}, and major cations, as well as microbial community members that are also incorporated into river waters (Hauptmann et al., 2016; Zarsky et al., 2018). The amount and nature of sediment/soil-associated microbial assemblages eroding into rivers may then influence local microbial diversity within these waters. This is supported by our analysis of local diversity in glacial rivers and Lake Hazen, where alpha diversity of microbial communities was significantly higher ($p<0.05$, Wilcoxon rank sum test, Bonferroni-Hochberg corrected) in glacial rivers than in Lake Hazen (Figure 2.6A). The rate at which taxa are incorporated into glacial rivers from the melting glaciers themselves and erosional materials downstream is likely greater than the rate at which local environmental sorting can take place due to shorter water residence times in these fast-moving rivers (i.e., the mass effects paradigm; Crump et al., 2004 & 2012). Once glacial river microorganisms are deposited into Lake Hazen, selection along environmental gradients can occur over a longer time scale (Peter and Sommaruga, 2016), where water residence time in Lake Hazen is often up to 90 years (Lehnherr et al., 2018). Using Venn diagrams to visually

display richness and the number of shared OTUs between sites, glacial rivers appear to contain many more unique OTUs not present in downstream Lake Hazen, whose overall richness decreased further during the spring (Figure A2). Because taxonomic composition and alpha diversity of glacial river microbial communities were relatively stable over the course of the melt season, we hypothesized that glacial rivers are simply passive conduits for microorganisms that then seed downstream systems where they are subsequently selectively sorted (Hauptmann et al., 2016, Zarsky et al., 2018; Cameron et al, 2017) (Figure A1 & 2.7).

The spring Lake Hazen microbial communities were significantly correlated with turbidity, Ca^{2+}, SO_4^{2-}, and PN, along with temperature, (Table 2.2) which are characteristic of glacial inputs and mineral weathering (Wadham et al., 2010; Vargas et al., 2017). Association of increased concentrations of some of these chemical components (such as SO_4^{2-}) immediately under ice cover, settling out of residual glacial river-derived physicochemistry and continued weathering over winter and spring months thus serve to structure the composition of microbial communities along physicochemical gradients. These parameters were strongly correlated with community structure but only explained 34.3% of community variability (Table 2.2), suggesting strong environmental and species selection likely driven by stratification of the lake over the winter. In contrast, the summer Lake Hazen microbial communities were best correlated with temperature, DIC, turbidity and TP, components that vary strongly with glacial input and seasonality (Table 2.2). These parameters only explained 18.1% of total community variation (Table 2.2), suggesting that other processes occurring primarily along the shoreline of Lake Hazen select for specific summer microbial community members. It has been previously suggested that microbial communities, especially those immediately downstream of glacial melt input, are shaped by turbidity, which can modify both water temperature and light penetration (Peter and Sommaruga,

2016). Microbial communities in Lake Hazen may be predominately structured along a spatio-temporal turbidity gradient. Interestingly, the Lake Hazen shoreline indicator species included *Polaromonas*, which were otherwise only found in turbid waters within the glacially fed portions of Lake Hazen.

Indicator microorganisms in the spring and summer Lake Hazen water column were strongly correlated with similar glacially-associated physicochemical parameters that exhibited strong seasonality. These conditions thus produced seasonally-specialized communities (Table 2.3). Additionally, even though the Lake Hazen shoreline and summer water column were heavily impacted by the high influx of glacial river-sourced microorganisms, sediments and other physicochemical parameters, the Lake Hazen shoreline microbial community itself appeared to be relatively resilient towards these inputs and is thus likely composed of microorganisms specialized for the shoreline environment (Figure 2.4B). We therefore suggest that species sorting may initially begin along the shores of Lake Hazen, which acts as a transition zone for incoming glacial inflows, prior to dispersion throughout the remainder of the lake and its depths (Monard et al., 2016). Through the subsequent winter and spring, microorganisms unsuited to the physicochemical environment in Lake Hazen are selected against, and phyla such as Elusimicrobia and Chloroflexi, only minor components of glacial input, emerge amongst the top three taxa (Figure 2.4B). Whereas very little has been documented regarding the ecological role of microorganisms in the phylum Elusimicrobia, microorganisms in the phylum Chloroflexi are capable of anoxygenic photosynthesis, nitrogen transformation, and the biological transformation of nitrogen rich DOM (Newton et al., 2011; Denef et al., 2016), which may be important for the overall water quality of Lake Hazen. These microorganisms are often found nearly exclusively in deep stratified lakes, similar to Lake Hazen (Newton et al., 2011).

Overall, microorganisms exported from glacial rivers have the potential to destabilize resident community structuring in Lake Hazen, but selective forces within the lake appear to be enforced on them due to the comparatively long water residence time within the lake. Hence, there is likely a strong taxonomic, and by extension, functional resiliency of the Lake Hazen microbiome, as was also found for the microbial communities characterizing the bottom sediments of this lake (Ruuskanen et al., 2018). Indeed, the biologically active components of the Lake Hazen bottom sediments were comprised of similarly dominating phyla as observed for the watercolumn (Ruuskanen et al., 2018), suggesting that glacially-sourced microbial species surviving the environmental sorting process within the Lake Hazen watercolumn eventually deposit, populate and subsequently regulate biogeochemical processes occurring within the lake sediments. Despite the rapid environmental changes that this lake has undergone in response to recent climate warming (Lehnherr et al., 2018), it is possible that the overall microbiology of the lake itself has remained relatively stable over time, despite greater inputs from both glacial melt, and to a lesser extent, permafrost thaw. Conceivably then, microbially-driven biogeochemical processes have likewise remained relatively stable over time. Coupled with metatranscriptomic studies, long-term surveys of lake microbiology over both finer and longer-term timescales will be able to resolve this hypothesis further for better future predictions.

With the exception of Skeleton Lake in spring under ice, the freshwater compartments in the permafrost thaw continuum were weakly, albeit significantly, correlated with Na^+, K^+, and DOC (Table 2.2), the concentrations of which generally increased as water moved downstream of the continuum, where elevations in sodium and potassium levels may be explained by evaporative processes. This permafrost continuum was characterized by uniformly low amounts of total phosphorous and more biologically important, phosphate, as in other permafrost-sourced

freshwaters (Burpee et al., 2015). This lack of readily available phosphate has been implicated in shifting to the use of DOC in these environments for microbial respiration (Burpee et al., 2015) rather than microbial reproduction, which produces greenhouse gases like carbon dioxide and methane. As such, the permafrost thaw continuum and the microbial communities found therein may be more sensitive than Lake Hazen to further warming, and indeed, both Skeleton Lake and its adjacent post ponds have been determined to be sources of methane (Emmerton et al., 2016). DOC and Na^+ concentrations were the sole physicochemical parameters that were found to correlate with overall microbial community structure throughout the permafrost thaw continuum. These parameters explained only 27.6% of overall community variability among the sites (Table 2.2).

While water residence times in the permafrost thaw continuum are longer than in the glacial rivers, other key ecological factors not encompassed by the physicochemical parameters assessed, such as the presence of wetland vegetation, high productivity (Keatley et al., 2008), and evaporation in these shallower systems, are likely also drivers of microbial community composition. Microbial community structuring throughout the various compartments of the permafrost thaw continuum generally did not mirror the distribution of physicochemical parameters observed in the corresponding bi-plots, although microorganisms are often closely tied to the associated redox potential and physicochemistry of individual sites (Ruuskanen et al., 2018). This is particularly evident for Skeleton Lake microbial communities in the spring and summer. General increases in alpha diversity in sites sequentially downstream (Figure 2.6B) from Skeleton Lake during the summer months is likely due to the fact that Skeleton Lake presents a uniquely selective environment, characterized by high microbial predation levels by grazing zooplankton, biofilm formation in the shallows, and increased microbial competition.

Indeed, many members of the microbial community that develop in Skeleton Lake during the summer are likely unsuited to downstream environments if they get transferred there (Hauptmann et al, 2016). Microorganisms surviving the selection process downstream are predominantly structured according to the nutrient and water retention capacity of the wetlands that form along Skeleton Creek (Knox et al., 2008; Evans et al., 2017).

