Illuminating the dynamic rare biosphere of the Greenland Ice Sheet's Dark Zone

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Abstract (201 words)

The low-albedo Dark Zone of the Greenland Ice Sheet is the largest region of contiguous bare terrestrial ice in the Northern Hemisphere. Dark Zone microbial processes play an important role in driving extensive darkening and amplified melting, yet the dynamics of those consortia have not been fully identified. Here we present joint 16S rRNA gene and 16S rRNA (cDNA) comparison of input (snow), storage (cryoconite), and output (supraglacial stream water) habitats across the Dark Zone over the melt season. Our analysis reveals that all three Dark Zone communities have a preponderance of rare taxa exhibiting high protein synthesis potential (PSP). Furthermore, taxa with high PSP represent highly connected "bottlenecks" within community structure, consistent with their roles as metabolic hubs. Finally, low abundance-high PSP taxa affiliated with *Methylobacterium* within snow and stream water suggest a novel role for *Methylobacterium* in the carbon cycle of Greenlandic snowpacks, and importantly, the export of potentially active methylotrophs to the bed of the Greenland Ice Sheet. By comparing the dynamics of bulk and potentially active microbiota in the Dark Zone of the Greenland Ice Sheet we provide novel insights into the mechanisms and impacts of the microbial colonization of this critical region of our melting planet.

INTRODUCTION

Microbes that colonize snow and ice surfaces live at the critical interface between the atmosphere and cryosphere (Budyko, 1969). Their potential to darken glacier surfaces and thereby amplify melt (Nordenskiöld, 1870, Takeuchi, 2002, Lutz *et al.*, 2016, Cook *et al.*, 2017, Stibal *et al.*, 2017, Ryan *et al.*, 2018), has been a long standing question that has recently been identified by the IPCC (AR5) as requiring urgent attention (IPCC, 2014). The "Dark Zone" of the Greenland Ice Sheet is a conspicuous band of low albedo bare ice that covers some 10,000 km^2 of the western ablating margin of the ice sheet. Surface melt rates of up to eight metres (water equivalent) per year have been observed here, representing a major component to the Greenland Ice Sheet's negative mass-balance and contributor to global sea-level rise (Wientjes & Oerlemans, 2010, Wientjes *et al.*, 2011, Ryan *et al.*, 2016, Van Tricht *et al.*, 2016). It is a biologically active surface (Hodson *et al.*, 2010, Cook *et al.*, 2012) where extensive microbial colonization drives regional surface albedo reduction and enhanced ablation (Stibal *et al.*, 2017, Tedstone *et al.*, 2017, Ryan *et al.*, 2018). Microbial processes associated with Greenland's dark ice surface also contribute to the cycling and hydraulic export of microbial biomass (Cameron *et al.*, 2017, Dubnick *et al.*, 2017), organic carbon (Stibal *et al.*, 2010, Bhatia *et al.*, 2013, Musilova *et al.*, 2017), and nutrients (Bhatia *et al.*, 2013, Hawkings *et al.*, 2016) in significant quantities to downstream englacial, subglacial, and proglacial hydrological networks and ecosystems which ultimately drain to the coast. Thus, microbial processes on the Greenland Ice Sheet may influence biogeochemical cycling in downstream habitats, be they subglacial or proglacial ecosystems.

Previous studies of microbial diversity within the Dark Zone have focused on supraglacial communities within granular microbe-mineral aggregates termed cryoconite (Edwards *et al.*, 2014, Cameron *et al.*, 2015, Musilova *et al.*, 2015, Stibal *et al.*, 2015) and glacier algae (Yallop *et al.*, 2012, Lutz *et al.*, 2018). These studies employed transects (e.g.(Edwards *et al.*,

2014, Lutz *et al.*, 2018), or used pooled cryoconite material (Musilova *et al.*, 2015, Stibal *et al.*, 2015), thereby limiting detailed information regarding temporal bacterial community stability. Few studies have directly addressed the diversity of snowpack bacteria across the region (Cameron *et al.*, 2014). Despite the vast scale of this microbial habitat created by seasonal snowmelt (Ryan *et al.*, 2019), nothing is known of microbial temporal dynamics within the snowpack of the Greenland Ice Sheet. Furthermore, although fluvially-exported microbiota from the ice sheet surface may influence downstream biogeochemical processes such as subglacial methane cycling (Dieser *et al.*, 2014, Lamarche-Gagnon *et al.*, 2019), to our knowledge, the microbial diversity and functional potential of supraglacial meltwater exported from the Dark Zone remains undocumented. We sought to address these gaps in our knowledge by presenting an integrated study of community structure, connectivity, and its functional potential within three principal bacterial habitats found within the Dark Zone: snow (input), cryoconite hole (storage) and runoff (output).

