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Key Points:
- The subglacial environment provides a first-order control on the microbial assemblages exported in glacial meltwater
- Water source (i.e., supraglacial or extraglacial) and subglacial residence time are second-order controls on the microbial assemblages exported in glacial meltwater

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Hydrological controls on glacially exported microbial assemblages

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Abstract The Greenland Ice Sheet (GrIS) exports approximately 400 km^3 of freshwater annually to downstream freshwater and marine ecosystems. These meltwaters originate in a wide range of well-defined habitats that can be associated with very different physical environments within the ice sheet, ranging from oxygenated surface environments that are exposed to light and supplied with nutrients from atmospheric/aeolian sources to subglacial environments that are permanently dark, isolated from the atmosphere, and potentially anoxic. Hydrological conditions in the latter likely favor prolonged rock-water contact. The seasonally evolving hydrological system that drains meltwaters from the GrIS connects these distinct microbial habitats and exports the microbes contained within them to downstream ecosystems. The microbial assemblages exported in glacier meltwater may have an impact on downstream ecosystem function and development. We explored how the seasonal development of a glacial drainage system influences the character of microbial assemblages exported from the GrIS by monitoring the seasonal changes in hydrology, water chemistry, and microbial assemblage composition of meltwaters draining from a glacier in southwest Greenland. We found that the microbial assemblages exported in meltwaters varied in response to glacier hydrological flow path characteristics. Whether or not meltwaters passed through the subglacial environment was the first-order control on the composition of the microbial assemblages exported from the glacier, while water source (i.e., supraglacial or extraglacial) and subglacial residence times were second-order controls. Glacier hydrology therefore plays a fundamental role in determining the microbial exports from glaciated watersheds.

Plain Language Summary Glacier meltwater volume is increasing as the result of a warming climate. Much of this water is transported beneath the glacier. We examined the impact of passing through the subglacial environment on the microorganisms in the water: these microorganisms are the only living things in this environment and have the potential to strongly influence downstream marine ecosystems. We found that passing through the subglacial environment changed the microbial communities and that the amount of time spent and the source of the water were secondary, but still important, influences on the microbial communities leaving the glacier. These findings, combined with previous observations that subglacial environments around the world host similar microbial communities, indicate that meltwater that passes beneath glaciers may house similar microbial communities and that these communities have the potential to affect downstream ecosystems.

1. Introduction

Once thought to be devoid of active microbial life, glacier systems are now known to harbor productive and diverse microbial assemblages [*Anesio and Laybourn-Parry*, 2012; *Boetius et al.*, 2014] that can be exported to downstream ecosystems in meltwater. The microbial exports from glacier systems can influence downstream ecosystem processes [e.g., *Battin et al.*, 2004], biodiversity [*Wilhelm et al.*, 2013], and the biogeochemistry [*Logue et al.*, 2004; *Battin et al.*, 2009; *Singer et al.*, 2012] and microbial ecology of fjord surface waters [*Gutiérrez et al.*, 2014; *Cameron et al.*, 2016]. The microbial characteristics of glacier runoff is of importance since downstream ecosystems support particularly high rates of primary productivity [*Rysgaard et al.*, 2012], contain genetically isolated populations [*Sköld et al.*, 2003], function as refugia for cold-water species [*Wesslawski et al.*, 2011], and are important feeding grounds and critical habitats for a number of marine mammals and seabirds [*Kuletz et al.*, 2003; *Mathews and Pendleton*, 2006; *Arimitsu et al.*, 2012].

Some of the best documented microbial glacier habitats are those that are present on the glacier surface in wet snow, cryoconite holes, supraglacial lakes, and streams (supraglacial environment) [*Hodson et al*., 2008] and at the glacier bed in the pore waters of saturated till, water-filled cavities, and large subglacial channels and lakes (subglacial environment) [*Tranter et al*., 2005; *Christner et al*., 2014]. These habitats vary widely in terms of physical conditions, including the presence/absence of light energy and meltwater, the type/quantity of sediment available, allochthonous input of nutrients and microbes, redox conditions, temperature, seasonality, and meltwater residence times. These physical differences can give rise to site-specific microbial assemblages and biogeochemical processes. The most notable difference in microbial assemblages is the presence of photosynthetic species in the supraglacial environment [*Anesio et al*., 2009; *Hodson et al*., 2010] and chemosynthetic organisms in the permanently dark, sediment-rich subglacial environment [*Boyd et al*., 2014; *Christner et al*., 2014]. However, even within the subglacial environment, microbial assemblages consist of both locally distinct [*Bhatia et al*., 2006; *Lanoil et al*., 2009] and globally distributed species [*Foght et al*., 2004; *Tranter et al*., 2005; *Bhatia et al*., 2006; *Lanoil et al*., 2009]. The hydrological networks that export the ~400 km^3 of meltwater from the Greenland Ice Sheet (GrIS) to coastal environments each year [*Tedesco et al*., 2013] evolve over the course of a melt season and dictates the proportion of water that is exported from microbial habitats within the supraglacial and subglacial systems and the meltwater residence times in each. Most surface meltwater on the GrIS is derived from snow and ice melt on the glacier surface (supraglacial environment), where it may be routed through snow and firn, cryoconite holes, and/or supraglacial or ice-marginal lakes and streams. In these environments, microbial cells and nutrients are largely supplied by atmospheric/aeolian sources, and organisms are exposed to light. While some regions of the GrIS are cold based, and supraglacial meltwater drains directly into the proglacial system, polythermal outlet glaciers draining the GrIS are believed to have similar hydrology to that of smaller, temperate, and polythermal alpine glaciers [*Bartholomew et al*., 2011; *Bhatia et al*., 2011; *Chandler et al*., 2013]. In these systems, the accumulation of summer meltwaters on the glacier surface can induce the hydrologically driven propagation of fractures and initiate connections between the surface and bed of the glacier via crevasses and moulins [*Boon and Sharp*, 2003] and drive the seasonal development of subglacial drainage.

