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Research Article

RESEARCH ARTICLE

Freshwater microbial community diversity in a rapidly changing High Arctic watershed

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One sentence summary: High Arctic freshwater microbial communities are hydrologically interconnected over broad spatial scales throughout the Lake Hazen watershed (northern Ellesmere Island, Nunavut, Canada).

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ABSTRACT

Current models predict increases in High Arctic temperatures and precipitation that will have profound impacts on the Arctic hydrological cycle, including enhanced glacial melt and thawing of active layer soils. However, it remains uncertain how these changes will impact the structure of downstream resident freshwater microbial communities and ensuing microbially driven freshwater ecosystem services. Using the Lake Hazen watershed (Nunavut, Canada; 82°N, 71°W) as a sentinel system, we related microbial community composition (16S rRNA gene sequencing) to physicochemical parameters (e.g. dissolved oxygen and nutrients) over an annual hydrological cycle in three freshwater compartments within the watershed: (i) glacial rivers; (ii) active layer thaw-fed streams and waterbodies and (iii) Lake Hazen, into which (i) and (ii) drain. Microbial communities throughout these freshwater compartments were strongly interconnected, hydrologically, and often correlated with the presence of melt-sourced chemicals (e.g. dissolved inorganic carbon) as the melt season progressed. Within Lake Hazen itself, water column microbial communities were generally stable over spring and summer, despite fluctuating lake physicochemistry, indicating that these communities and the potential ecosystem services they provide therein may be resilient to environmental change. This work helps to establish a baseline understanding of how microbial communities and the ecosystem services they provide in Arctic watersheds might respond to future climate change.

Keywords: Arctic; freshwaters; watersheds; Lake Hazen; glacial rivers; microbial ecology; soil active layer streams; biogeochemistry

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INTRODUCTION

Recent 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 (Representative Concentration Pathway (RCP) 8.5 with Coupled Model Intercomparison Project Phase 5 (CMIP5)) (Stocker *et al.* 2013). These changes will not only have substantial impacts on the Arctic hydrological cycle due to increased snowfall, glacial melt and active layer thaw, but also result in the flux of nutrients and pollutants previously archived in ice and soils to downstream freshwater systems (Rydberg *et al.* 2010; Hood *et al.* 2014). These fluxes are important because freshwater ecosystems across Arctic landscapes (i.e. lakes, wetlands and rivers) have unique biogeochemistry and levels of productivity (Vincent *et al.* 2012; Emmerton *et al.* 2016a), intricately balanced food webs and energy flows (Wrona *et al.* 2016) and important hydrological linkages with the surrounding landscape (Newton *et al.* 2011). Collectively, freshwaters are important sentinel systems for environmental change (Adrian *et al.* 2009). Although freshwater microorganisms are critical mediators in the mineralization of organic matter and biogeochemical cycling of nutrients and contaminants (Barkay, Kroer and Poulain 2011; Newton *et al.* 2011; Graham *et al.* 2016), we know very little about how microbial communities are affected by physicochemical variability across High Arctic freshwaters due to seasonal glacial melt and active layer thaw.

The Lake Hazen watershed, located on northern Ellesmere Island, Nunavut, represents an interconnected and diverse freshwater system composed of three basic compartments: (i) glacially sourced rivers draining the northern Ellesmere Icefield, (ii) active layer thaw-fed sub-catchments and (iii) Lake Hazen, the High Arctic's largest lake by volume, which receives input from river and sub-catchment compartments at the heart of the watershed. The Lake Hazen watershed has experienced alterations due to climate change over recent decades, including soil warming and a 10-fold increase in glacial melt since 2007 (Lehnherr *et al.* 2018), with yet unknown consequences for the microbial ecology of downstream systems.

Over the course of two sampling seasons encompassing an annual hydrological cycle, from early summer to the following spring, we studied microbial community composition and corresponding physicochemical properties in each of the three freshwater compartments. We also explored the flow of microbial community members through river and sub-catchment compartments and investigated how these contributed to overall Lake Hazen microbiota.

To date, there are few data addressing the variability of High Arctic freshwater microbial communities. Currently, only a single study has used amplicon sequencing to assess microbial connectivity across a Canadian High Arctic freshwater watershed (located on Ward Hunt Island) (Comte *et al.* 2018). Most other studies concern smaller lakes (Crump, Amaral-Zettler and Kling 2012), glaciers, snow, marine or permafrost systems (Skidmore, Foght and Sharp 2000; Harding *et al.* 2011; Maccario, Vogel and Larose 2014; McCann *et al.* 2016; Ribicic *et al.* 2018). To the best of our knowledge, this work is among the first watershed-scale studies linking freshwater microbial community composition to sourcing and physicochemical properties within and between various freshwater compartments in the High Arctic. Hence, we here provide a baseline understanding of the composition and drivers of freshwater microbial community dynamics across a large and rapidly changing High Arctic watershed.

MATERIALS AND METHODS

Site description

The Lake Hazen watershed is located within Quttinirpaaq National Park on northern Ellesmere Island, Nunavut, Canada (81.8°N, 71.4°W). The watershed is 7516 km^2 in area, 40.9% of which is covered by outlet glaciers of the northern Ellesmere Icefield (Lehnherr *et al.* 2018; St. Pierre *et al.* 2019). The watershed is a polar semi-desert receiving little precipitation throughout the year (~95 mm), ~65% of which falls as snow between September and May with the remainder as rain in the summer (St. Pierre *et al.* 2019). Snowmelt on the lake surface and adjacent landscape occurs over a 1–2-week period in early June. Meltwaters from the outlet glaciers of the northern Ellesmere Icefield are by far the dominant hydrological input to the lake, flowing up to 42 km into Lake Hazen along its northwestern shore between mid-June and the end of August (St. Pierre *et al.* 2019). Lake Hazen itself has a surface area of 544 km^2 and maximum depth of 267 m, making it the world's largest High Arctic lake by volume (51.4 km^3) (Lehnherr *et al.* 2018). Lake Hazen is typically ice-covered most of the year, but in recent years it has gone ice-free almost annually by late July/early August to mid-September. The Ruggles River (~29 km), which drains Lake Hazen, flows year-round into Chandler Fjord on the northeastern coast of Ellesmere Island. Small permafrost and ground-ice fed streams are found across the landscape but are hydrologically inconsequential at the watershed scale relative to the glacial rivers. For example, near the Lake Hazen base camp is an 'active layer thaw-fed continuum'. This continuum consists of active layer thaw seeps 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, Douglas and Smol 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. An in-depth table of all of our sampling sites can be found in Table S1 (Supporting Information).

Sampling of snow and snowmelt

On 20 and 21 May 2017, composite snowpack samples were obtained along parallel transects spanning the frozen Lake Hazen surface and the nearby landscape (Fig. 1). Corresponding snowmelt samples were obtained at the beginning of June 2017 at the very start of melt from the deltas of the Blister and Abbé rivers and from Skeleton Creek.

Sampling of glacial inflows

Over a 7-week period (2 July and 9 August 2016) encompassing the initial onset of glacial melt, the height of melt and the slowing of the melt season, we sampled the Snowgoose and Blister glacial rivers weekly at their deltas to quantify temporal changes in microbial community structure and physicochemical parameters. No biofilms were observed within these rivers throughout the field season. Although these two rivers were not the major glacial inputs into Lake Hazen, they were sampled frequently due to their ease of access. We also accessed the Henrietta Nesmith and Gilman rivers, the two most important hydrological inputs twice in 2016, collecting samples that coincided with the peak of glacial melt and the subsequent slowing flow.