During the spring, Skeleton Lake communities became more taxonomically similar, but structurally different, to the rest of the communities found throughout the rest of the permafrost thaw continuum during the summer (Figure 2.4C&D). Although the physicochemistry of Skeleton Lake during the spring is clearly distinct (Figure 2.2C), the reduction in eukaryotic predators, ice cover and subsequent consumption of oxygen in both the water column and sediments (Wisniewski et al., in preparation) likely mediate stronger selective forces on microbial community composition and structure relative to summertime conditions. Selective environmental conditions during the spring then result in low levels of alpha diversity (Figure 2.6B) in Skeleton Lake, most notably leading to a marked decrease in photosynthetic microorganisms (Figure 2.4D), similar to a diatom study of the same site conducted by Keatley et al., (2008). Differences in the presence of photosynthetic microorganisms within watercolumn microbial communities between spring and summer conditions here also follow the same differences observed in the microbial assemblages populating the sediments of this lake (Ruuskanen et al., 2017). Here, distinct losses in the proportions of photosynthetic microorganisms, such as those belonging to cyanobacteria, were evident in Skeleton lake during the spring, replaced by more heterotrophic taxa such as Bacteroidetes and Proteobacteria. Communities within this particular sub-catchment within the Lake Hazen watershed are likely structured according to the overall effects of evaporative processes (Emmerton et al., 2016),

competition and predation amongst microbial assemblages unique to lake, ponds, and wetlands fed by permafrost thaw.

To summarize, we have identified distinct microbial communities in each of the three main freshwater compartments found in the Lake Hazen watershed (i.e., glacial rivers, a permafrost thaw continuum, and Lake Hazen). These distinct microbial communities were not always strongly influenced by the physicochemical parameters of the sites in which they were found. Glacial river microbial communities appear to be structured most strongly by mass effect dynamics, whereby the rate at which taxa entering these high-flow rivers from glaciers and sediment/soil erosion is likely greater than the rate at which local environmental sorting can take place (Nino-Garcia et al., 2016). The microbial communities that get exported to Lake Hazen then likely undergo selective species sorting, after which a more specialized and stable long-term Lake Hazen microbial community persists over subsequent winter and spring months.

In contrast, permafrost continuum microbial communities were found to be only weakly influenced by the physicochemical parameters we measured. Communities here are likely thus influenced more by competitive interactions and strong evaporative processes that impact shallower bodies of water (Granger and Hedstrom, 2010), suggesting that this smaller, yet still important freshwater system may be more vulnerable to climate change, with corresponding fluctuations in greenhouse gas cycling and other important biogeochemical cycling (Emmerton et al., 2016). Overall, the microbial communities that get exported to Lake Hazen likely undergo selective species sorting, after which a more specialized and stable long-term Lake Hazen microbial community persists. However, given the ten-fold increase in glacial melt that the Lake Hazen watershed has experienced and the subsequent reduction in water residence time of up to

70% (Lehnherr et al., 2018), the stability of the Lake Hazen microbiome and consequent ecological processes are uncertain.

2.4 FIGURES AND TABLES

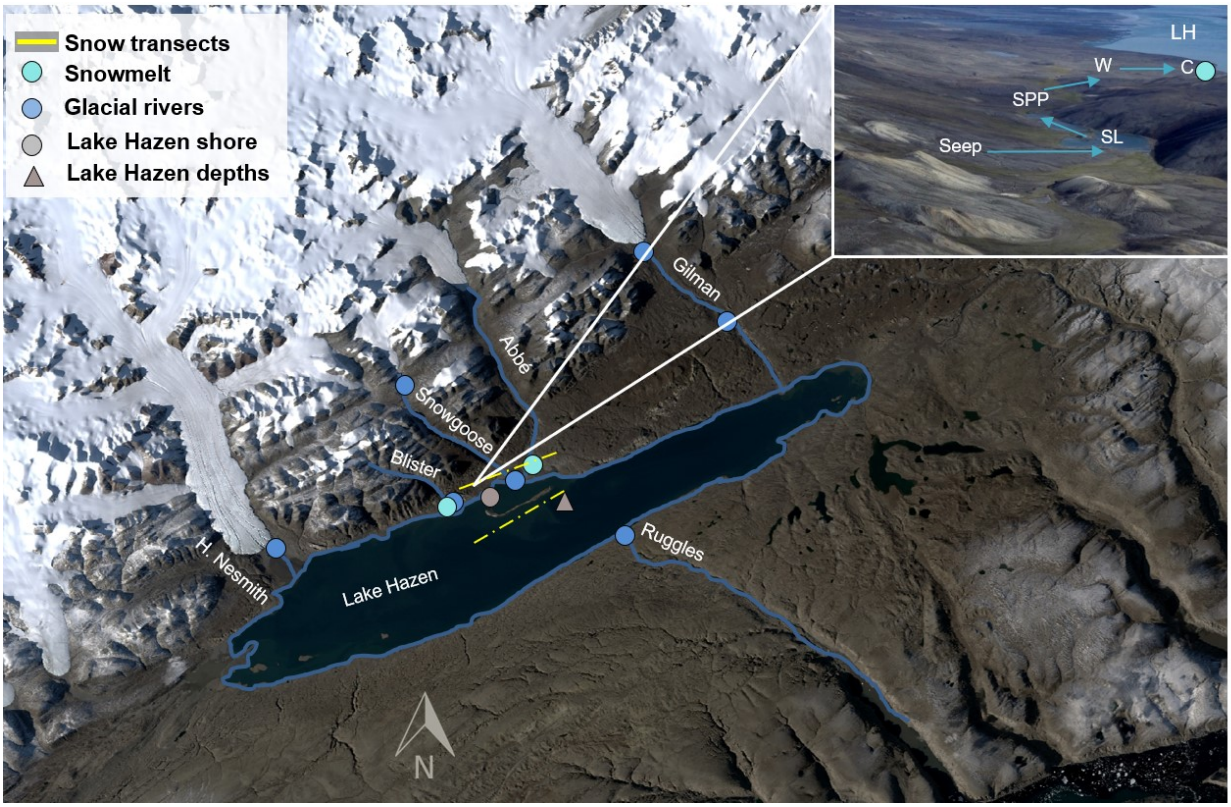


Figure 2.1. Map of the Lake Hazen watershed located on northern Ellesmere Island, Nunavut, delineating sample sites and sample type during field seasons spanning July-August 2016 and May 2017. Yellow lines delineate parallel transects over which snow samples were collected during May 2017, while light blue nodes depict snowmelt samples obtained over the same 2017 season. Dark blue nodes depict glacial river sampling sites (rivers which are delineated in blue and labelled) over the summer melt season spanning July-August 2016 and some of July 2017. H. Nesmith: Henrietta Nesmith river, Blister: Blister Creek, Snowgoose: Snowgoose River. Lake Hazen shoreline samples were obtained as represented by the grey node and water column depths were obtained from Lake Hazen as represented by the grey triangle. Inset shows the location of the permafrost thaw continuum and the various sub-compartments found within. Arrows demonstrate the sequential flow of water between the sub-compartments of the permafrost thaw continuum. Seep: Skeleton seep, SL: Skeleton Lake, SPP; Skeleton Post Ponds, W: wetlands, C: Skeleton Creek, LH: Lake Hazen.

Table 2.1. Relevant physicochemical parameters measured for various freshwater sub-compartment within the Lake Hazen watershed. Although parameters were measured on a weekly basis for sites accessed during the summer, the measurements have been averaged to reflect conditions within each freshwater compartment (Lake Hazen summer samples,(including the shoreline, summer depths and outflow to the Ruggles) Lake Hazen spring samples (spring depths and outflow to Ruggles), river samples and the permafrost thaw continuum samples (seep, SL, spring: Skeleton Lake during the spring, SL, summer: Skeleton Lake during the summer, post ponds, wetland, and creek) as a whole. Standard error is shown. Parameters measured include: Temperature, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), sulfate, turbidity, oxygen, total dissolved solutes (TDS), nitrate and nitrite, particulate nitrogen (PN), total phosphorous (TP), sodium and potassium, calcium, and iron. < D.L: below detection limit of 0.002 mg/L. NA: not enough sample for analysis.