The subglacial drainage system of polythermal glaciers drainage the GrIS can include permanent elements as well as elements that form, grow, and change structure over the course of a melt season and then collapse over winter. There are two end-members of subglacial drainage system structure, "distributed" and "channelized" networks [*Fountain et al*., 1998, and references therein]. Distributed or multithread drainage networks dominate the subglacial environment in the winter and early in the melt season, when runoff is largely derived from basal melt (from geothermal or frictional heat), groundwater, and/or water stored at the bed in a preceding summer. Such networks typically lack large, well-defined channels, and water passes through them relatively slowly following inefficient pathways where it is under high pressure [*Fountain et al*., 1998]. These pathways include water films, till pore waters, and poorly interconnected, water-filled cavities.

Large channels develop and form a more efficient channelized drainage system as the flux of supraglacial meltwater delivered to the glacier bed increases during the melt season. These systems receive meltwaters from both the supraglacial environment and regions of the bed with distributed drainage and promote rapid transit to the terminus [*Chandler et al*., 2013]. Efficient hydrological connectivity with the glacier surface allows channelized drainage systems to experience weather-related or diurnal discharge-related fluctuations in water pressure, which can result in reversals of the pressure gradient between the channelized and distributed drainage systems and bidirectional exchanges of water between the two systems [*Hubbard et al*., 1995].

We hypothesized that seasonal changes in both hydrological routing and water sources would yield strong intraseasonal differences in the makeup of microbial assemblages exported from the GrIS to downstream ecosystems in meltwaters. Here we examine how the seasonal evolution of meltwater sources and subglacial drainage system properties affects the character of the microbial assemblages exported in runoff from a sector of the Greenland Ice Sheet (GrIS). We monitored meltwater discharge, hydrochemistry, and the composition of the microbial assemblages contained in waters exiting the GrIS throughout a single melt season. We found a significant change in the microbial assemblage composition exported in meltwaters as they were routed through the subglacial system. In addition, microbial assemblages in the proglacial stream became more similar to those in ice sheet surface waters as a more efficient, channelized subglacial drainage system developed and as high channel discharges and water pressures restricted export of waters and microbes from the distributed component of the subglacial drainage system.

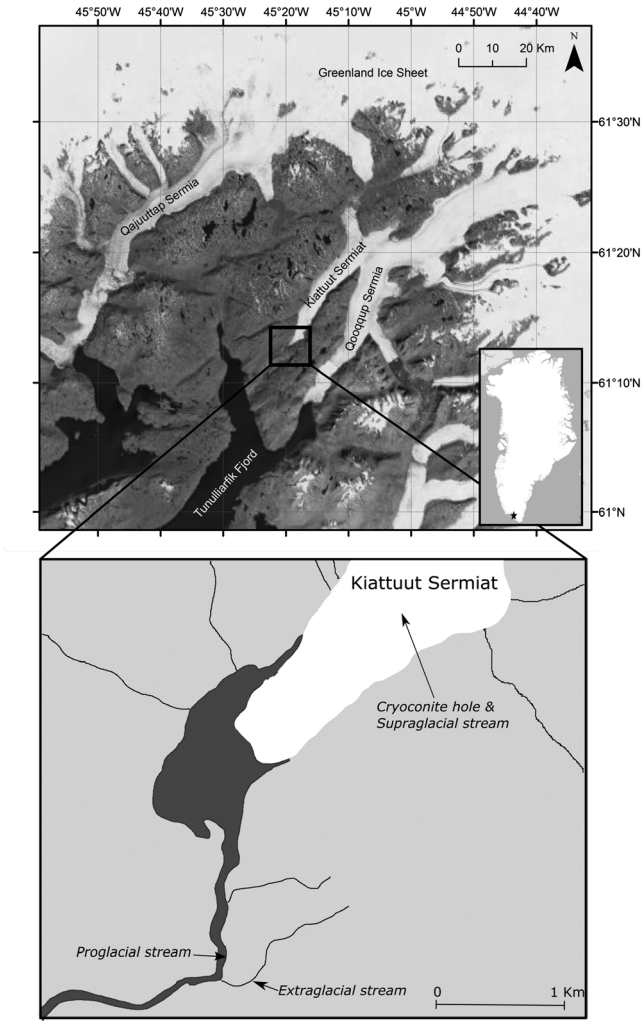

Figure 1. Study site including Kiattuut Sermiat (white), its proglacial lake/stream (dark grey) and extraglacial streams (black), and sample locations (italics).

2. Materials and Methods

2.1. Field Site

Kiattuut Sermiat (KS; Figure 1) is a 36 km^2 glacier in southern Greenland that terminates on land approximately 9 km from Tunulliarfik Fjord [*Hawkings et al*., 2016]. The glacier is located in a mountainous region with a bedrock geology dominated by crystalline granite and diorite, pyroxene-biotite monzonite, and basaltic intrusions [*Henriksen et al*., 2009]. The surrounding landscape contains podzolic soils [*Jones*, 2010] which support a vegetation cover of lichens, shrubs, sedges, and herbs. KS has an active subglacial drainage system fed by runoff from both the glacier surface and surrounding hills. It currently terminates in a small proglacial lake (0.5 km^2).

2.2. Sample Collection and Processing

Fieldwork was completed at KS from 1 May to 9 August 2013 and, in summary, included (1) continuous hydrometric monitoring (water level and electrical conductivity, EC) on the proglacial stream, (2) daily biogeochemical (major ions, nutrients, pH, and EC) and suspended sediment sampling on the proglacial stream, (3) microbial (approximately biweekly) sampling of the proglacial stream, and (4) microbial and biogeochemical spot sampling of waters from cryoconite holes and both supraglacial and extraglacial (draining nonglaciated upland areas) streams (Figure 1).