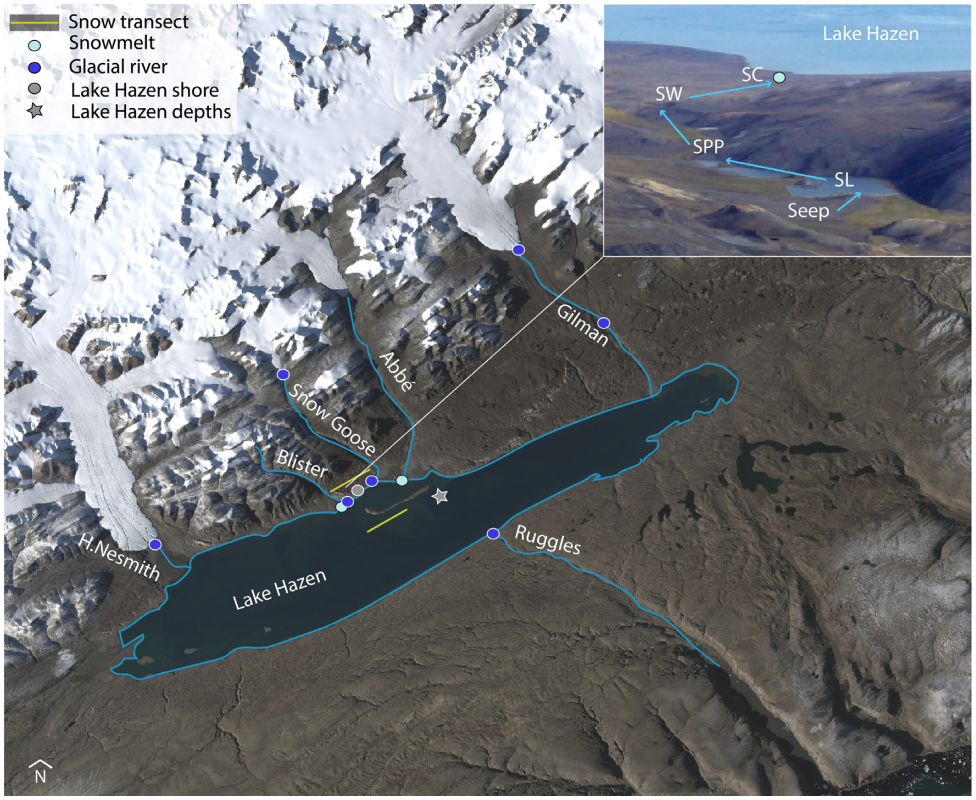

Figure 1. Map of the Lake Hazen watershed located on northern Ellesmere Island, Nunavut, delineating sample sites and sample type during field seasons spanning July–August 2016 and May 2017. Yellow lines delineate parallel transects over which snow samples were collected during May 2017, whereas light blue nodes depict snowmelt samples obtained over the same 2017 season. Dark blue nodes depict glacial river sampling sites (rivers that are delineated in blue and labeled) 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 gray node and water column depths were obtained from Lake Hazen as represented by the gray star. Inset shows the location of the active layer thaw-fed continuum and the various sub-compartments found within. Arrows demonstrate the sequential flow of water between the sub-compartments of this continuum. Seep: Skeleton seep, SL: Skeleton Lake, SPP: Skeleton post-ponds, SW: Skeleton wetlands, SC: Skeleton Creek.

Sampling of the Skeleton active layer thaw-fed continuum

Over the same 7-week period in July and August 2016, we sampled five sites within the active layer thaw-fed continuum weekly (Fig. 1). Similar to glacial melt sampling, our sampling of the continuum coincided with the onset of active layer thaw water flow through to the height of thaw. We obtained five seep water samples once the active layer had begun thawing, and two wetland and creek samples once enough thaw water was present to fill Skeleton Lake and allow overflow discharge downstream to Lake Hazen. A total of seven Skeleton Lake shoreline samples were obtained throughout the 2016 thaw season (once weekly). 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 physicochemical parameters. Water samples here were taken at depths of 1.5 m (below the ice) and 4 m (bottom waters) during spring and summer sampling.

Sampling of Lake Hazen

To assess the contribution of glacial and active layer thaw inflows to the microbial community and physicochemical properties of Lake Hazen nearshore waters, we sampled Lake Hazen nearshore water between the Snowgoose and Blister rivers and Skeleton creek outflows weekly in 2016 (Fig. 1). On 9 August 2016, we also obtained samples from the Lake Hazen water column at depths of 1, 15 and its maximum depth of 250 m. 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 it again in late May 2017 at depths immediately below the surface of the ice (~2 m), at 5, 10, 15, 25, 50, 100, 200, 235, 240, 245 and 250 m. Additionally, samples were obtained from the Ruggles River just as it exited Lake Hazen during both summer seasons and spring.

Sampling for microbial and physicochemical analyses

Snow samples for microbial analyses were collected in duplicate, from the same sampling location, using a clean stainless-steel snow corer, immediately placed into sterile 5 L Whirl-Pak Giant-Size sample bags (*Nasco* Whirl-Pak, Fort Atkinson, WI, USA), and maintained at −20°C until further processing. Snow samples for water chemistry analyses were collected the same way but stored in large Ziploc freezer bags pre-tested for contamination. For microbial community analyses in flowing and standing waters, duplicate water samples (600 mL each) from the same site were collected into Whirl-Pak Thio-Bags (*Nasco* Whirl-Pak, Fort Atkinson, WI, USA) specialized for microbial water sampling, with the thiosulfate tablet aseptically removed. Bags were rinsed three times with site water prior to being filled. Samples were transported to the Lake Hazen/Quttinirpaaq Field

Laboratory clean room for further processing within a few hours of collection. Samples were maintained in the dark and at 4°C prior to processing. Duplicate samples were pooled at the operational taxonomic unit (OTU) stage, where little difference in count was observed.

Microorganisms and associated particles from water/snow samples were filtered onto a 0.2 μm polycarbonate membrane inserted into a glass vacuum filter holder and attached to a Büchner flask and filter pump (25 psi). Filters were immediately preserved with 1 mL of RNAlater (Fisher Scientific) in sterile Eppendorf tubes and kept frozen at −20°C until subsequent DNA extraction.

At each water sampling site, we used an EXO2 multiparameter sonde (YSI Incorporated, Yellow Springs, OH, USA) to measure turbidity, dissolved oxygen (DO) concentration, pH, temperature and electrical conductivity. Additional water samples were collected into pre-cleaned HDPE bottles for analyses of general chemistry including particulate nitrogen (PN), total dissolved phosphate (TDP), nitrate–nitrite (NO_3^- and NO_2^-), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved solids (TDS), major ions and trace metals. These samples were also processed in the Lake Hazen/Quttinirpaaq Field Laboratory clean room using established clean chemistry protocols. Processed samples were maintained in the dark at ∼5°C or frozen until they were analyzed at the Biogeochemical Analytical Service Laboratory (BASL, University of Alberta, Edmonton, Alberta) using standard analytical protocols.