Site	Temp ºC	DIC mg-C/L	DOC mg/L	SO_4^{2-} mg/L	Turbidity FNU	O_2 mg/L	TDS mg/L	NO_2^-+NO_3^- mg/L	PN mg/L	TP mg/L	Na^+& K^+ mg/L	Ca^{2+} mg/L	Fe mg/L
Hazen, spring	2.75 ±0.29	8.14 ±0.19	0.576 ±0.02	11.4±0.21	0.710 ± 0.06	9.50 ± 1.4	60.7 ±3.7	0.220 ± 0.009	0.150±0.004	0.13±0.004	0.94±0.02	21.98±0.26	0.15±0.017
Hazen, summer	4.34±0.59	8.00 ±1.1	0.610 ±0.16	9.87 ± 1.8	10.36 ± 6.7	12.4 ± 14	44.8 ±8.9	0.130 ± 0.01	0.250 ± 0.04	0.14± 0.01	1.31±0.04	13.99±1.71	0.35±0.037
Minor rivers	6.28 ± 1.3	4.20 ±0.75	0.610±0.016	23.1 ± 4.9	475 ± 281	12.0±0.27	63.40±10	0.250 ± 0.02	0.48 ± 0.08	0.36± .010	0.93±0.10	8.07± 2.55	0.27± 0.01
Major rivers	1.88± 0.50	4.20 ±0.60	0.61 ± 0.04	6.21 ± 1.2	64.3 ± 18	14.0±0.15	28.7 ±4.7	0.167 ± 0.014	0.38 ± 0.100	0.36±0.060	0.93±0.24	8.07± 1.44	0.27±0.002
Seep	6.88 ±0.32	1.17±0.61	0.636 ±0.11	132 ±30	15.2 ± 7.5	11.1 0.19	221 ±31	0.027 ± 0.007	0.089±0.004	0.402±0.40	3.86±1.01	49.2 ±8.1	0.021±0.002
SL, spring	1.65 ±0.06	1.52 ±0.28	8.18 ±0.090	NA	1.75± 0.33	2.61±0.63	822 ±12	0.007 ± 0.005	0.034±0.006	0.0065±0.0003	0.01±0.0	130 ±0.48	NA
SL, summer	14.0 ±0.87	1.66 ±0.53	2.16 ±1.0	270 ± 4.6	8.04 ± 2.7	10.9±0.29	444 ±3.7	< D.L	00.048±0.007	0.003±0.0003	9.18±0.45	88.6 ± 0.17	0.0088±0.0008
Post ponds	12.8 ± 1.8	1.81 ±0.45	4.80 ± 0.40	246 ± 2.6	13.6 ± 6.6	6.52±1.93	414 ±5.0	< D.L	0.359 ±0.17	0.0083 ±0.003	7.94±0.075	85.5 ± 0.75	0.0088±0.0060
Wetland	8.38 ±1.2	2.44 ±1.2	4.85 ±0.75	257 ±7.0	0.640 ±0.16	11.4±0.58	501 ±8.8	< D.L	0.0051±0.003	0.005 ±0.0005	7.57±0.39	105 ±0.13	0.107 ±0.078
Creek	9.74 ±4.2	2.46 ±1.3	5.70 ±0.10	272 ±5.0	0.700 ±0.00	11.18±1.1	505 ±15	< D.L	0.77 ±0.76	0.005 ±0.0005	8.21±0.08	112 ±2.8	0.0087±0.0002
Snow	< 0	NA	0.513 ± 0.05	1.82 ± 0.48	NA	NA	21.5 ±3.2	0.0406 ± 0.003	0.188 ± 0.05	0.170 ± 0.04	0.032±0.01	11.0 ± 3.1	1.77 ± 0.50
Snowmelt	0.069±0.4	7.00 ±0.90	4.80 ± 3.7	14.9 ± 2.3	1.80±0.25	1.14±0.02	258 ± 153	0.0985 ± 0.002	0.161 ± 0.04	0.030± 0.02	NA	NA	NA

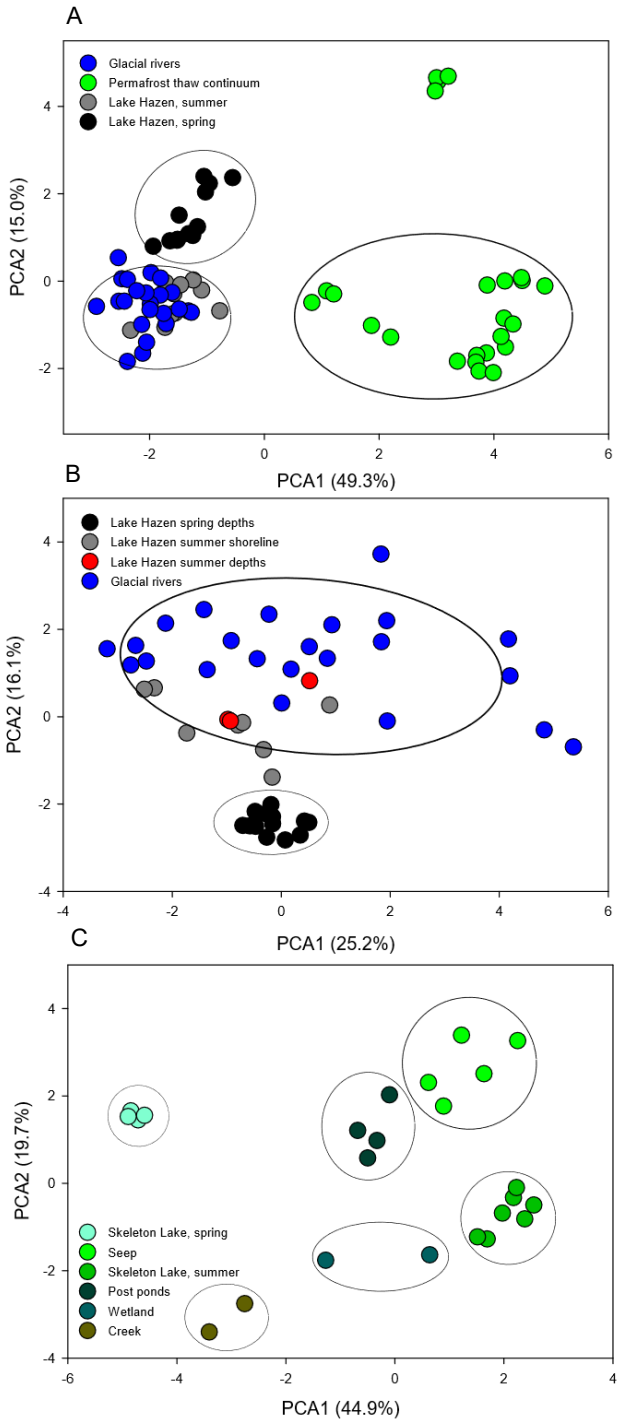


Figure 2.2. Principal Component Analyses of relevant physicochemical parameters measured at each freshwater compartment sampled. Ordinations, with 95% confidence intervals, are based on Euclidian distances of physicochemical parameters per freshwater compartment shown in Table 2.1. A: the three watershed compartments B: glacial rivers & Lake Hazen. C: permafrost continuum