A continuous discharge record was generated for the KS proglacial stream using multiple water level loggers (two HOBO® Onset water level loggers and two Druck wired pressure transducers), installed in the upper 3 km of the river. Water level data at 10 min intervals were converted to discharge estimates using a rating curve generated from 51 instantaneous discharge measurements derived from rhodamine dye-dilution experiments as described by *Bartholomew et al*. [2011] and *Hawkings et al*. [2016]. Temperature-compensated electrical conductivity **(**EC) records were collected using Campbell Scientific sensors with 10 min resolution. pH was measured continuously using an ISFET (ion-selective field-effect transistor) Honeywell Durafet® pH sensor, calibrated monthly using low ionic strength pH 4.01 and pH 6.96 Reagecon buffers. The pH and EC of discrete samples were measured using a Beckman Coulter ϕ 470 series handheld meter with the pH sensor calibrated daily using National Institute of Standards and Technology-certified Fisherbrand™ pH 7 and 10 buffers.

Proglacial meltwater samples were collected daily using a three times sample-rinsed 1 L high density polyethylene (HDPE) bottle (Nalgene®). This water was used for analyses of suspended sediment concentration, dissolved organic carbon (DOC), dissolved phosphorus species (dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP)), dissolved nitrogen (NO_3^-, NH_4^+, and dissolved organic nitrogen

(DON)), and major ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, Cl^-, and F^-). Additional samples were collected from the supraglacial and extraglacial environments throughout the melt season. Separate collection bottles were used for each sample site. For suspended sediment concentration, a known water volume was filtered through a preweighed 47 mm 0.45 μm Whatman® cellulose nitrate filter paper using a polyethersulfone filtration unit (Nalgene®). Papers were later oven dried overnight and reweighed. Samples for analysis of $\delta^{18}O$, δ^2H, and DOC were filtered through a 0.7 μm sample-rinsed syringe filter (Whatman® GD/X filter with GF/F membrane) and collected into either a dry 1.5 mL Chromacol™ glass vial (isotopes) or a precombusted and three times sample-rinsed 30 mL borosilicate glass vial (DOC). Samples for DIP, DOP, NH_4^+, and NO_3^- were filtered through 0.45 μm syringe filters (Whatman® GD/XP polyethersulfone) into three times sampled-rinsed 30 mL HDPE bottles (Nalgene®) and stored frozen near −20°C until analysis [*Hawkings et al.*, 2016]. Similarly, major ion samples were filtered through 0.45 μm syringe filters (Whatman® GD/XP polyethersulfone) into three times sample-rinsed plastic 30 mL HDPE Nalgene® bottles but were refrigerated until analysis.

Samples for microbial analyses were collected in sterile Nasco Whirl-Pak™ sample bags and filtered within 2 h through a three times sample- rinsed glass filtration kit and a Pall Supor® 47 mm diameter, 0.2 μm pore size, sterile filter paper. The filter paper was retrieved using acid-washed and rinsed forceps and placed into an autoclave-sterilized Eppendorf tube with 1 mL RNALater (Qiagen). Samples were frozen immediately and stored at −20°C until analysis.

2.3. Chemical Indices

Differences in the geochemical histories of waters emerging from the glacier were detected using four simple chemical indices:

$$D:M = \frac{\left(Ca^{2+} + Mg^{2+}\right)}{\left(Na^{+} + K^{+}\right)} \tag{1}$$

where concentrations are in μeq L^{-1}

$$\mathrm{SMF} = \frac{SO_4^{2-}}{\left(SO_4^{2-} + HCO_3^{-}\right)} \tag{2}$$

where concentrations are in μeq L^{-1}

$$\rho CO_2 = \log_{10}\left[HCO_3^{-}\right] - \mathrm{pH} + K_H + K_1 \tag{3}$$

where concentrations are mol, K_H is $10^{-1.12}$ mol L^{-1} amt^{-1}, and $K_1 = 10^{-6.6}$ mol L^{-1} amt^{-1}.

1. The ratio of divalent to monovalent cations (D:M; equation (1)), which is a proxy for the relative contributions of silicate and carbonate weathering to the total solute load. Na^+ and K^+ are assumed to be derived primarily from silicate weathering and Ca^{2+} and Mg^{2+} primarily from carbonate weathering [*Tranter et al.*, 2002; *Wadham et al.*, 2010]. Silicate weathering has relatively slow reaction kinetics and is thought to occur primarily in the distributed drainage system, while carbonate weathering, which has relatively rapid reaction kinetics, dominates the solute load of waters that pass rapidly through the channelized system [*Tranter et al.*, 2002]. As a result, waters from distributed drainage networks typically yield low divalent to monovalent cation ratios, while those from channelized drainage systems typically yield high divalent to monovalent cation ratios [*Wadham et al.*, 2010].
2. The sulfate mass fraction of the total anion load (SMF; equation (2)) provides a measure of the fraction of HCO_3^- that is derived from sulfide oxidation coupled with carbonate dissolution. As meltwaters from distributed drainage networks have more prolonged access to freshly comminuted reactive sulfide minerals than waters from channelized systems, they typically have higher SMF values than those from channelized drainage networks [*Wadham et al.*, 1998, 2004; *Tranter et al.*, 2002]. Sulfide oxidation coupled with carbonate dissolution (equation (1)) results in SMF = 0.5. Carbonation reactions (where aqueous CO_2 provides the proton source equation (2)) dominate weathering in channelized systems where meltwater residence times are short and result in SMF < 0.5. Sulfide oxidation coupled with silicate weathering and/or precipitation of carbonate (equation (3)) yields SMF > 0.5.
3. The partial pressure of CO_2 with which the waters appear to be in equilibrium (ρCO_2; equation (3)) provides a measure of the degree to which waters appear to be in equilibrium with atmospheric

CO_2 and indicates the balance between CO_2 diffusion into/out of solution and the rate of CO_2 drawdown via chemical weathering processes [*Raiswell*, 1984; *Raiswell and Thomas*, 1984]. Waters in equilibrium with atmospheric CO_2 have a ρCO_2 of $10^{-3.5}$ atm. Higher ρCO_2 values are possible if the rate of proton supply exceeds the rate of consumption, for example, if there is an additional source of H^+ beyond the dissolution of atmospheric CO_2 in water (e.g., from oxidation of organic carbon or sulfide minerals). In contrast, lower ρCO_2 values occur when rates of CO_2 consumption by chemical weathering processes exceed rates of CO_2 diffusion into solution [*Sharp*, 1991]. Differences in proton supply and consumption between distributed and channelized systems tend to suggest that high ρCO_2 waters are characteristic of distributed systems, while low ρCO_2 waters are associated with channelized systems.