Processing of samples for microbial analyses

Microbial DNA was extracted from duplicate filters from all sites using the DNeasy PowerSoil kit (Qiagen). We followed the extraction procedure recommended by the manufacturer, except for three modifications: (i) prior to DNA extraction, excess RNAlater was removed from the filters via centrifugation at 10 000 g for 10 s, because RNAlater may impede the efficiency of DNA extraction from environmental samples (McCarthy *et al.* 2015); (ii) 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 (iii) 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 then determined using a Qubit fluorometer (model 2.0, using the 1× HS dsDNA kit), with concentrations ranging between ∼0.1 and 15 ng/μL to serve as template for PCR, depending on the water source, and time of sampling.

Sequencing and computational analyses

The 16S rRNA genes were amplified from randomized samples using universal prokaryotic primers 515F-Y (Parada, Needham and Fuhrman 2016) and 926R (Quince *et al.* 2011). Each primer also contained a six-base index sequence for sample multiplexing as well as flow cell binding and sequencing sites (Bartram *et al.* 2011). The PCR mix (25 μL total volume) contained 1× ThermoPol buffer, 0.2 μM forward primer, 0.2 μM reverse primer, 200 μM dNTPs, 15 μg bovine serum albumin, 0.625 U Hot Start *Taq* 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 s, 50°C for 30 s, 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 instrument (Illumina Inc., CA, USA) using a 2 × 250 cycle MiSeq Reagent Kit v2 (Illumina Canada Inc). The number of reads per sample can be found in Table S2 (Supporting Information). The MiSeq reads were demultiplexed using MiSeq Reporter software version 2.5.0.5. Each read pair was assembled using the paired-end assembler for Illumina sequences (PANDAseq; Masella, Bartram and Truszkowski 2012) with a quality threshold of 0.9 and assembled reads were analyzed using Quantitative Insights Into Microbial Ecology (QIIME; Caporaso *et al.* 2010a) managed by automated exploration of microbial diversity (AXIOME) version 1.5 pipeline (Lynch *et al.* 2013). Sequences were clustered into OTUs with 97% sequence similarity, using the 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.* 2010a). Singletons and low abundance OTUs were removed. Chimeric sequences were removed using UCHIME (Edgar *et al.* 2011). 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, using the most recent release (13_8) (McDonald *et al.* 2012). Raw sequences have been uploaded to the NCBI database with accession numbers PRJNA505817 and PRJNA504462.

For all 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 *et al.* 2016) in R (R core team 2013). Here, the definition of indicator species (i.e. OTUs) were those microorganisms found primarily 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 ≤ 0.05. Because we did not want to be too stringent, as the water bodies within the Lake Hazen watershed are quite interconnected, we chose an indicator value of 0.7 (1.0 being the highest value), meaning that 70% of microbial species within an OTU are present at all sites of a specific group (for example, glacial rivers). The *P*-value determines the statistical significance of the indicator value assigned to an OTU using random permutations. 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).

Beta diversity, or the diversity among the different freshwater compartments sampled throughout the watershed, was visualized using non-metric multidimensional scaling (NMDS) ordinations of OTU data converted to a Bray–Curtis dissimilarity matrix and plotted in SigmaPlot (Systat Software, San Jose, CA). The significance of observed site clusters within the ordination were calculated using global ADONIS, pairwise PERMANOVA (permutational multivariate analysis of variance) tests (using Benjamini–Hochberg corrected *P*-values) and betadisper functions in Vegan.

Principal component analyses (PCA) 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 R FactoMineR and factoextra packages (Le, Josse and Husson 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. These parameters were first tested for multicollinearity by calculating the variance inflation factor (VIF) in R. All VIFs were <5, suggesting little multicollinearity between variables. Euclidean distances were calculated for environmental variables, whereas OTU data were converted to a Bray–Curtis dissimilarity matrix. Distance-based redundancy analyses (db-RDA) 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 were visualized using phyloseq (McMurdie and Holmes 2014).

RESULTS

Physicochemical characteristics

We identified three physicochemically distinct freshwater compartments within the Lake Hazen watershed, based on 95% confidence intervals (Table S4, Supporting Information; Fig. 2A): (i) glacial rivers and Lake Hazen during the summer, (ii) the active layer thaw continuum and (iii) Lake Hazen during the spring. Points falling outside of the 95% confidence intervals represent the physicochemical environment of Skeleton Lake under complete ice cover. We obtained snow and snowmelt samples representing two other freshwater compartments within the Lake Hazen watershed, but as we were not able to collect comparable physicochemical properties, such as turbidity, DO and DIC, we did not include these samples in analyses here. Parameters such as calcium (Ca^{2+}), sulfate (SO_4^{2-}) and nitrate–nitrite (NO_3^- & NO_2^-) contributed most to the first principal component (PC1; Fig. 2A; Table S4, Supporting Information), whereas DIC, particulate nitrogen (PN), and temperature contributed most to the second principal component (PC2; Table S4, Supporting Information). Samples collected during the summer from the Lake Hazen water column at 1, 15 and 250 m depth grouped with glacial river inflow samples, as did Lake Hazen shoreline samples (Fig. 2B). With the formation of ~2 m thick ice cover on the lake over the winter and spring months, the water column exhibited reverse temperature stratification (Figure S1, Supporting Information). In the absence of glacial melt and active layer thaw inputs to the lake, DO declined with depth, reflecting heterotrophic activity under the ice and at the sediment bed, along with reduced photosynthetic capability in the water column during ice cover, and thus a distinct environment within the Lake Hazen spring water column (Fig. 2B) forms.

In contrast to glacially derived freshwaters in the watershed, the PCA revealed distinct environments within each subcompartment of the active layer thaw-fed continuum (Fig. 2C; Table S6, Supporting Information). Sodium (Na^+), potassium (K^+) and Ca^{2+} loaded highest on PC1, whereas DIC, DOC and TDS concentrations loaded highest on PC2 (Table S6, Supporting Information). In the spring, the physicochemistry of Skeleton Lake becomes distinct from that in the summer (Fig. 2C), particularly as the lake stratifies during the spring. The lack of physicochemical similarity between Skeleton Lake in the summer and its neighboring downstream ponds, wetland and creek (Fig. 2C) suggests different controls on biogeochemical processes within each compartment.