The sequencing of 16S rRNA genes and 16S rRNA (reverse transcribed as cDNA) from co-extracted DNA and rRNA represents a common strategy within microbial ecology. Its application for the discrimination of "total" and "active" bacterial communities has been subject to critique, with limitations in the equivalence of rRNA and "activity" highlighted by Blazewicz et al ((Blazewicz *et al.*). With the caveat that ratios between 16S rRNA (cDNA) and 16S rRNA genes are indicative of protein synthesis potential (PSP; (Blazewicz *et al.*, 2013), rather than unequivocal quantitative evidence of contemporaneous growth, the technique offers the potential for insights into the responses of taxa to rapidly fluctuating environments. For example, within alpine proglacial streams which experience considerable diurnal fluctuation in temperature and discharge, joint 16S rRNA gene and 16S rRNA (cDNA) sequencing revealed that rare taxa were over-represented in the 16S rRNA (cDNA) population (Wilhelm *et al.*, 2014). Within the austere and isolated environs of the Dark Zone

in summer, solar radiation, air temperature, melt intensity, and stream discharge all fluctuate with high periodicity, typically diurnally, and hence well within the typical doubling times of supraglacial microbes (Anesio *et al.*, 2010, Williamson *et al.*, 2018). How the bacterial communities of the Dark Zone respond to these fluctuations remains poorly understood, and the potential for these rare taxa to disproportionately influence community structure is unknown.

In this study, we evaluate the temporal dynamics of bacterial communities from snowpack, cryoconite and stream water habitats in the Dark Zone of the Greenland Ice Sheet using analysis of both 16S rRNA gene and 16S rRNA (cDNA). This was performed by sampling at weekly intervals, in June and July 2014, to incorporate the transition from early season melt onset to snow-free exposed ice surface.

MATERIALS AND METHODS

Methods summary

Sampling took place on the western ablating margin of the Greenland Ice sheet, within the Dark Zone and adjacent to the Kangerlussuaq (K-) transect S6 automatic weather station (AWS), at 67°05'N, 49°23'W; 1020 m asl (FIGURE 1). Cryoconite, snow and water from supraglacial meltwater streams was collected in triplicate, on seven sampling occasions, at weekly intervals between June 19 and July 31 2014. Independent sites, within a 25m^2 area were used for each sampling occasion. A total of 62 samples were collected and chemically preserved using Soil Lifeguard (MO BIO Laboratories, Solana, CA, USA). Samples were then frozen within three weeks. Upon return to the home laboratory, community DNA and RNA was co-extracted from snow and meltwater samples previously concentrated on 0.22 µm Sterivex GP polyethersulfone filters (Millipore, MA, USA) using a modified

PowerWater® Sterivex™ DNA Kit and from cryoconite using a PowerBiofilm™ RNA Isolation Kit (MO BIO Laboratories) prior to 16S rRNA gene and 16S rRNA (cDNA) quantitative PCR (qPCR) and V3-V4 region MiSeq (Illumina, Cambridge, UK) sequencing (Supplementary Table 1). All sequence data are available on EBI-SRA under the study accession number PRJNA318626. The methods employed for sample archival, nucleic acid extraction, qPCR, sequencing, and data processing are detailed in full as **supplementary methods.**

RESULTS

Bacterial 16S rRNA gene and 16S rRNA (cDNA) quantification

Quantitative PCR was used to analyse the amplifiable copy number of 16S rRNA genes and 16S rRNA in DNA and cDNA samples (Figure 2). The amplifiable copy number of 16S rRNA genes appeared highly consistent across the sampling period (Figure 2A) with weekly averages of 2.4 - 4.5 × 10^5 amplifiable copies of the 16S rRNA gene per gram dry weight of cryoconite. The amplifiable copy number of the 16S rRNA pool fluctuated, ranging between a weekly average of 2.3 × 10^7 (week 4) and 1.6 × 10^9 (week 2) per gram dry weight of cryoconite. The ratio between 16S rRNA gene and 16S rRNA (cDNA) amplifiable copy number is interpreted as a marker of the overall bacterial PSP. Average cryoconite bacterial PSP showed high (1:88, week 4) to extremely high ratios (1:5000, week 2) throughout the study period. This is indicative of a high level of potential activity relative to biomass within the cryoconite bacterial communities sampled.