4. The EC of meltwaters is a proxy for the solute concentration in meltwaters. EC tends to be higher in distributed drainage networks, where prolonged contact between water and highly reactive minerals yields higher solute concentrations and lower in dilute supraglacial and channelized subglacial systems where the duration of rock-water contact is limited.

2.4. Analytical Procedures

Major cation (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and anion (Cl^-, NO_3^-, and SO_4^{2-}) concentrations were measured using a Thermo Scientific™ Dionex™ capillary ICS-5000 fitted with simultaneous anion and cation columns, with HCO_3^- estimated as the charge deficit [*Hawkings et al*., 2015]. DOC and TN were measured via high-temperature combustion (680°C) using a Shimadzu TOC-V_{CSN}/TNM-1 analyzer. DIP and DOP were measured using a LaChat QuickChem® 8500 series 2 flow injection analyzer as in *Hawkings et al*. [2016], with DOP determined after acid persulfate digestion. NH_4^+ was also measured using a LaChat QuickChem® 8500 series 2 flow injection analyzer, as described by *Wadham et al*. [2016]. Oxygen and hydrogen isotopes were measured using a Los Gatos Research liquid-water isotope analyzer following the procedure described by *Lis et al*. [2008].

DNA extraction from preserved filters was performed using a FastDNA® SPIN KIT following the manufacturer's protocol. DNA was extracted from duplicate filter papers collected from each sampling site and combined prior to library preparation. Library construction included a two-step polymerase chain reaction (PCR) protocol designed to prepare amplicons for IonTorrent Personal Genome Machine sequencing. In the first step, the V3 variable region was targeted using a 341 forward primer (5′ -CCTACGGGAGGCAGCAG- 3′) carrying a universal GLENN tag (5′ -CAGTCGGGCGTCATCA- 3′; developed by Travis Glenn, http://www.gvsu.edu/dna/universal-primer-tag-6.htm) and a 518 reverse primer (5′ -ATTACCGCGGCTGCTGG- 3′) carrying a trP1 adapter (5′ -CCTCTCTATGGGCAGTCGGTGAT- 3′). Bar codes to allow multiplexing of samples were added in the second step, which used the matching GLENN universal tag carrying a bar-coded forward primer and the matching trP1 adapter as the reverse primer. PCR conditions were as follows: 95°C for 4 min, followed by 10 cycles of 95°C for 15 s, 65°C for 30 s, and 72°C for 30 s; the next 20 cycles included 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s; finally, the last elongation step was performed at 72°C for 10 min. Subsamples were mixed in equal concentrations and purified using UltraClean GelSpin DNA Extraction Kit. Samples were amplified in triplicate in the first step, combined, and amplified in triplicate and combined in the second step to control for PCR biases. Negative control blanks were extracted and processed along with the samples, and no PCR product or sequences were obtained from these blanks. The subsequent sequencing protocol was performed using Ion Torrent technology with the Ion PGM™ Sequencing 200 Kit v2 with the Ion 316 Chip v2.

2.5. DNA Sequence Processing and Statistical Analyses

Raw sequences were processed using the Mothur 454 SOP (v. 1.33.3) [*Schloss et al*., 2009, 2011, http://www.mothur.org/wiki/454_SOP]. Briefly, sequences were initially subject to quality control parameters that discarded those sequences (i) below a quality score of 25, (ii) shorter than 100 bp, (iii) containing primer and barcode mismatches, and (iv) containing homopolymers longer than 8 bp. Alignment of sequences was performed using the SILVA reference database. Sequences were then preclustered for further noise reduction as recommended [*Pruesse et al*., 2007; *Huse et al*., 2010; *Schloss*, 2010; *Schloss et al*., 2011]. Chimeras were removed using the UCHIME implementation within Mothur [*Edgar et al*., 2011; *Schloss et al*., 2011]. Operational Taxonomic Units (OTUs) were assigned using a 97% sequence similarity definition with the average neighbor clustering algorithm. OTUs were then classified to the highest taxonomic resolution possible (i.e., phylum, class, family, and/or genus level) using the Ribosomal Database Project classifier

(train set 9) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). Those sequences corresponding to chloroplast, mitochondria, Eukarya, or unknown were removed; furthermore, those OTUs represented by only a single sequence were removed.

Processed sequences were subsampled to lower than the smallest library size to allow identical sequencing depth for each sample before further alpha and beta diversity analyses [*Gihring et al*., 2012]. For the complete data set (supraglacial, extraglacial, and proglacial samples), each sequence data set was subsampled to 1000 sequences, while for the sample group including only the proglacial samples, each sequence data set was subsampled 2500 sequences.

Taxon (OTU) richness was measured in Mothur using observed richness and estimated richness with Chao1 nonparametric richness estimator; overall diversity was estimated using inverse Simpson's index (1/*D*) as well as the Shannon diversity index (*H*), which are nonparametric diversity estimators that consider both species richness and species evenness [*Shannon*, 1947; *Simpson*, 1949; *Chao*, 1984; *Chao and Shen*, 2003].