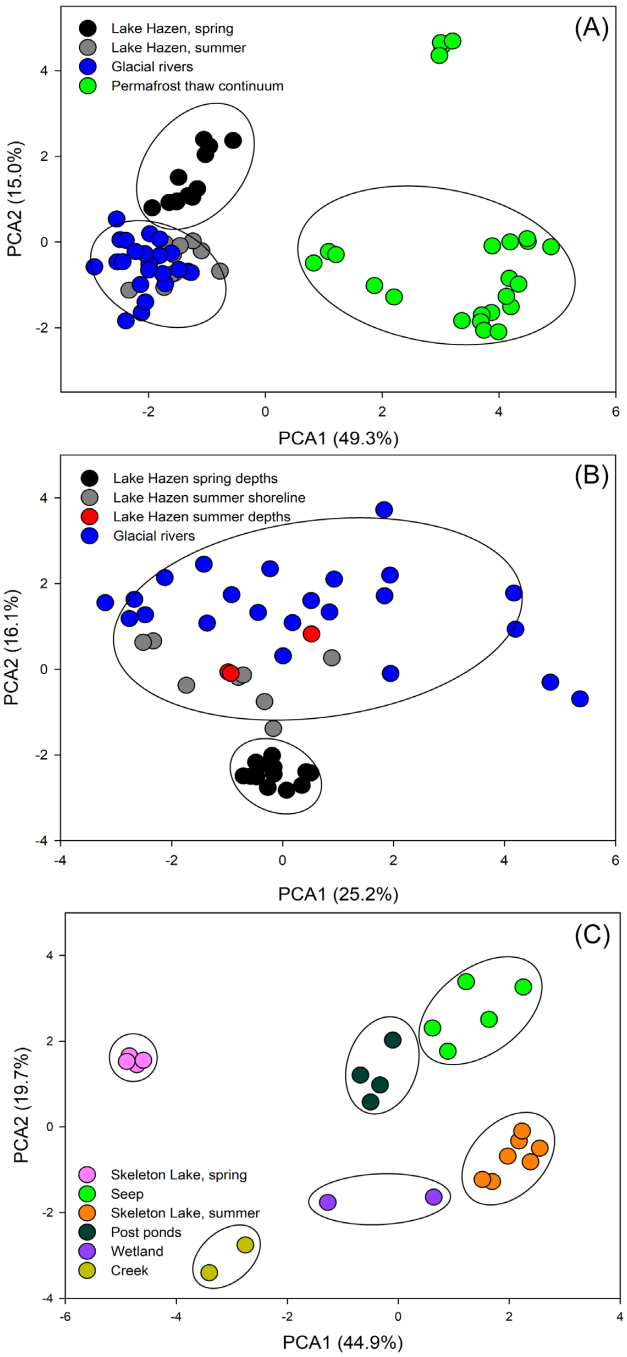

Figure 2. Principal component analyses of relevant physicochemical parameters measured at each freshwater compartment. Ordinations, with 95% confidence intervals, are based on Euclidian distances of physicochemical parameters per freshwater compartment as shown in Tables S4–S6 (Supporting Information). Percent variation captured by axes is shown. **(A)** The three watershed compartments; **(B)** glacial rivers and Lake Hazen; **(C)** active layer thaw-fed compartments.

Freshwater microbial communities

We identified five significantly distinct microbial communities associated with snow, snowmelt, glacial rivers, Lake Hazen and the active layer thaw-fed continuum, as demonstrated

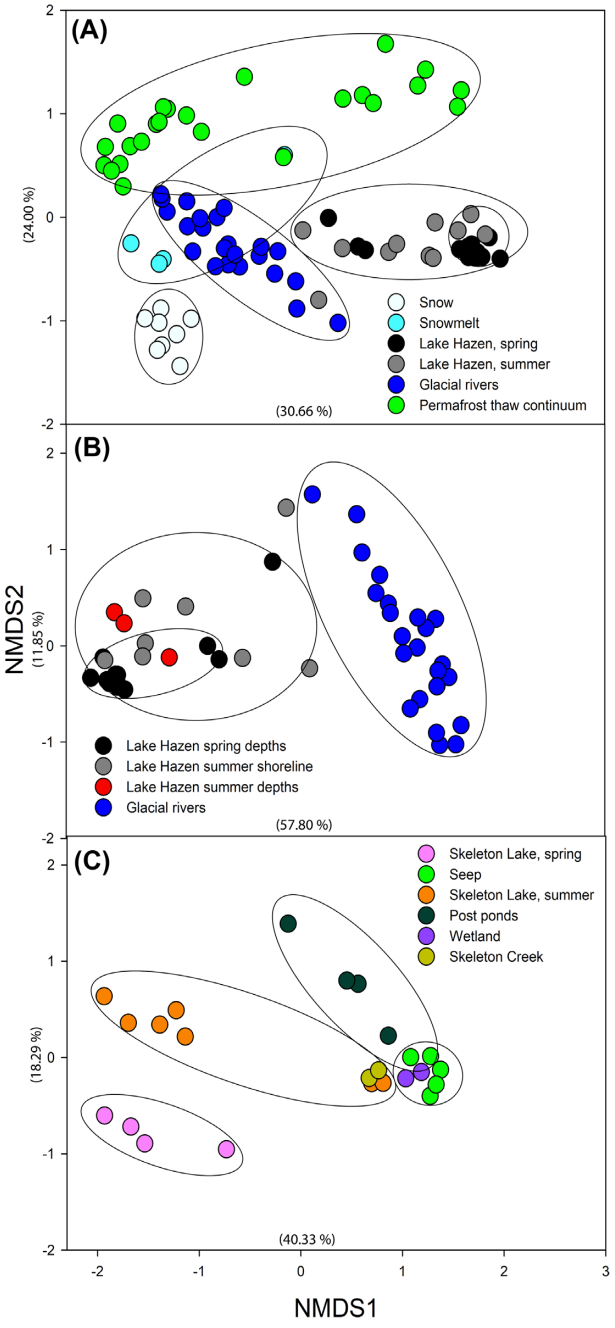

Figure 3. Microbial community patterns in freshwater compartments within the Lake Hazen watershed. NMDS ordinations based on non-transformed OTU abundances. In all panels, sample site is indicated by color, and numbers in brackets on the axes represent the r^2 values. **(A)** NMDS ordination of all freshwater 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 active layer thaw-fed continuum. Stress: 0.06.H

by the five discrete 95% confidence intervals (Fig. 3A). Lake Hazen spring microbial communities were significantly different from summer samples ($P < 0.05$, non-parametric PERMANOVA); however, microbial communities at different depths within a seasonal profile were highly similar to each other (Kruskal–Wallis test, $P > 0.05$; Fig. 3B). Microbial communities in glacial rivers and the Lake Hazen shoreline displayed relatively strong inter-sample similarity (Figure S2, Supporting Information), but there were distinct community-level differences between the microbiomes of glacial rivers and the Lake Hazen water column (Fig. 3B). Microbial community diversity, both in terms of observed and Shannon diversity, was significantly higher in both snow and snowmelt than in Lake Hazen, regardless of season ($P < 0.05$, Wilcoxon rank sum test, Bonferroni-Hochberg corrected; Fig. 4A), as expected. Further, microbial community diversity was also significantly higher ($P < 0.05$, Wilcoxon rank sum test, Bonferroni-Hochberg corrected; Fig. 4A) in the glacial rivers than in Lake Hazen throughout the year ($P < 0.05$, Wilcoxon rank sum test, Bonferroni-Hochberg corrected; Fig. 4A), whereas diversity between spring and summer Lake Hazen communities did not differ significantly (Fig. 4A). The microbial communities of Lake Hazen and the glacial rivers were dominated by members of the phyla Proteobacteria, Bacteroidetes and Actinobacteria (Fig. 5B). Waters along the Lake Hazen shoreline contained distinct microbial populations, with proportionally higher relative abundances of Elusimicrobia and Chloroflexi compared to glacial rivers (Fig. 5B). The relative abundance of OTUs affiliated with these phyla increased in the spring Lake Hazen water column samples. Microbial taxa in the glacial rivers did not significantly vary either temporally or between the different rivers over the sampling period (Fig. 5B). We include a more in-depth look at the relative abundance of classes belonging to Proteobacteria and Bacteroidetes in Figure S5 (Supporting Information).