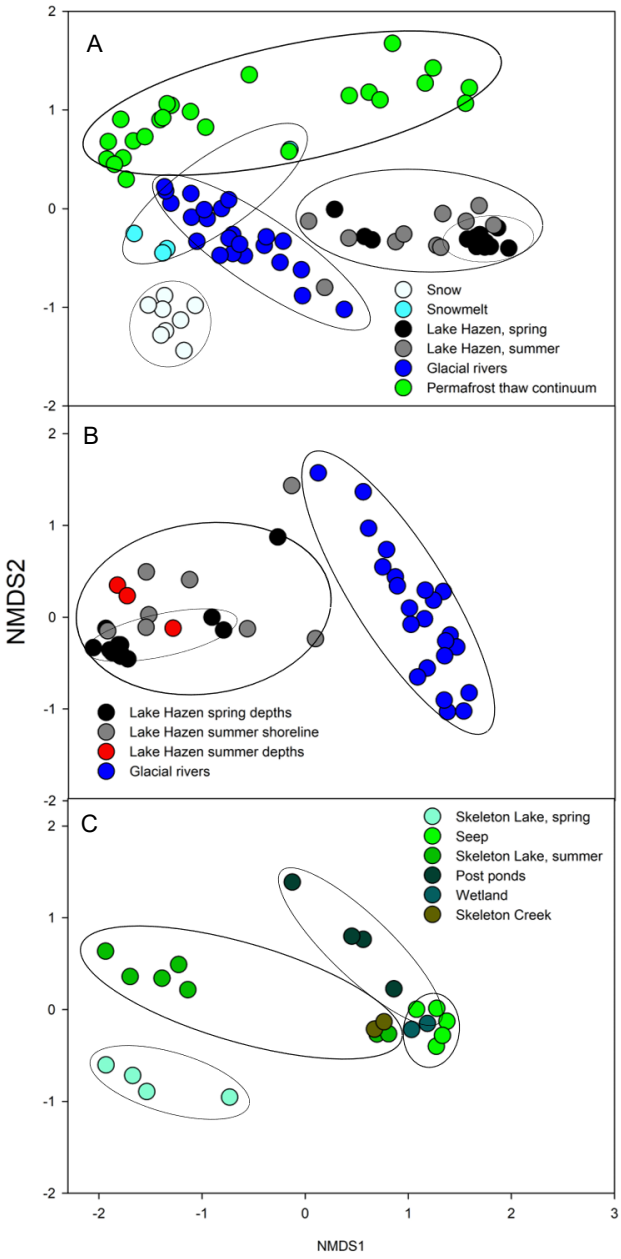
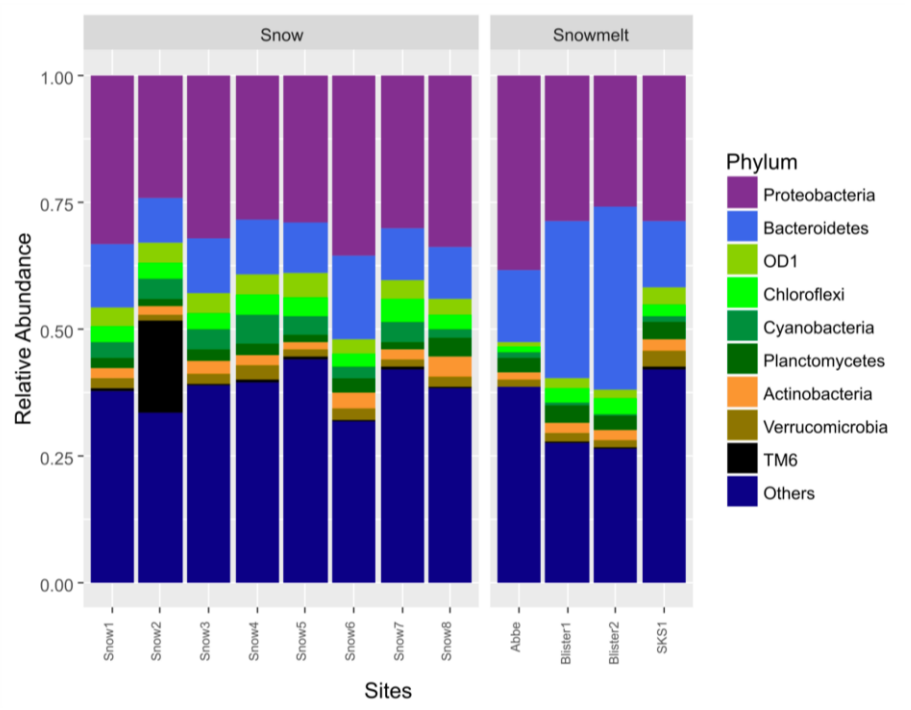
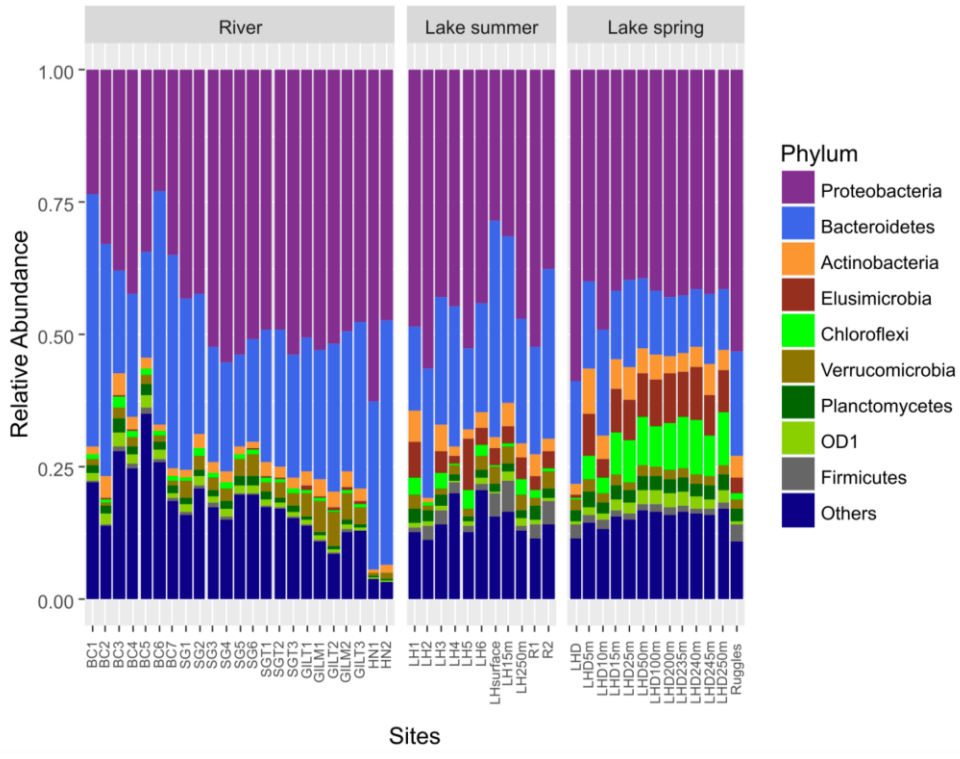


Figure 2.3. Microbial community patterns according to freshwater compartment within the Lake Hazen watershed. NMDS ordinations based on non-transformed OTU abundances. In all panels, sample site is indicated by colour, where green denotes permafrost thaw samples, blue denotes glacial run off samples, black and grey represent Lake Hazen (spring and summer, respectively), and brown and light blue represent snow and snowmelt, respectively. A: NMDS ordination of all freshwater compartments within the Lake Hazen watershed. Stress: 0.126. B: NMDS ordination of freshwater samples corresponding to Lake Hazen and glacial river samples. Stress: 0.107. C: NMDS ordination of freshwater sub-compartments within the permafrost thaw continuum. Stress: 0.06.

A


Snow
Snowmelt
1.00
0.75
0.50
0.25
0.00
Relative Abundance
Snow1
Snow2
Snow3
Snow4
Snow5
Snow6
Snow7
Snow8
Abbe
Blister1
Blister2
SKS1
Sites
Phylum
Proteobacteria
Bacteroidetes
OD1
Chloroflexi
Cyanobacteria
Planctomycetes
Actinobacteria
Verrucomicrobia
TM6
Others

B


River
Lake summer
Lake spring
1.00
0.75
0.50
0.25
0.00
Relative Abundance
BC1
BC2
BC3
BC4
BC5
BC6
BC7
SG1
SG2
SG3
SG4
SG5
SG6
SGT1
SGT2
SGT3
GILT1
GILM1
GILT2
GILT2
GILT3
HN1
HN2
LH1
LH2
LH3
LH4
LH5
LH6
LHsurface
LH15m
LH250m
R1
R2
LHD
LHD5m
LHD10m
LHD15m
LHD25m
LHD50m
LHD100m
LHD200m
LHD235m
LHD240m
LHD250m
Ruggles
Sites
Phylum
Proteobacteria
Bacteroidetes
Actinobacteria
Elusimicrobia
Chloroflexi
Verrucomicrobia
Planctomycetes
OD1
Firmicutes
Others