Beta diversity was examined using PC-ORD (version 6) [*McCune et al*., 2002] where changes in bacterial assemblage composition were visualized using nonmetric multidimensional scaling (NMS) ordination using 100 runs of real data. To determine if axes created were significantly better than would be obtained by chance, a Monte Carlo test of 100 runs on randomized data was also performed. Based on three distinct hydrological periods, we grouped samples in the NMS ordination into "Early Season", "Transition Period", and "Late Season" groups and determined whether there were significant changes in assemblage composition between these groups using the multiresponse permutation procedure (MRPP) [*McCune et al*., 2002]. Three statistical values are obtained in MRPP analysis: the test statistic (*T*) describing separation between groups, where greater separation is implied as values become more negative; within-group agreement (*A*), which is a chance-corrected value where $A = 0$ when within-group heterogeneity equals that expected by chance, $A < 1$ when within-group heterogeneity is greater than expected by chance, and $A = 1$ (maximum value) when all samples within a group are identical; and a *p* value for overall and multiple comparisons [*McCune et al*., 2002].

The relationship between physical/chemical variables and changes in bacterial assemblage composition were determined in PC-ORD using Mantel tests; Mantel tests were conducted using independent runs for each environmental parameter. To determine specific changes in biogeochemical or microbial parameters, we used analysis of variance (ANOVA) with Tukey's post hoc HSD for multiple comparisons.

3. Results

3.1. Hydrology/Hydrochemistry

Three distinct hydrological periods were defined on the basis of major shifts in the discharge, electrical conductivity, suspended sediment content, and chemical composition of the proglacial stream waters at KS. We use these shifts to define three distinct runoff periods that we refer to as the Early Season, Transition Period, and Late Season (Figure 2). Although there is some variability in the exact dates on which distinct shifts in each parameter occur, the shifts are present in all records and, in each case, are synchronous to within a 2 day period (Figure 2).

The Early Season (prior to 6 June) was characterized by low proglacial discharge (<10 m^3 s^{-1}) with little diurnal variability, even though daytime high air temperatures were above 0°C and there was a strong diurnal air temperature signal. The chemistry of these waters was distinct from that of waters leaving the glacier in subsequent periods. It is characterized by the lowest average ratio of divalent to monovalent cations (5.91) and the highest mean values of SMF (0.16), ρCO_2 ($10^{-3.90}$ atm), and EC (49.6 μS cm^{-1}; ANOVA, $p < 0.05$), indicating long transit times. Only during this period did suspended sediment concentrations increase consistently over time (from ~0.04 g L^{-1} to ~0.22 g L^{-1}).

The Transition Period lasted from 7 June to approximately 26 June. Its onset was marked by a "spring event" [*Mair et al*., 2004], including a distinct peak in discharge (43 m^3 s^{-1}) and suspended sediment concentration (0.22 g L^{-1}) (Figure 2). Waters draining from the glacier during this interval showed a consistent increase in the ratio of divalent to monovalent cations (5.59 to 6.81) and consistent decrease in ρCO_2 ($10^{-4.36}$ to $10^{-5.20}$ atm), SMF (0.18 to 0.08), and EC (39.7 to 24.6 μS cm^{-1}) (Figure 2), indicating that meltwater residence times decreased as the flux of dilute supraglacial meltwaters increased.

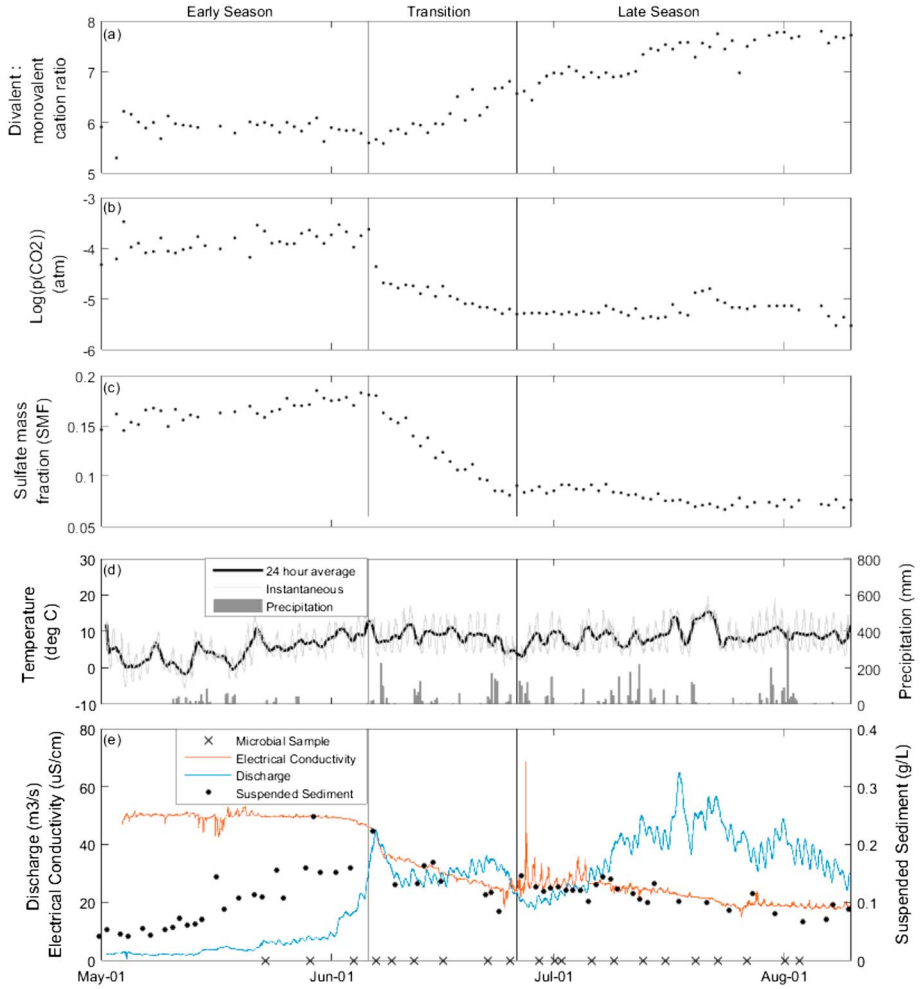

Figure 2. (a) Divalent (Mg^{2+} + Ca^{2+}) to monovalent (K^{+} + Na^{+}) cation ratios, (b) partial pressure of CO_2 with which the water appears to be in equilibrium, (c) sulfate mass fraction (SMF), (d) air temperature and precipitation [from *Cappelen*, 2015], and (e) discharge, electrical conductivity, suspended sediment, and microbial sampling dates of proglacial stream waters. Vertical lines distinguish the Early Season, "Transition", and Late Season hydrological phases.