Indicator species analysis suggested that communities associated with snow contained the largest proportion of unique OTUs (Fig. 6; Table S11, Supporting Information). Glacial river indicator taxa included members from the order Xanthomonadales, Verrucomicrobiales, Flavomicrobiales and Burkholderiales (Fig. 6). Lake Hazen spring indicator microorganisms were dominated by taxa associated with Caldilineales, Bacteroidales, and Rhodobacterales, among others. Lake Hazen during the summer contained indicator OTUs only affiliated with the order Burkholderiales (Fig. 6).

The microbial communities from the seep, post-ponds, and wetlands were similar ($P > 0.05$, non-parametric PERMANOVA; Fig. 3C) despite the physical distance between sites (Fig. 1), and were dominated by OTUs affiliated with the phyla Proteobacteria, Bacteroidetes, and Verrucomicrobia (Fig. 5C). We include a more in-depth look at the relative abundance of classes belonging to Proteobacteria and Bacteroidetes in Figure S5 (Supporting Information). Distinct microbial communities were identified in Skeleton Lake between spring and summer. Alpha diversity generally increased downstream of Skeleton Lake, with the lowest local diversity occurring in Skeleton Lake during the spring (Fig. 4B). There were no indicator species detected among the sub-compartments of the active layer thaw-fed continuum, relative to all of the water compartments sampled within the watershed.

Environmental relationships

Microbial community composition was significantly and positively correlated to specific physicochemical characteristics ($P < 0.05$). Glacial river microbial community composition was best correlated with DO, major ion concentrations, and temperature, cumulatively explaining 37.1% of the total community variation (Table 1). Spring Lake Hazen microbial communities were significantly correlated with turbidity, Ca^{2+}, SO_4^{2-}, PN and temperature (Table 1), which explained 34.3% of community variability (Table 1). In contrast, summer Lake Hazen microbial communities were best correlated with temperature, DIC, turbidity, and TP, components that vary strongly with glacial input and

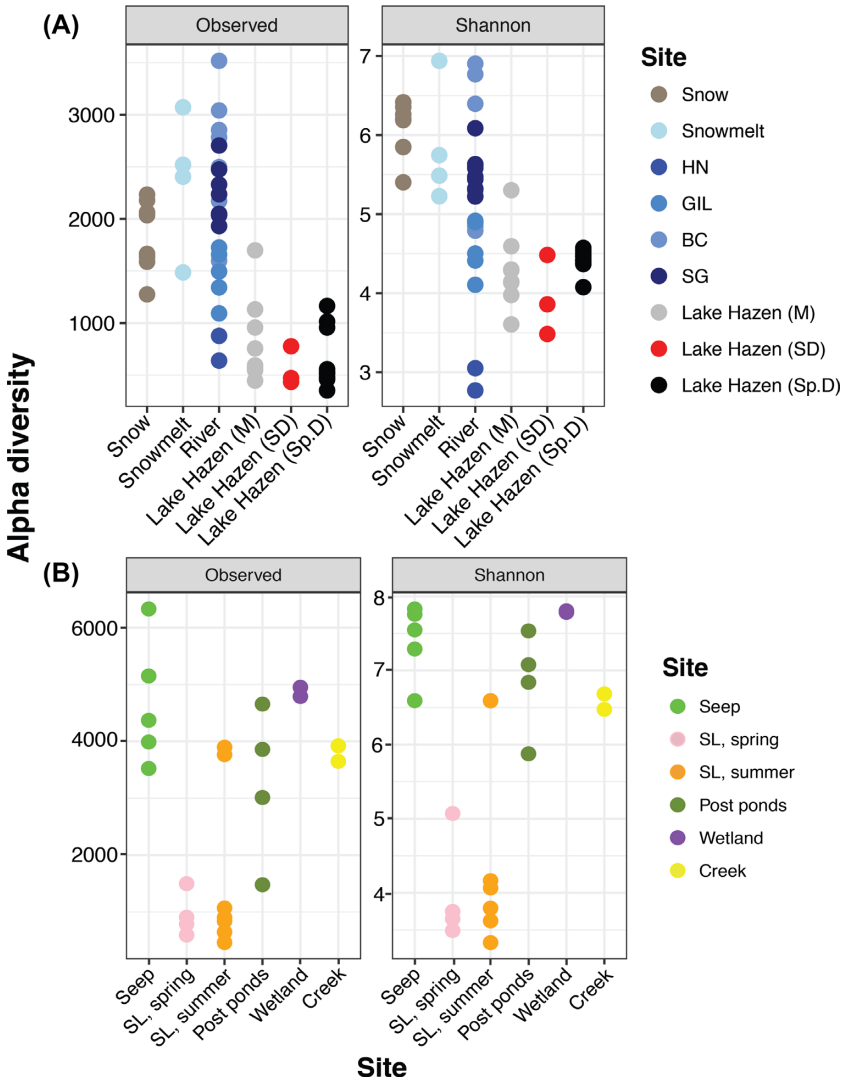

Figure 4. Alpha diversity metrics for specific freshwater compartments within the Lake Hazen watershed. Based on a rarefied dataset with 10 109 sequences each. In both panels, specific sample type is indicated by color. **(A)** Observed OTUs and Shannon indices for snow and glacially sourced freshwaters, organized by compartment. Lake Hazen (M) refers to shoreline samples, Lake Hazen (SD) refers to Lake Hazen summer depths and Lake Hazen (Sp.D) refers to Lake Hazen spring depths. **(B)** Observed OTUs and Shannon indices for compartments within the active layer thaw-fed continuum, arranged from upstream to downstream.

seasonality (Table S3, Supporting Information). These parameters only explained 18.1% of total community variation (Table 1). Indicator taxa in the spring and summer Lake Hazen water column were strongly correlated with physicochemical parameters associated with glaciers, and which exhibited strong seasonality (Table 2). With the exception of Skeleton Lake in spring under ice, the freshwater compartments in the active layer thaw-fed continuum were weakly, albeit significantly, correlated with Na^+, K^+ and DOC (Table 1), the concentrations of which generally increased as water moved downstream of the continuum. These parameters explained 27.6% of overall community variability among sites (Table 1).