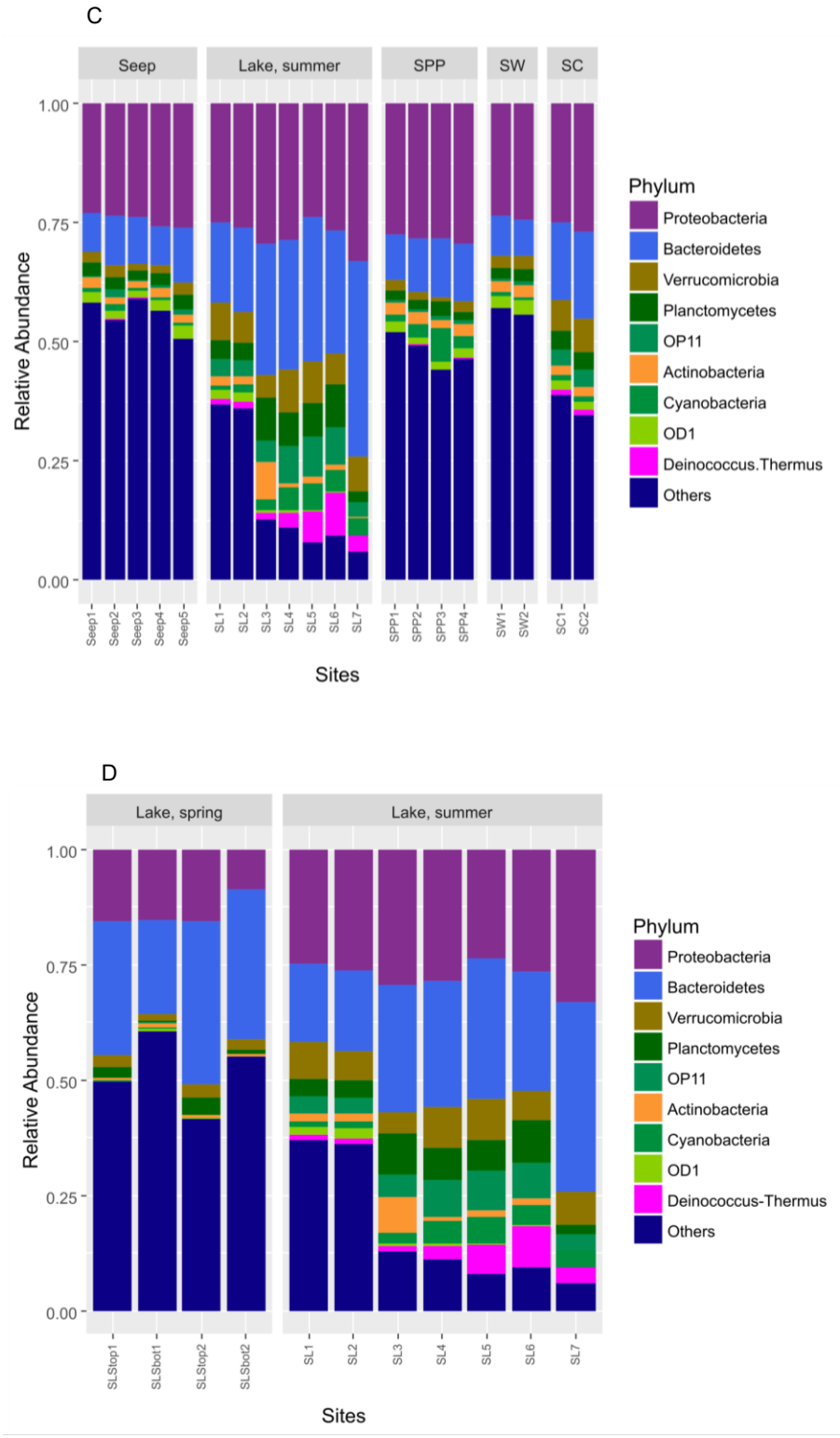


Figure 2.4. Relative abundances of the top 10 phyla found within specific watershed compartments. Samples are arranged in chronological order from left to right for each panel, per site (x axis). OTU assignations to taxonomic designations are based on the RDP database,

defined at 97% similarity thresholds. Colours denoting the presence of specific phyla is kept uniform for all panels. A: Taxonomy for snow and snowmelt samples. B: Taxonomy for glacial river and Lake Hazen samples. C: Taxonomy for permafrost thaw continuum samples. D: Taxonomy for permafrost thaw communities in Skeleton Lake in the summer vs spring.

Table 2.2. BIOENV and db-RDA analysis of total Lake Hazen (spring, n= 13, summer, n= 11) glacial rivers (n= 22) and permafrost thaw continuum (n= 24) microbial communities with relevant physicochemical parameters. All environmental parameters, except for temperature, were logarithmically scaled and the centered prior to correlational analysis.

Freshwater compartment	Physicochemical parameter	Spearman's rho	Significance	% total adjusted variation explained
Glacial rivers	Temperature, O_2, SO_4, Na and K,	0.619	0.040	37.1
Lake Hazen, spring	Temperature, SO_4, turbidity, PN, Ca	0.756	0.003	34.3
Lake Hazen, summer	Temperature, DIC, turbidity, TP	0.395	0.011	18.1
Permafrost continuum	DOC, Na+K	0.367	0.005	27.16

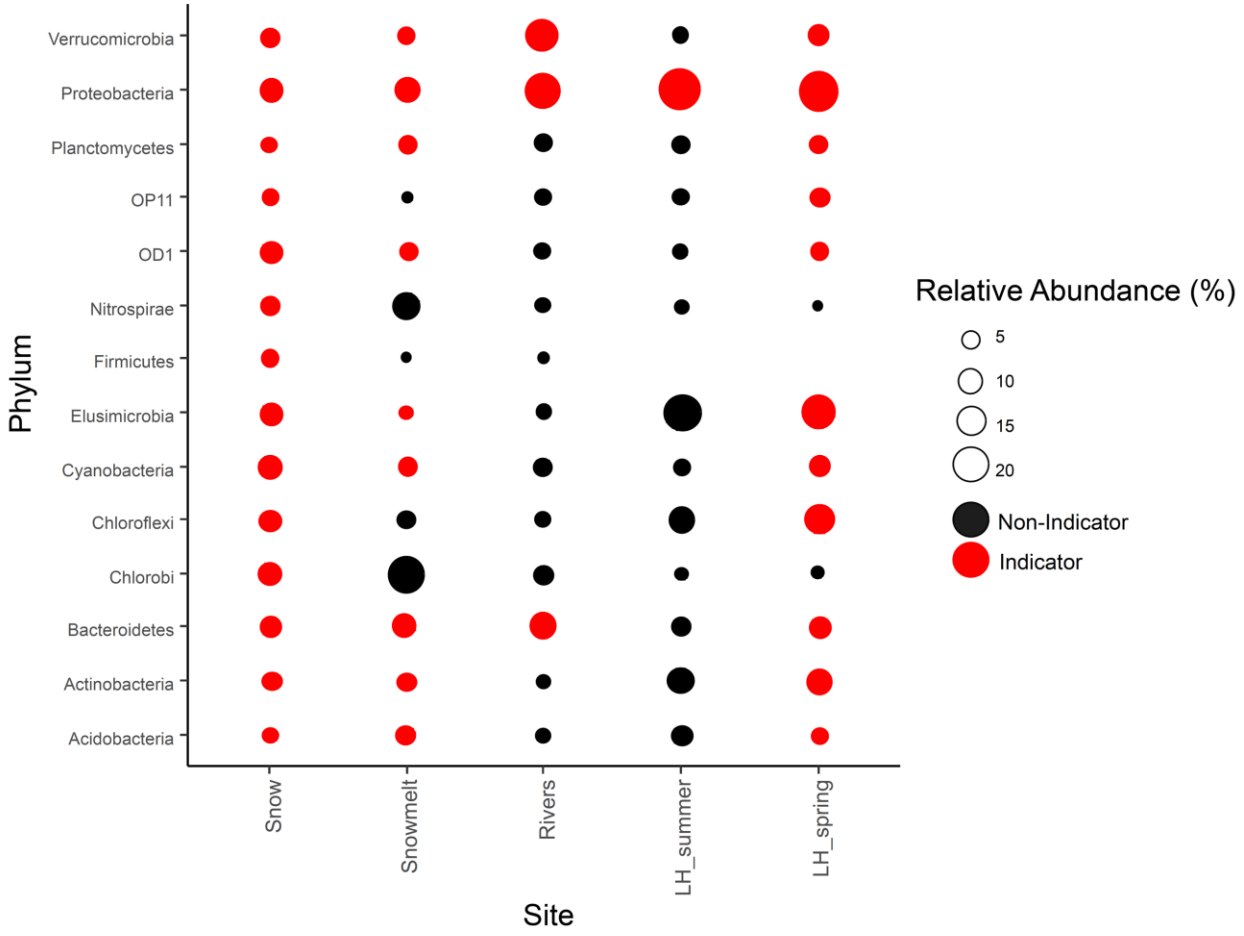


Figure 2.5. Bubble plot showing relative abundance of indicator phyla found within various freshwater compartments within Lake Hazen. Those dots that are red represent OTUs assigned to particular phyla that are indicators (Indicator value ≥ 0.7, p-val <0.05) for a particular site, whereas those that are black contain OTUs assigned to phyla that are not indicators for specific sites. Rivers: all glacial river samples. LH_summer: Lake Hazen summer samples. LH_spring: Lake Hazen spring samples. No indicator microorganisms were found to be attributed to the permafrost continuum.