The Late Season extended from approximately 26 June to the end of sampling in early August. Discharge reached a seasonal peak in mid-July (64 m^3 s^{-1}) in response to the high daytime temperatures (Figure 2) and then receded (to 25 m^3 s^{-1} in mid-August). Throughout this period, clear diurnal variations in discharge were superimposed upon longer-term trends. Suspended sediment concentrations generally decreased over time, from 0.15 g L^{-1} to 0.09 g L^{-1}, as in the Transition Period. Late Season waters had chemical signatures distinctly different from those of waters sampled during the Early Season and Transition Period. They had the highest average ratio of divalent to monovalent cations (7.32), and the lowest average values for ρCO_2 ($10^{-5.23}$ atm), SMF (0.078), and EC (22.2 μS cm^{-1}) (ANOVA, $p < 0.05$), indicating a strong influence of surface waters and short subglacial residence times.

3.2. Microbiology

To estimate coverage of diversity, Good's coverage indicated high coverage within all samples collected, with the lowest sample having 84% coverage (data not shown). However, we propose some caution with respect to these results as both rarefaction analysis and the relatively high number of Chao1-estimated OTUs compared to observed OTUs imply that increased sampling may lead to discovery of new OTUs and increased diversity.

Microbial assemblages in the proglacial stream waters were significantly different from those in waters sampled from the extraglacial and supraglacial environments throughout the melt season (Figure 3a,

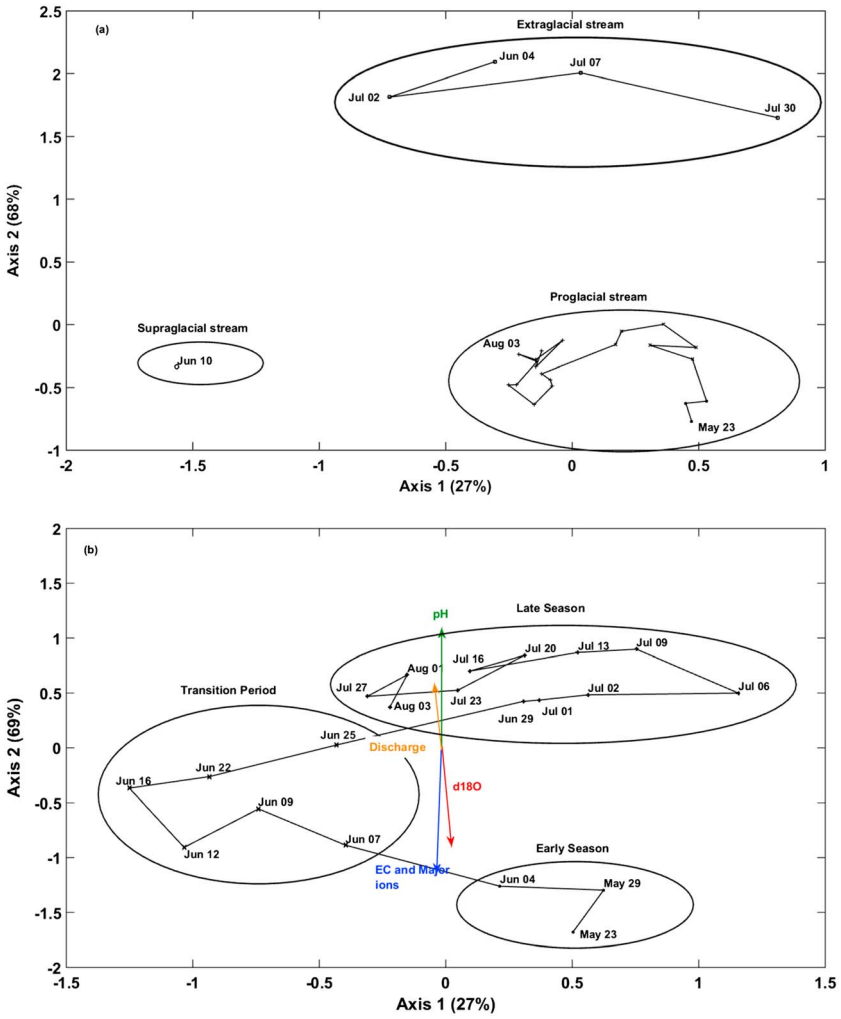

Figure 3. Microbial assemblage composition determined by nonmetric multidimensional scaling (NMS) ordination of Bray-Curtis distance measure using 16S rRNA gene sequencing (stress = 0.06). Values in parentheses next to axis labels indicate the percent of variance in the distance matrix explained by each axis. Multiresponse permutation procedure (MRPP) reveals significant differences between (a) proglacial, supraglacial, and extraglacial microbial assemblages (1000 subsamples) and (b) Early Season, Transition Period, and Late Season proglacial stream microbial assemblages (2500 subsamples). Colored arrows represent significant correlations with physical parameters using Mantel tests ($P < 0.001$).

$t = -12.96$; $A = 0.15$; $p < 0.001$). The composition of the microbial assemblage in the proglacial stream waters was remarkably stable throughout the melt season (Figure 3a). For example, the variability in microbial assemblage composition among the 21 proglacial stream samples collected over a 10 week period is approximately the same as that found among the assemblages from the four extraglacial stream samples that were collected over a 4 week period (Figure 3a). This stability in proglacial stream microbial assemblage composition occurred despite the large inferred changes in subglacial residence times, flow paths, and water sources over the melt season. These hydrological changes include a more than 100-fold increase in discharge, a ninefold increase in suspended sediment concentration, a decrease in electrical conductivity from ~50 μS cm^{-1} to 20 μS cm^{-1}, an increase in the divalent to monovalent cation ratio from ~6 to 8, a drop in the SMF from ~0.15 to 0.07, and a drop in the partial pressure of CO_2 (ρCO_2) from ~10^{-4} atm to 10^{-5} atm (Figure 2).