DISCUSSION

Microbial communities and their response to environmental parameters

Glacial river microbial communities were best correlated with DO, major ion concentrations, and temperature (Table 1). Variability in these parameters often results from changes in flow rate and the mobilization of source materials (including terrestrial microbial inoculum), rather than specific constraints, such as turbidity, on microbial metabolic potential (Hauptmann *et al.* 2016; Zarsky *et al.* 2018). The rate at which taxa are sourced from the glaciers and via bank erosion is likely greater than the rate at which local environmental sorting can take place in fast-flowing rivers with short residence times (i.e. the mass effects paradigm; Crump *et al.* 2004; Crump, Amaral-Zettler and Kling 2012). Once glacial river microorganisms are deposited into Lake Hazen, which has water residence times of 25–90 years (Köck *et al.* 2012; Lehnherr *et al.* 2018), selection along environmental gradients can occur over longer time scales (Peter and Sommaruga 2016). This is supported by our analysis of local diversity whereby the alpha diversity of microbial communities was significantly higher in glacial rivers than in Lake Hazen ($P < 0.05$, Wilcoxon rank sum test, Bonferroni-Hochberg corrected) (Fig. 4A). Similar to a study conducted by Sheik *et al.* (2015) of an Alaskan glacier system, the taxonomic composition and alpha diversity of glacial river microbial communities were relatively stable over the course of the melt season (Figs 4A and 5B). Therefore, these glacial rivers are likely only passive conduits for

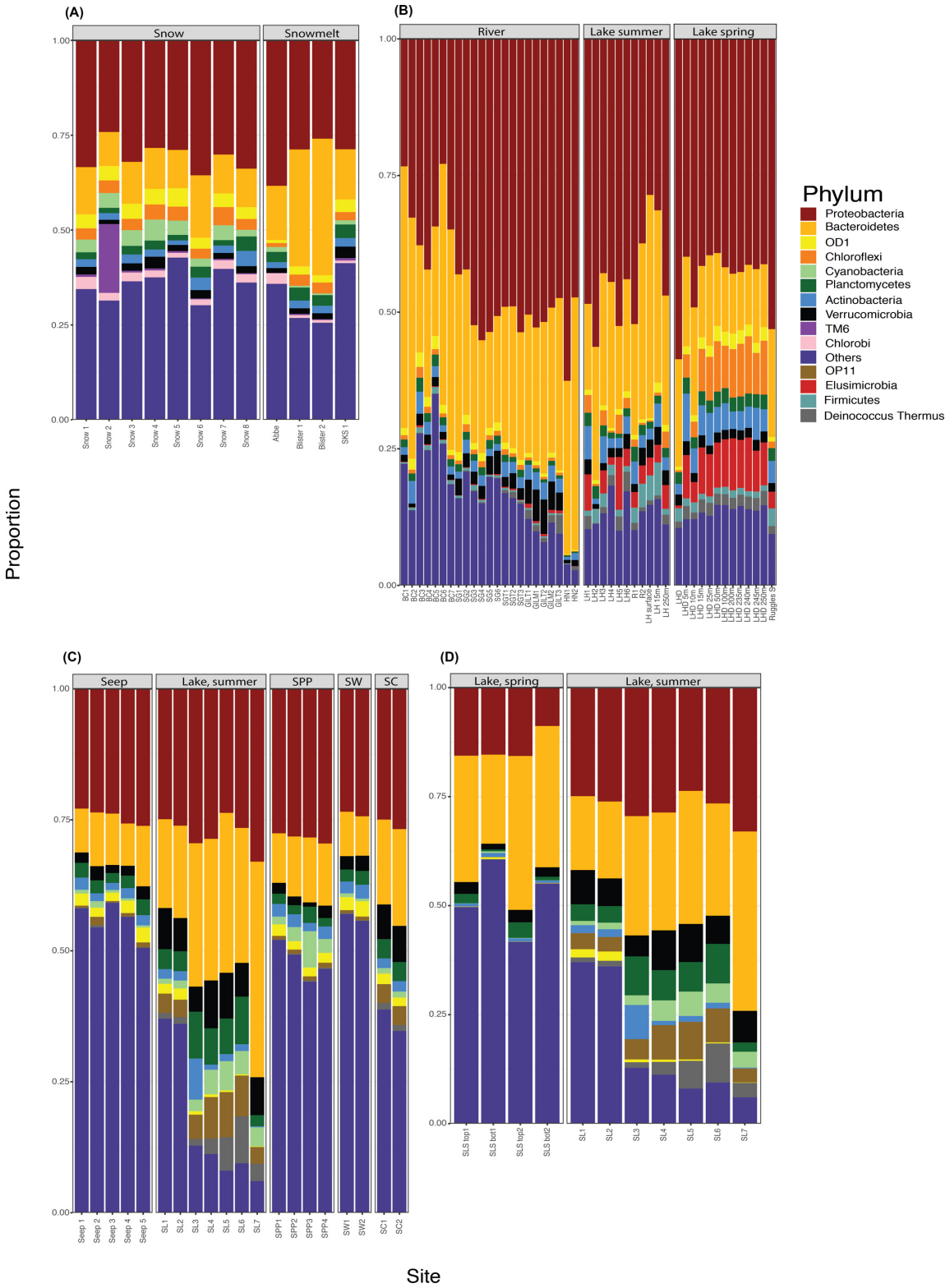

Figure 5. Relative abundances of the top 10 phyla identified within specific watershed compartments. Samples are arranged in chronological order from left to right for each panel, per site (*x* axis). OTU attributions to taxonomic designations are based on the RDP database, defined at 97% similarity thresholds. Colors denoting the presence of specific phyla are uniform across all panels. **(A)** Taxonomy for snow and snowmelt samples. The numbering scheme refers to the order in which they were sampled: as a transect on both the landscape (numbers 1–4), and on Lake Hazen's surface (numbers 5–8) or by time, for snow and snowmelt, respectively. **(B)** Taxonomy for glacial river and Lake Hazen samples, with LH referring to Lake Hazen shoreline samples and LHD referring to Lake Hazen spring water column samples (i.e. taken at depth). **(C)** Taxonomy for active layer thaw-fed continuum samples, where SL is Skeleton Lake, SPP is post-pond, SW is wetland and SC is for creek samples. **(D)** Taxonomy for active layer thaw-fed communities in Skeleton Lake in the summer (SL) versus spring (SLS, where top and bottom refer to different portions of this lake's water column; 2 and 4 m, respectively).