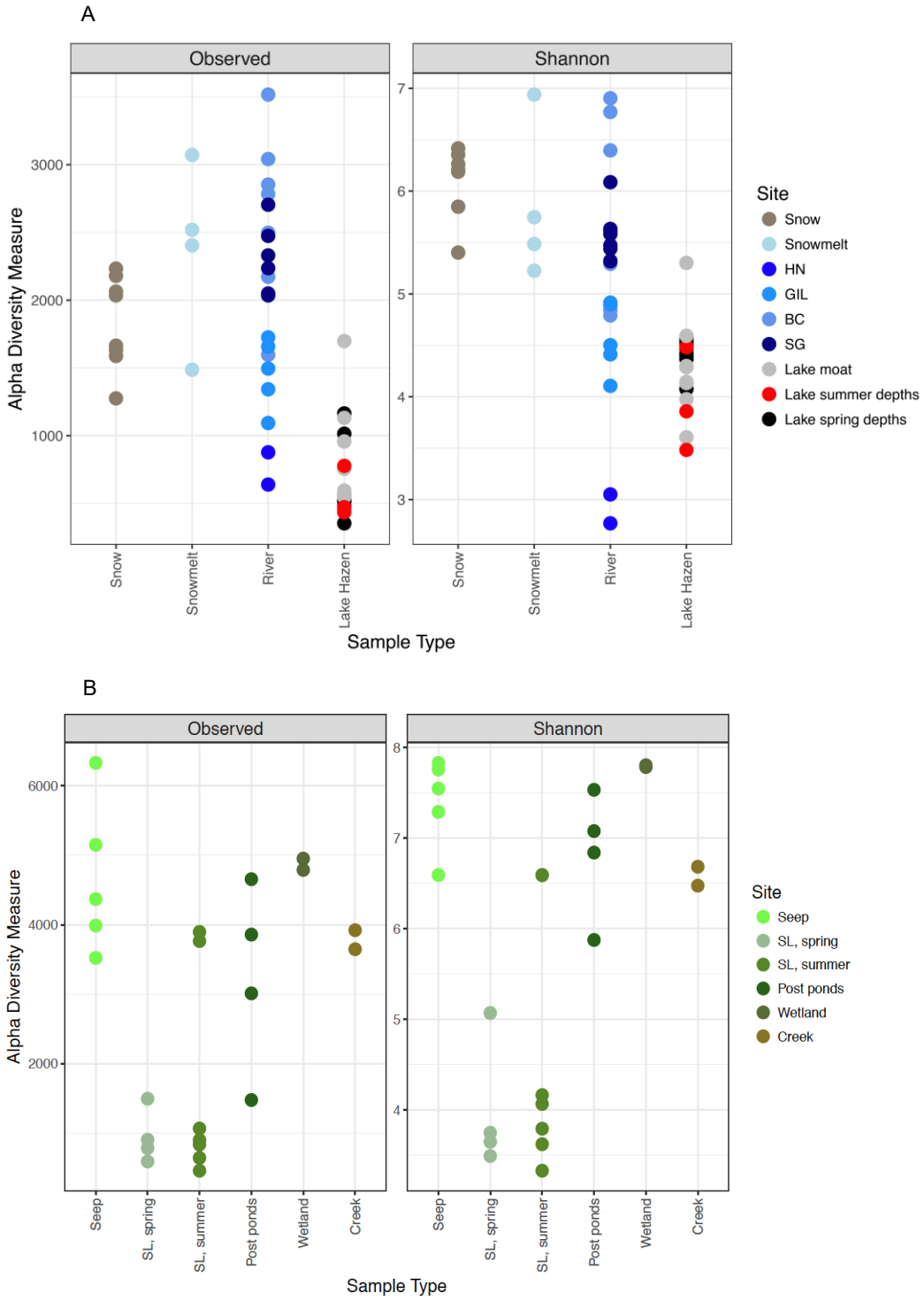


Figure 2.6. Alpha diversity metrics for specific freshwater compartments within the Lake Hazen watershed. Based on rarefied dataset containing uniform depth of 10109 sequences. In both panels, specific sample type is indicated by colour**. A:** Observed and Shannon Diversity for snow and glacially-sourced freshwaters, organized by compartment**. B:** Observed and Shannon Diversity, organized by sequential compartments within the permafrost thaw continuum.

Table 2.3. BIOENV analysis of Lake Hazen (spring n= 13, summer n=11) and glacial river (n= 22) microbial indicator communities with relevant physicochemical parameters. All environmental parameters, except for temperature, were logarithmically scaled and the centered prior to correlational analysis.

Freshwater compartment	Physicochemical parameter	Spearman's rho	Significance
Glacial rivers	Temperature, O_2, sampling date, DIC, TDS, NO_2+NO_3, PN, Na+K	0.7000	0.006
Lake Hazen, spring	Temperature, SO_4, turbidity, PN, Ca	0.8036	0.002
Lake Hazen, summer	Temperature, SO_4, PN, Ca	0.7787	0.002

3 CONCLUSIONS

3.1 Research Conclusions

In Chapter 2, I presented the composition of microbial communities and physicochemical parameters found within three basic freshwater compartments of the Lake Hazen watershed and discussed how they varied over an annual hydrological cycle between summer 2016 and spring 2017. These compartments consisted of: 1) glacial rivers; 2) a permafrost thaw continuum, consisting of a seep, and downstream lakes, ponds, wetlands and creeks and; 3) Lake Hazen. Each of these compartments had distinct microbial communities but were not always strongly influenced by the physicochemical parameters of the sites from which they were isolated. Glacial river microbial communities appeared to be structured most strongly by mass effect dynamics, whereby the rate at which taxa entering these high-flow rivers from glaciers and subsequent erosional material was likely greater than the rate at which local environmental sorting could take place (Niño-Garcia et al., 2016). Microbial community structuring here was most strongly correlated with sulfate, dissolved oxygen, temperature and sodium concentrations, suggesting that incorporation of erosional materials, and associated weathered components into glacial waters as they rush across the landscape brings with it terrestrially-derived microbial assemblages. The microbial communities that then get exported to Lake Hazen subsequently undergo selective species sorting according to physicochemical parameters, such as sulfate and turbidity, from which a specialized Lake Hazen microbial community develops. This microbial community consists of three dominating phyla, including well characterized members from Proteobacteria and Chloroflexi, and the not-so-well described members from Elusimicrobia. Microorganisms belonging to phylum Chloroflexi in particular are capable of anoxygenic

photosynthesis, commonly found in deep stratified lakes, which may be important for autochthonous carbon production within Lake Hazen over winter and spring months.

In contrast, microbial communities found along the permafrost continuum were only weakly influenced by the physicochemical parameters we measured, most importantly sodium and dissolved organic carbon concentrations, which are groundwater and productivity measures, respectively. This suggests that microbial communities there were likely influenced more by competitive interactions and strong evaporative processes that impact shallower bodies of water (Granger and Hedstrom, 2010). We posit that the smaller, yet still important, freshwater compartments along the thaw continuum may be more vulnerable to fluctuations in water availability and temperatures caused by climate change, with important implications on biogeochemical cycling (Emmerton et al., 2016). While both glacial rivers and, to a lesser extent, permafrost thaw streams, contributed microbial assemblages, nutrients and other chemical parameters downstream to Lake Hazen, the Lake Hazen microbiome was a taxonomically, and by extension, functionally stable system in which the most influential selector for microbial community structuring was a long water residence time (25-90 years).

3.2 Future Research Directions

We acknowledge the limitations of this study, in that it is only a baseline characterization that spanned a single summer and the following spring season. To obtain improved insight into the stability of the Lake Hazen microbiome, and the potential ecological responses of freshwater microbial communities to fluctuating water quality as glacial melt and permafrost thaw intensify under predicted warming, it would be ideal to sample continuously over at least two more hydrological cycles. While 16s rRNA gene sequencing provides useful understanding of the

freshwater microbial communities found within the watershed and their potential relationships with the freshwater physicochemistry, metagenomics would be useful to understand the functional capacity of the microbial community. Because metatranscriptomics would further provide a clearer picture of the microbial activity within freshwater compartments of a watershed, such a survey is recommended for future studies. Implementation of these suggestions, however, are challenging to carry out in such remote sites, with limited scientific infrastructure and high associated logistical costs.

Further, in the analysis presented herein, we have observed that overall glacial river microbial communities contained similar taxonomic structure that did not significantly fluctuate over time or between sites, as evidenced by very few glacial-river specific microbial "species" or operational taxonomic units (OTUs) according to an indicator species analysis. As glaciers within the Lake Hazen watershed are cold-based, we believe there to be minimal microbial community inputs to rivers from the subglacial environment. Most of the microbial inoculum must therefore originate from erosion of river banks, and secondarily, from potential supraglacial routing. Sampling soil in a transect parallel and /or perpendicular to various glacial rivers throughout the watershed for subsequent microbial 16S rRNA surveys would allow us to trace the movement and establishment of microbiota from the landscape into Lake Hazen over time, and to assess how important sediment microbial communities are as seeds of biodiversity to High Arctic freshwater systems.