Although microbial assemblage composition in the proglacial stream was generally stable throughout the melt season, it is possible to recognize three statistically significant clusters of microbial assemblages corresponding to the Early Season, Transition Period, and Late Season hydrological periods (MRPP; $T = -10.59$; $A = 0.21$; $p < 0.001$, Figure 3b). The changes in microbial assemblage composition between

the three periods appear to involve a shift from a "subglacial" assemblage to one more similar to assemblages from the extraglacial and supraglacial samples. The meltwater microbial assemblage composition shifted toward that of the extraglacial environment during a short period from 6 to 16 June (the beginning of the Transition Period; Figure 3b, Axis 2). Another, more dramatic, shift occurred between 16 and 29 June when the proglacial stream assemblage composition became more characteristic of the supraglacial environment (Figure 3b, Axis 1). These compositional shifts were associated with changes in discharge, pH, major ion content and composition (total IN, SO_4^{2-}, NO_3^-, Na^+, Mg^{2+}, K^+, HCO_3^-, F^-, Cl^-, and Ca^{2+}), and $\delta^{18}O$ (Mantel tests; $p < 0.001$; Figure 3b). There were no significant correlations with other parameters such as deuterium excess (indicative of melt-freeze processes) or the concentrations of suspended sediment, DON, DOC, DIP, or DOP. However, the occurrence of seven of the 10 most dominant classified OTUs found in the proglacial waters (*Acetobacteraceae*, *Flavobacteriaceae*, *Comamonadaceae*, *Rhizobiaceae*, and unclassified families belonging to the phylum *Proteobacteria*, *Actinobacteria*, and the order *Sphingobacteriales*) was significantly ($p < 0.05$) and positively correlated with suspended sediment concentration.

4. Discussion

4.1. Hydrological Regime

Early Season meltwaters displayed a chemical signature characteristic of both relatively long rock-water contact (e.g., silicate weathering) and weathering processes that involve reactive sediments (e.g., sulfides and carbonates). This suggests the existence of a relatively inefficient subglacial drainage system, with water sources that might include basal ice melt (from geothermal or frictional heating), supraglacial snowmelt from the current season that was routed slowly through the subglacial system, and/or meltwater from the end of the previous melt season that was retained in the subglacial environment over winter.

High runoff volumes and diurnal hydrograph signatures suggest that channelized drainage was established by the onset of the Transition Period (approximately 7 June). The seasonal peak in suspended sediment concentration in the proglacial stream coincides with the first major peak in discharge of the melt season (Figure 2), suggesting that this was likely a period of high basal water pressure, reduced basal drag, increased basal motion by sliding and/or sediment deformation, and reorganization of the subglacial drainage system [*Mair et al*., 2004; *Swift et al*., 2005]. The water chemistry suggests a growing contribution from dilute supraglacial waters that passed rapidly through an increasingly efficient subglacial drainage system during this period. However, the stream water chemistry does show a continuing but diminishing contribution to the solute load from slow reactions and reactive minerals (such as sulfide oxidation coupled to carbonate and silicate dissolution), suggesting drainage from areas of the bed with an inefficient distributed meltwater drainage system.

The chemical signature of Late Season waters, which began to emerge on approximately 24 June and continued to do so until the end of the sampling period, suggests an increasing proportion of water draining through an efficient, channelized subglacial drainage network. These waters are likely sourced predominantly from supraglacial snow and ice melt and are routed relatively quickly and efficiently across the largely snow-free lower elevations of the glacier surface. During transit through the channelized subglacial drainage network, these relatively dilute meltwaters are involved in chemical weathering reactions, such as the carbonation of carbonates, which have relatively quick reaction kinetics and occur in the absence of proton supply from sulfide oxidation. However, there is also evidence for continued drainage from distributed elements of the subglacial drainage system throughout the Late Season. This is particularly obvious at times when surface melt inputs to the channelized drainage system were exceptionally low (e.g., between 24 June and 5 July). Persistent drainage from regions of the bed with distributed drainage systems, even during seasonal peak discharge conditions, has been described previously [*Tranter et al*., 2005] and observed elsewhere on the GrIS [*Bhatia et al*., 2011; *Hawkings et al*., 2016].

The Early Season, Transition Period, and Late Season subglacial drainage regimes reported here imply strong intraseasonal variability in the sources of water and solute exported from the glacier and in subglacial water residence times and the rock-water contact that occurs during passage through the glacier. These hydrological characteristics are typical of many warm-based and polythermal glacier systems and are consistent with observations elsewhere on the GrIS [*Bartholomew et al*., 2010, 2011; *Bhatia et al*., 2011; *Chandler et al*., 2013].

4.2. First-Order Control on Exported Microbial Assemblages: Subglacial Drainage

The proglacial stream at KS had microbial assemblages that differed significantly from those found in supraglacial and extraglacial source waters, suggesting that the passage of water through the subglacial environment was a first-order control on the microbial assemblages exported from the glacier. The microbial assemblages in proglacial stream waters remain distinct from source waters (i.e., extraglacial and supraglacial), even during periods of high discharge in the late season when meltwaters were routed relatively quickly through the subglacial system. Therefore, changes in the microbial assemblages contained in meltwaters as they were routed from the supraglacial system through the subglacial system must have also occurred relatively rapidly. Changes in the microbial assemblages contained in meltwater in the subglacial environment likely resulted from the mixing of surface-derived waters with subglacial delayed-flow waters that were routed slowly through distributed drainage networks. The microbial contributions from delayed-flow waters are likely to contain both distinct microbial assemblages and high microbial abundances [*Sharp et al*., 1999; *Foght et al*., 2004].