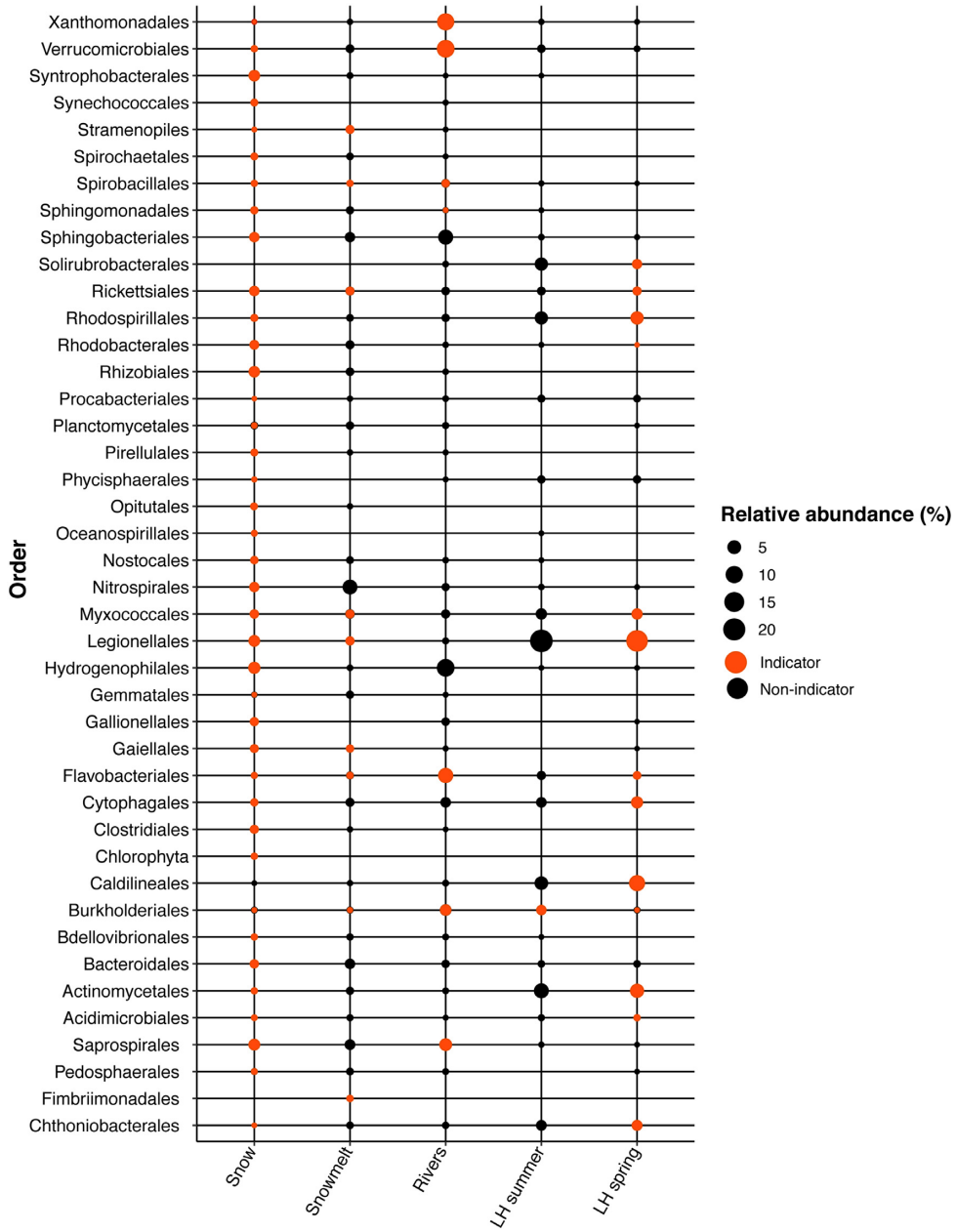

Figure 6. Bubble plot showing relative abundance of indicator orders found within various freshwater compartments within Lake Hazen. Red dots represent OTUs assigned to particular orders that are indicators (i.e. indicator value ≥ 0.7, $P < 0.05$) for a specific site, whereas those that are black contain OTUs assigned to orders 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 associated with the active layer thaw-fed continuum. For a more detailed look into which OTUs are assigned to which order, per freshwater compartment, please see Table S10 (Supporting Information).

microorganisms sourced from bank erosion, snowmelt and/or the glacial system (Cameron *et al.* 2017). These can seed downstream freshwater systems where they are selectively sorted, as in Arctic and other watersheds (Sheik 2015; Hauptmann *et al.* 2016; Zarsky *et al.* 2018).

Further evidence of connectivity in this watershed, in this case between snowmelt and glacial rivers, is shown by a minor seeding signal between these two compartments (Figure S3, Supporting Information; Fig. 3A). Snow has been previously demonstrated to be an important source of microbial assemblages, linking downstream ecosystems together (Comte *et al.* 2018). We found snow to have the highest microbial diversity amongst the sampled sites (Fig. 4A), indicating that its matrix can support a complex variety of microorganisms that can then shape the communities downstream. However, we did not find a strong linkage between snow and snowmelt communities (Figure S3, Supporting Information), even though both hosted similar communities (Fig. 3A), likely because the resulting snowmelt community reflects the extreme bottleneck that new environmental conditions can initiate (Schmidt and Lipson 2004) (warmer temperatures, increased soil-microbe contact) as snow melts. Indeed, snow contained a large number of unique microorganisms, not seen in other compartments of the watershed (Fig. 6). Spring Lake Hazen microbial

Table 1. BIOENV and db-RDA analysis of total Lake Hazen (spring, $n = 13$, summer, $n = 11$) glacial rivers ($n = 22$) and active layer thaw-fed continuum ($n = 24$) microbial communities with relevant physicochemical parameters. All environmental parameters, except for temperature, were logarithmically scaled and then centered prior to correlational analysis.

Freshwater compartment	Physicochemical parameter	Spearman's rho	Significance	Adjusted variation explained (%)
Glacial rivers	Temperature, O_2, SO_4, Na^+ and K^+	0.62	0.04	37.1
Permafrost continuum	DOC, Na^+ and K^+	0.37	0.005	27.2
Lake Hazen, spring	Temperature, SO_4, turbidity, PN, Ca	0.76	0.003	34.3
Lake Hazen, summer	Temperature, DIC, turbidity, TP	0.4	0.011	18.1

Table 2. 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 then 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	0.7	0.006
Lake Hazen, summer	Temperature, SO_4, PN, Ca	0.78	0.002
Lake Hazen, spring	Temperature, SO_4, turbidity, PN, Ca	0.8	0.002

communities were strongly and significantly correlated with parameters that are linked to glacial inputs and mineral weathering (Table 1), suggesting strong environmental and species selection driven by chemical stratification (e.g. DO, turbidity) (Wadham *et al.* 2010; Vargas *et al.* 2017) of the lake over winter (Figure S1, Supporting Information). In contrast, the summer Lake Hazen microbial communities were best correlated with temperature, DIC, turbidity, and TP, components that varied strongly with glacial input and seasonality (Table 1). These parameters only explained 18.1% of total community variation (Table 1), suggesting that other processes occurring primarily along the shoreline of Lake Hazen selected for specific summer microbial community members. Microbial communities immediately downstream of glaciers can be shaped by turbidity, which can modify both water temperature and light penetration (Peter and Sommaruga 2016). Microbial communities in Lake Hazen during the summer may be predominately structured along a spatio-temporal turbidity gradient. For example, *Polaromonas*, one of the Lake Hazen shoreline indicator species, was otherwise only found at the very bottom of the lake during the summer, where turbidity currents brought glacial meltwaters directly to the depths of the lake.

Even though the Lake Hazen shoreline and summer water column were impacted by the high influx of glacial meltwater, sediment and other physicochemical parameters, the Lake Hazen shoreline microbial community was relatively resilient and was thus likely composed of microorganisms specialized for the shoreline environment (Fig. 5B). 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 winter and spring, microorganisms unsuited to the physicochemical environment in Lake Hazen were selected against, and phyla that were only minor components of glacial input (i.e. Elusimicrobia and Chloroflexi) increased in relative abundance to become dominant taxa (Fig. 5B). 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). The biologically active Lake Hazen sediments were composed of similarly dominating phyla, such as Proteobacteria, Bacteroidetes, and Chloroflexi (Ruuskanen *et al.* 2018). These also dominated in the water column, suggesting that glacially sourced microbial species surviving the environmental sorting process within the Lake Hazen water column may eventually deposit, populate, and subsequently regulate biogeochemical processes within lake sediments. Additionally, a large proportion of microorganisms (~40% of the total rarefied community) that belong to the family Comamonadaceae existed within glacial rivers in the summer and Lake Hazen waters throughout the year. Members of this family can be putatively affiliated with iron reducers and denitrifiers (Maintinguer *et al.* 2013; Willems 2014). Given that Lake Hazen has been found to be a strong sink for dissolved inorganic nitrogen (St. Pierre *et al.* 2019), the presence of these microorganisms, along with microbial members from the phylum Chloroflexi, suggests that denitrification may account for a portion of nitrate removal processes within Lake Hazen (despite the fact that denitrification has been thought to be limited in cold environments (Palacin-Linzabe, Camarero and Catalan 2018)), thus influencing important nutrient cycling processes and overall aquatic productivity.