While we attempted cell counting in the field to help establish the number of microbial cells being transported downstream from glacial rivers and permafrost thaw sources into Lake Hazen, we were unsuccessful in capturing an accurate picture of cell abundance over the course of the melt season. In these environments, it was impossible to differentiate suspended particulates

from microbial cells, hampered by the fact that the microbial biomass was so low. For future work, we recommend a sequential filtration system, where water is first filtered through a 1µm filter, then through a 0.6µm, a 0.4µm and finally a 0.2µm filter, to capture only planktonic microorganisms. This final filter should then be stained with SYBR green nucleic stain to visualize intact microbial cells under an epifluorescent microscope. This procedure will enable one to capture planktonic microbial cells found within glacial rivers, permafrost thaw streams, and in lake waters themselves. This procedure will, however, overlook microbial cells adhered to sedimentary particles found within waters, which may be quite common in these turbid systems.

Regardless of these current deficiencies, my research makes a significant contribution to our overall understanding of Arctic freshwater microbial ecology and their relationships with the associated physicochemical parameters found within multiple interconnected freshwater compartments. The work presented here sets the stage for future studies to assess further the impacts of climate change on fragile ecoregions within the Arctic, including the Lake Hazen watershed.

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APPENDIX

Table A1. Metadata for samples obtained at various sites throughout the Lake Hazen watershed.

Site	Description	Latitude	Longitude	Date sampled
Snow_1	Snow, terrestrial surface	81.8	71.3	5/12/2017
Snow_2	Snow, terrestrial surface	81.8	71.5	5/12/2017
Snow_3	Snow, terrestrial surface	81.8	71.5	5/12/2017
Snow_4	Snow, terrestrial surface	81.0	71.5	5/12/2017
Snow_5	Snow, lake ice surface	81.8	70.4	5/13/2017
Snow_6	Snow, lake ice surface	81.8	70.7	5/13/2017
Snow_7	Snow, lake ice surface	81.8	71.1	5/13/2017
Snow_8	Snow, lake ice surface	81.8	71.4	5/13/2017
Abbe	Snowmelt	81.9	70.4	6/9/2017
Blister_1	Snowmelt	81.8	71.5	6/6/2017
Blister_2	Snowmelt	81.8	71.5	7/6/2017
SKS_1	Snowmelt	81.8	71.3	9/6/2017
LHD	Lake Hazen spring	81.5	70.4	5/23/2017
LHD_5m	Lake Hazen spring	81.5	70.4	5/23/2017
LHD_10m	Lake Hazen spring	81.5	70.4	5/23/2017
LHD_15m	Lake Hazen spring	81.5	70.4	5/25/2017
LHD_25m	Lake Hazen spring	81.5	70.4	5/25/2017
LHD_50m	Lake Hazen spring	81.5	70.4	5/25/2017
LHD_100m	Lake Hazen spring	81.5	70.4	5/25/2017
LHD_200m	Lake Hazen spring	81.5	70.4	5/25/2017
LHD_235m	Lake Hazen spring	81.5	70.4	5/26/2017
LHD_240m	Lake Hazen spring	81.5	70.4	5/26/2017
LHD_245m	Lake Hazen spring	81.5	70.4	5/26/2017
LHD_250m	Lake Hazen spring	81.5	70.4	5/26/2017
Ruggles_S	Ruggles, spring	81.8	70.4	5/28/2017
SLS_top1	Skeleton Lake, spring	81.8	71.5	5/13/2017
SLS_bot1	Skeleton Lake, spring	81.8	71.5	5/13/2017
SLS_top2	Skeleton Lake, spring	81.8	71.5	5/16/2017
SLS_bot2	Skeleton Lake, spring	81.8	71.5	5/16/2017
LH_1	Lake Hazen, summer	81.8	71.4	7/2/2016
LH_2	Lake Hazen, summer	81.8	71.4	7/8/2016
LH_3	Lake Hazen, summer	81.8	71.4	7/17/2016
LH_4	Lake Hazen, summer	81.8	71.4	7/23/2016
LH_5	Lake Hazen, summer	81.8	71.4	7/29/2016
LH_6	Lake Hazen, summer	81. 8	71.4	8/5/2016
LH_surface	Lake Hazen, summer	81.5	70.4	8/9/2016

LH_15m	Lake Hazen, summer	81.5	70.4	8/9/2016
LH_250m	Lake Hazen, summer	81.5	70.4	8/9/2016
R_1	Ruggles, summer	81.8	70.4	7/11/2016
R_2	Ruggles, summer	81.8	70.4	8/2/2016
BC_1	Minor river	81.8	71.5	7/2/2016
BC_2	Minor river	81.8	71.5	7/8/2016
BC_3	Minor river	81.8	71.5	7/16/2016
BC_4	Minor river	81.8	71.5	7/23/2016
BC_5	Minor river	81.8	71.5	7/29/2016
BC_6	Minor river	81.8	71.5	8/4/2016
BC_7	Minor river	81.8	71.5	7/16/2017
SG_1	Minor river	81.8	71.2	7/3/2016
SG_2	Minor river	81.8	71.2	7/8/2016
SG_3	Minor river	81.8	71.2	7/16/2016
SG_4	Minor river	81.8	71.2	7/23/2016
SG_5	Minor river	81.8	71.2	7/30/2016
SG_6	Minor river	81.8	71.2	8/5/2016
SG_7	Minor river, proglacial stream	81.9	71.9	7/21/2017
SGT_1	Minor river, proglacial stream	81.9	71.9	7/11/2016
SGT_2	Minor river, proglacial stream	81.9	71.9	8/1/2016
GILT_1	Major river, proglacial stream	82.1	70.3	7/11/2016
GILM_1	Major river, mid river	82.0	69.8	7/11/2016
GILT_2	Major river, proglacial stream	82.1	70.3	8/2/2016
GILM_2	Major river, mid river	82.0	69.8	8/2/2016
GILT_3	Major river, proglacial stream	82.1	70.3	7/20/2017
HN_1	Major river, proglacial stream	81.8	72.0	8/2/2016
HN_2	Major river, proglacial stream	81.8	72.0	7/21/2017
Seep_1	Permafrost continuum	81.4	71.5	7/14/2016
Seep_2	Permafrost continuum	81.4	71.5	7/17/2016
Seep_3	Permafrost continuum	81.4	71.5	7/25/2016
Seep_4	Permafrost continuum	81.4	71.5	7/31/2016
Seep_5	Permafrost continuum	81.4	71.5	7/11/2016
SL_1	Permafrost continuum	81.8	71.5	7/3/2016
SL_2	Permafrost continuum	81.8	71.5	7/9/2016
SL_3	Permafrost continuum	81.8	71.5	7/14/2016
SL_4	Permafrost continuum	81.8	71.5	7/25/2016
SL_5	Permafrost continuum	81.8	71.5	7/31/2016
SL_6	Permafrost continuum	81.8	71.5	8/6/2016
SL_7	Permafrost continuum	81.8	71.5	7/14/2017
SPP_1	Permafrost continuum	81.5	71.2	7/14/2016
SPP_2	Permafrost continuum	81.5	71.2	7/25/2016

SPP_3	Permafrost continuum	81.5	71.2	7/31/2016
SPP_4	Permafrost continuum	81.5	71.2	8/6/2016
SW_1	Permafrost continuum	81.5	71.2	7/31/2016
SW_2	Permafrost continuum	81.5	71.21	8/6/2016
SC_1	Permafrost continuum	81.8	71.4	7/31/2016
SC_2	Permafrost continuum	81.8	71.4	8/6/2016