Areas of the glacier bed away from major channels provide microbial habitats that are permanently dark and cold, relatively isolated from the atmosphere, and which allow prolonged rock-water contact. These environments support psychrotolerant or psychrophilic organisms that acquire nutrients and energy from reactions involving minerals or organic compounds derived from both underlying and entrained debris and bedrock [*Sharp et al*., 1999; *Bottrell and Tranter*, 2002; *Foght et al*., 2004; *Wadham et al*., 2008, 2012], which is different from those found in surface environments where light and oxygen are more abundant. Remote areas of the bed may tend toward anoxia because oxidation of sulfides and organic matter can consume the oxygen available in these relatively closed systems [*Wadham et al*., 2012]. As a result subglacial environments host microbial species capable of nitrate, iron, or sulfate reduction and methanogenesis [*Skidmore et al*., 2000; *Boyd et al*., 2010; *Yde et al*., 2010; *Stibal et al*., 2012; *Wadham et al*., 2012; *Telling et al*., 2015].

Entrainment of distinctive microbes that reside in both sediments and water within the subglacial system may change the composition of the microbial assemblage in emergent meltwaters. While glacier ice, snow, cryoconite hole waters, and supraglacial stream water typically contain bacterial cell concentrations on the order of 10^4 cells mL^{-1} [*Säwström et al*., 2002; *Foreman et al*., 2007; *Anesio et al*., 2010], basal ice and subglacial water can contain cell concentrations 1–2 orders of magnitude higher [*Karl et al*., 1999; *Sheridan et al*., 2003; *Gaidos et al*., 2004; *Skidmore et al*., 2005]. More than 0.03 g L^{-1} sediment was acquired by meltwaters in the subglacial environment at KS, and subglacial microbial concentrations are positively correlated with sediment concentrations in basal ice and meltwater [*Sharp et al*., 1999] and are orders of magnitude higher in subglacial sediment than in basal ice [*Sharp et al*., 1999; *Foght et al*., 2004; *Tung et al*., 2006]. At other sites, the GrIS subglacial environment hosts between 10^5 cells g^{-1} sediment [*Lawson*, 2012] and up to the equivalent of 10^{11} cells g^{-1} dry weight silt [*Tung et al*., 2006], although the higher values may be an overestimate. Thus, entrainment of even small concentrations of subglacial water and/or sediment likely strongly influences the composition of the proglacial stream microbial assemblage.

4.3. Second-Order Control on Exported Microbial Assemblage: Source Water and Subglacial Residence Time

Despite the remarkably consistent microbial assemblage composition of proglacial stream waters at KS, there is still significant seasonal variability ($p < 0.001$; Figure 3b). Although this variability is relatively small compared to the changes that occur as supraglacial or extraglacial waters are routed through the subglacial system, it does suggest a second-order control on the nature of the microbial assemblages exported from KS.

Two independent lines of evidence suggest that seasonal variability in microbial exports from KS is a response to changes in the sources, flow routing, and subglacial residence time of emerging meltwaters. First, changes in microbial assemblage composition were significantly correlated with changes in pH, discharge rate, $\delta^{18}O$, EC, and major ion composition (Figure 3b), which are proxies for water sources and/or subglacial residence time. Second, the microbial assemblage found in Early Season proglacial stream waters was most distinct from the assemblages found in supraglacial and extraglacial waters, and it becomes more similar to that of extraglacial waters during the Transition Period and to supraglacial waters during the Late Season (Figure 3a). These shifts are consistent with inferred changes in the relative contributions to runoff of the different water sources over the melt season. Early Season proglacial stream waters likely

spent a prolonged period at the glacier bed, where viable microbial populations that are distinct from those found in supraglacial and extraglacial environments are known to exist [*Sharp et al*., 1999; *Foght et al*., 2004; *Skidmore et al*., 2005; *Lanoil et al*., 2009; *Yde et al*., 2010]. The shift in microbial assemblage composition during the Transition Period, toward one characteristic of extraglacial waters, coincides approximately with the timing of the spring freshet, when extraglacial snowmelt inputs to the subglacial system likely peaked. The subsequent shift in microbial assemblage composition toward that typical of supraglacial waters during the Late Season coincides approximately with the timing of peak supraglacial meltwater production.

5. Conclusion

We used next generation sequencing of bacterial small subunit rRNA genes to explore the microbial assemblage compositions recovered from a glacial drainage system in southwest Greenland. Drainage through the subglacial environment significantly altered the microbial assemblages exported in meltwater. Changes in assemblage composition occurred relatively rapidly relative to the subglacial residence times (i.e., significant changes occurred even during those parts of the season with the shortest subglacial residence times) and were consistent in nature (i.e., they resulted in relatively stable microbial assemblages in the proglacial stream). These results contribute to mounting evidence that suggests the presence of an active, endogenous microbial assemblage in the subglacial environment [*Skidmore and Sharp*, 1999; *Foght et al*., 2004; *Skidmore et al*., 2005; *Lanoil et al*., 2009; *Stibal et al*., 2012; *Wadham et al*., 2012]. They also confirm that the subglacial environment is a strong and stable source of distinct microbial assemblages to waters that pass through it [*Cameron et al*., 2016].

While passage (or not) through the subglacial drainage system is the first-order control on the composition of proglacial stream microbial assemblages, changes in water source and subglacial residence time have second-order effects. These explain small but significant shifts in the composition of microbial assemblages in the proglacial stream over a melt season and highlight the potential influence of water from supraglacial and extraglacial environments on the microbiology of proglacial and downstream environments. Although this study only examined the microbial assemblages exported during a single melt season from one glacier, and may not represent the full range of spatial and temporal conditions on the GrIS, it does suggest that a strong connection may exist between the microbial assemblages contained in proglacial stream and the hydrological regime of the glacial system.

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