The absence of indicator microorganisms in the active layer thaw-fed continuum, suggests the microbial community of this system may be highly interconnected with the rest of the freshwater compartments of the watershed (Fig. 6; Table S11, Supporting Information). With the exception of ice-covered Skeleton Lake in spring, the freshwater compartments in the active layer thaw-fed continuum were weakly, albeit significantly, correlated with Na^+, K^+ and DOC (Table 1). Gradual elevation of

DOC, Na^+ and K^+ concentrations from upstream to downstream (Figure S4, Supporting Information) may be due to evaporative processes, decomposition of *Carex* sp. sedges in the wetland, and groundwater seepage. Both Skeleton Lake and the adjacent post-ponds, wetland, and creek are sources of methane to the atmosphere (Emmerton *et al.* 2016a). Although methanogens were undetected in the water column in this study, they have been observed in Skeleton Lake's sediments (Ruuskanen *et al.* 2018). Methanotrophs were also present in these waters, although were not a major constituent of the community, indicating that some breakdown of methane potentially occurs.

Within the active layer thaw-fed continuum, the top 10 taxa were mostly related to potential denitrifiers within the family Comamonadaceae (Table S12, Supporting Information; as detected in Lake Hazen) and the phylum Verrucomicrobia. Although the latter phylum is poorly described, microorganisms within it may be capable of anaerobic metabolisms (Yoon 2014) and thus specially adapted to nutrient-rich wetlands and other productive freshwaters fed by active layer thaw-fed continuum.

Increases in alpha diversity downstream of Skeleton Lake during the summer months (Fig. 4B) are likely a result of the strongly selective environment in Skeleton Lake where zooplankton graze on microbes, biofilms form in shallow zones and where microbial competition is elevated. Indeed, many members of the microbial community in Skeleton Lake during the summer (Fig. 5C) are likely unsuited to downstream environments (Hauptmann *et al.* 2016). Microorganisms that survive the selection process in the different active layer thaw-fed compartments and flow into Lake Hazen are predominantly structured according to the nutrient and water retention capacity of the wetlands along Skeleton Creek (Knox *et al.* 2008; Evans, Martiny and Allison 2017).

During spring, Skeleton Lake had lower microbial diversity than in the summer and along the rest of the continuum (Fig. 5C and D). The distinct physicochemistry of Skeleton Lake (Fig. 2C) during springtime conditions was likely mediated by depleted DO concentrations due to loss of photosynthetic activity and possible increase in heterotrophic activity under ice cover. These conditions impose distinct selective forces on the microbial community relative to those in summer, resulting in low alpha diversity (Fig. 4B), limited photosynthetic bacteria (e.g. cyanobacteria; Fig. 5C and D) (Keatley, Douglas and Smol 2008), and higher relative abundance of heterotrophic taxa (e.g. Flavobacteriia from phylum Bacteriodetes and Betaproteobacteria; from phylum Proteobacteria, Figure S5, Supporting Information) in Skeleton Lake. In general, communities from this active layer thaw-fed continuum are likely structured according to competitive interactions and evaporative processes that impact shallower bodies of water (Granger and Hedstrom 2011), suggesting that the microbial community structures and biogeochemical cycles (Emmerton *et al.* 2016a,b) of this smaller freshwater environment, characteristic of many Arctic freshwater systems (Emmerton *et al.* 2016b) may be particularly vulnerable to climate change.

Future implications for microbial communities under a warming High Arctic climate

Our baseline characterization has identified distinct microbial communities in three main freshwater compartments of the Lake Hazen watershed: glacial rivers, an active-layer thaw-fed continuum and Lake Hazen during the spring. Glacial river microbial communities were most strongly controlled by mass effect dynamics, whereby the rate at which taxa entering these high-flow rivers from glacial and erosional environments is greater than the rate at which local environmental sorting can take place (Niño-Garcia, Ruiz-Gonzales and Del Giorgio 2016). In contrast, active layer thaw-fed communities were more influenced by evaporative processes characteristic of shallower bodies of water, delivering select communities to downstream Lake Hazen, even though these types of systems were not major hydrological contributors.

In the coming decades, High Arctic watersheds like that of Lake Hazen will continue to experience reduced ice and snow cover (Bring *et al.* 2016), increased permafrost thaw (Koven, Riley and Stern 2012) and glacial melt (Radic *et al.* 2013). In the case of the glacial rivers within the Lake Hazen watershed, more sediment-associated microbiota and physicochemistry may be incorporated and delivered to Lake Hazen downstream. This increased input, combined with potentially warmer winters and less ice cover duration annually, will not only decrease the water residence time of this lake (Lehnherr *et al.* 2018), but may also shift the unique microbial Lake Hazen signal to a transitional community shaped by turbidity, temperature and light penetration gradients. This could result in an overall decrease in phototrophy and an increase in denitrification and associated microbial pathways (Ansari 2016).

Increasing active layer thaw within the Lake Hazen watershed may also serve to increase the amount of previously archived organic nutrients available to resident microbial communities across the continuum. The microbial communities found within this continuum, including in the seep, lake, ponds, wetland and creek, may also experience increased evaporation as ambient temperatures increase and thus further selection will occur according to resulting sodium and potassium gradients. Eventually, this could result in a wetland continuum with a microbial community dominated by denitrifiers, methanogens and methanotrophs. As such, terrestrial and freshwater compartments across High Arctic watersheds may become increasingly connected, where unique microbial signatures previously belonging to distinct water compartments such as rivers, seeps, creeks, wetlands and lakes may be lost over time as physicochemical characteristics merge with the warming climate. Thus, as the Lake Hazen watershed encompasses a variety of freshwater systems that are interlinked over a large area, it acts as both a suitable model and sentinel system to assess how the composition of microbial communities and their relationships with various physicochemical conditions may shift and respond to climate change.

Microorganisms, as well as particulates and solutes exported from both glacial rivers and active layer-thaw systems, have the potential to destabilize resident microbial communities, with potentially important implications for biogeochemical processes in freshwater ecosystems. However, environmental selection pressures over relatively long water residence times in large water bodies, like Lake Hazen, may help to stabilize fluctuations in microbial inputs from glacial rivers and/or active layer thaw. Hence, there has likely been a strong taxonomic and, by extension, functional resiliency of the microbiome of Lake Hazen. It is yet unclear, however, whether there is a threshold to this resiliency, particularly as this work focuses on the 16S rRNA gene, which does not reliably link taxonomy to function. Nonetheless, if actions are not taken to curb global greenhouse gas emissions, we might expect an important loss of stability and resiliency in the microbiomes of Arctic freshwater ecosystems, with potentially important consequences for ecosystem function and services. Ongoing monitoring of this

watershed and others with shotgun metagenomics, along with the presence of indicator species previously identified in each watershed compartment would enable us to understand the impact that the climate change has on the microbial communities of High Arctic freshwater systems.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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