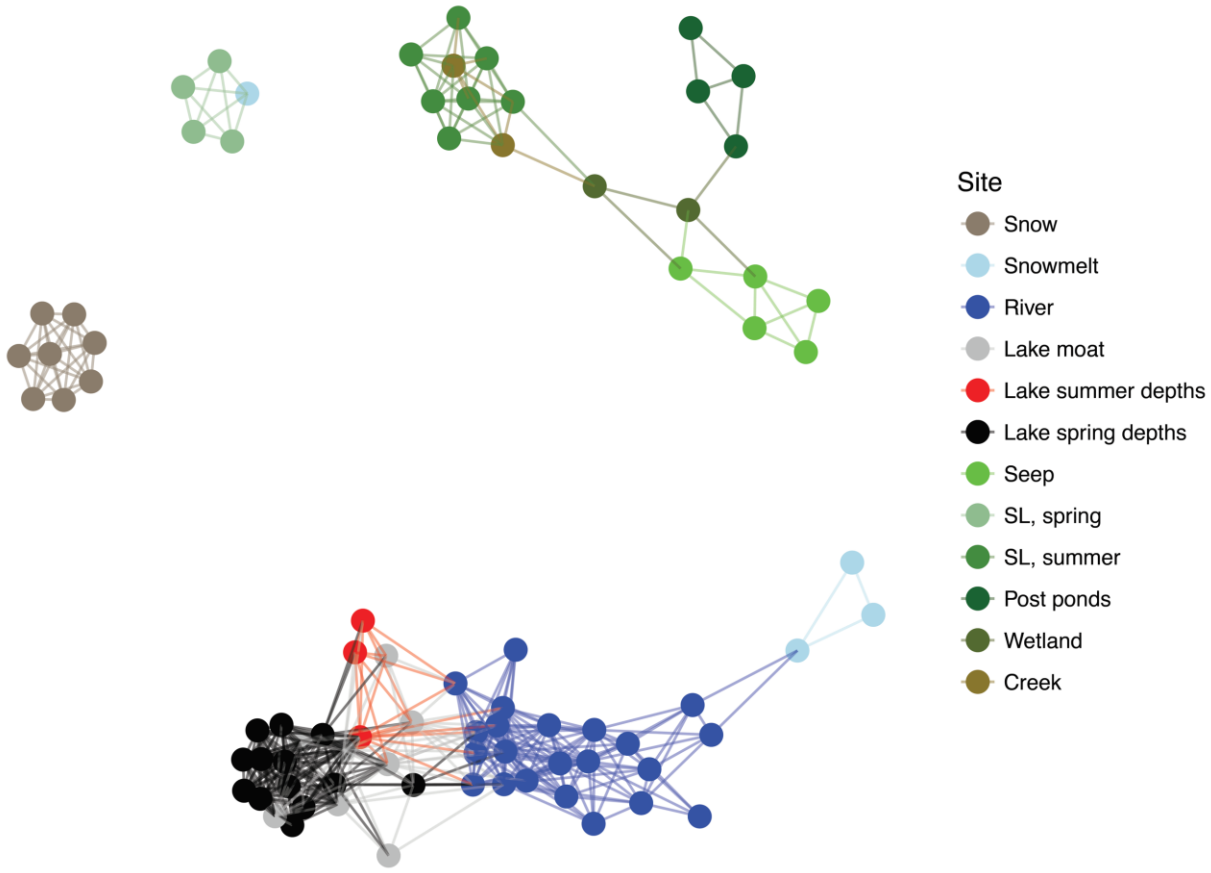

Figure A1. Interconnectivity of microbial communities from various sites throughout the Lake Hazen watershed. Network analysis is based on Bray-Curtis distances of non-transformed OTU abundances, with rare OTUs (occurring only once at less than 20 samples) removed. Maximum ecological distance between sites: 0.6.

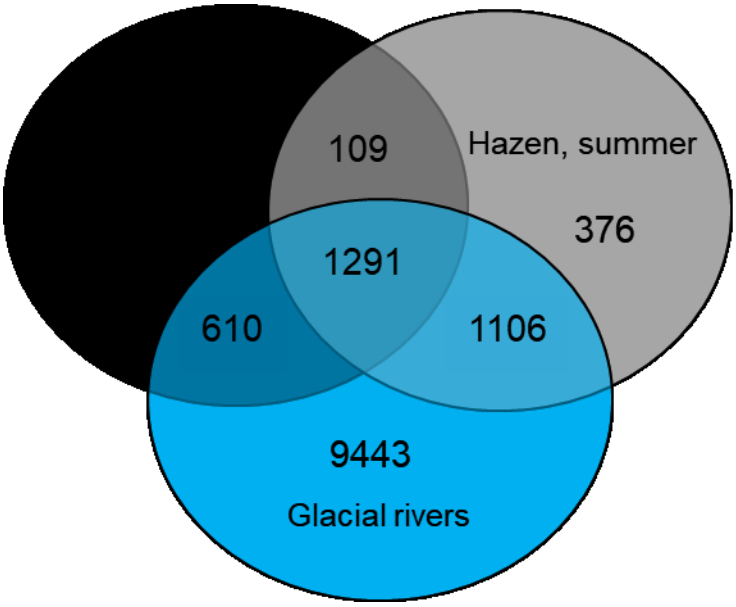

Figure A2. Venn diagrams of OTUs shared between glacial rivers and Lake Hazen during spring and summer. Figure completed using R package VennDiagram with rarefied, non-transformed OTUs.

Table A2. Contribution of physicochemical parameters onto Axis 1 and 2 Axis 1 and 2 of PCA biplot (Figure 2) for the permafrost continuum, glacial rivers, and Lake Hazen.

Parameter	Axis 1	Axis 2
NO_2+NO_3	10.2	0.450
Temperature	5.01	23.4
DOC	7.91	14.7
DIC	12.6	0.210
SO_4	14.9	0.00092
Dissolved O_2	3.04	9.50
PN	4.15	2.73
TDS	12.3	4.76
TP	2.86	3.74
Ca	5.49	25.5
Na+K	10.3	14.3
Fe	11.1	10.1

Table A3. Contribution of physicochemical parameters onto Axis 1 and 2 Axis 1 and 2 of PCA biplot (Figure 2) for Lake Hazen and glacial river samples.

Parameter	Axis 1	Axis 2
NO_2+NO_3	17.5	0.0096
Temperature	7.32	11.1
DOC	0.145	0.151
DIC	1.17	17.3
SO_4	18.0	0.147
Dissolved O_2	3.04	3.73
PN	0.146	4.28
TDS	16.5	4.28
TP	0.410	6.83
Ca	19.1	5.31
Na+K	6.14	3.75
Fe	0.550	10.1

Table A4. Contribution of physicochemical parameters onto Axis 1 and 2 of PCA biplot (Figure 2) for permafrost thaw continuum samples.

Parameter	Axis 1	Axis 2
Julian date	12.5	0.0254
Temperature	10.9	0.0006
DOC	5.02	17.3
DIC	1.93	23.7
SO_4	10.1	8.97
Dissolved O_2	9.61	1.67
PN	1.00	2.15
TDS	6.88	16.4
TP	0.0171	5.95
Ca	13.7	3.57
Na+K	14.5	0.115
Fe	6.92	3.49

Table A5. DB-RDA statistics for Glacial River samples (Table 3) Pseudo F statistic: 5.3, p=0.001. Total variation: 2.76, explanatory variables account for 45.7%, adj explained var: 37.1%.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.372	0.0618	0.0228	0.149
Cumulative explained var	37.2	43.4	45.7	60.6
Pseudo canonical correlation	0.912	0.824	0.735	0
Explained fitted var (cumulative)	81.5	95.0	100	

Table A6. DB-RDA statistics for permafrost thaw continuum samples (Table 3). Pseudo F statistic: 5.4, p=0.001 Total variation: 6.47, explanatory variables account for 33.9%, adj explained var: 27.6%.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.297	0.0419	0.0351	0.124
Cumulative explained var	29.67	33.9	69.0	81.4
Pseudo canonical correlation	0.975	0.658	0	0
Explained fitted var (cumulative)	87.6	100		

Table A7. DB-RDA statistics for Lake Hazen summer samples (Table 3). Pseudo F statistic: 1.6, p=0.12 Total variation: 1.54, explanatory variables account for 50.8%, adj explained var: 18.1%.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.294	0.141	0.0685	0.0045
Cumulative explained var	29.4	43.5	50.4	50.9
Pseudo canonical correlation	0.940	0.811	0.566	0.381
Explained fitted var (cumulative)	57.9	85.6	99.1	100

Table A8. DB-RDA statistics for Lake Hazen spring samples (Table 3). Pseudo F statistic: 3.1, p=0.048. Total variation: 0.97795, explanatory variables account for 50.7%, adj explained var: 34.3%.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.463	0.0307	0.0137	0.355
Cumulative explained var	46.3	49.3	50.7	86.2
Pseudo canonical correlation	0.763	0.735	0.791	0
Explained fitted var (cumulative)	91.3	97.3	100	