For the snow bacterial community (Figure 2B), weekly average amplifiable copies of the 16S rRNA gene increased from 3.0 × 10^4 copies per litre (water equivalent) in the first week of the study to 7.6 × 10^8 copies per litre (water equivalent) in the final week. Weekly average

amplifiable copies of 16S rRNA (cDNA) also increased from 4.5×10^4 copies per litre (water equivalent) in the first week of the study to 2.8×10^7 copies per litre (water equivalent) in the penultimate week. Weekly average bacterial PSP values for snow were consistently below equivalence, with exception of the first week (1:1.5 ratio).

Stream melt water bacterial communities (Figure 2C) exhibited 16S rRNA gene amplifiable copy numbers several orders of magnitude lower in week 1 (2.8×10^4 16S rRNA gene copies per litre) compared to the remainder of the study period (3.1 - 3.6×10^6 16S rRNA gene copies per litre). rRNA copy numbers were at least an order of magnitude higher (5.5×10^5 to 3.9×10^5 16S rRNA copies per litre) compared to the first week. Stream water bacterial PSP values varied between 0.3 and 2.8 during the study period. In summary, it is likely the stream water bacterial community was an admixture of quiescent and active taxa in transit from different sources (e.g. snowmelt, ice melt, cryoconite and other biofilms) from the ice sheet surface.

Community structure

The total number of reads obtained after sequence processing was 2,673,556 with a maximum of 130,001 reads (GrIScDNAcryo6.3), a minimum of 5 (GrISstream3.1) and a mean of 21,561 reads. Sequences were filtered and rarefied to 943 sequences per sample, resulting in the exclusion of 1 cryoconite DNA sample, 2 stream DNA samples, 3 cryoconite cDNA samples, 3 snow cDNA samples and 6 stream cDNA samples from downstream analysis. Sequences were clustered into 566 operational taxonomic units (OTUs) at 97% sequence similarity. 13.59 % of 16S rRNA gene OTUs and 14.40 % 16S rRNA (cDNA) OTUs were common to all three habitats.

Over 99 % of the sequences in the dataset from cryoconite hole, snow and stream water habitats were successfully assigned to the Greengenes taxonomy using UCLUST. .Non-

metric multidimensional scaling of OTUs ordinated both 16S rRNA gene and 16S rRNA (cDNA) profiles of the bacterial communities by habitat type (Figure 3A). These trends were confirmed by PERMANOVA of fourth-root transforms of Bray-Curtis distances of OTU relative abundance matrices. Highly significant differences were found between all habitat types (Pseudo$F$ = 13.8, p = 0.0001, Supplementary Table 2). Furthermore, highly significant differences are apparent between OTU composition of 16S rRNA gene and 16S rRNA (cDNA) profiles for each of the habitat types (Pseudo$F$ values = 5.3 - 22.5, p = 0.0001). The snow and stream communities revealed in 16S rRNA gene profiles (Pseudo$F$ = 2.6, p = 0.0001 and Pseudo$F$ = 2.4, p = 0.0001 respectively) were temporally dynamic at weekly sampling resolution, with highly significant differences. The 16S rRNA (cDNA) profiles of cryoconite and snow were significant and highly significantly different by week (Pseudo$F$ = 1.7, p = 0.02 and Pseudo$F$ = 3.0, p = 0.0004 respectively), while stream profiles are temporally stable. In contrast, while cryoconite exhibited highly significantly different 16S rRNA gene and 16S rRNA (cDNA) profiles, the 16S rRNA gene profiles were temporally stable. All PERMANOVA results are detailed in Supplementary Table 2.

Trends in taxonomic composition

Pronounced differences between 16S rRNA gene and 16S rRNA (cDNA) taxonomic profiles are apparent for each of the habitats (Figure 3B). Whereas 16S rRNA gene data reveal Actinobacteria, Bacteroidetes and Alphaproteobacteria are the major groups in cryoconite with a modest representation from Cyanobacteria, from the 16S rRNA (cDNA) data Cyanobacteria are the strikingly dominant group throughout the study period (Figure 3). Similarly, while the 16S rRNA gene profiles of snow reveal a transition between an Alphaproteobacteria dominated community to a Bacteroidetes dominated community during the study period, Alphaproteobacteria remains the dominant group within the 16S rRNA (cDNA) profile, with an increase in Acidobacteria in the final two weeks of the study.

Meanwhile, the discordance between 16S rRNA gene and 16S rRNA (cDNA) profiles are further mirrored in supraglacial streamwater, where Bacteroidetes and Betaproteobacteria were found to be in equitable dominance in the 16S rRNA gene dataset, and Alphaproteobacteria strongly dominated the 16S rRNA (cDNA) profiles.

16S rRNA gene sequencing

Trends in potential activity and relative abundance

The strikingly distinctive 16S rRNA gene and 16S rRNA (cDNA) profiles were further investigated to reveal taxonomic groups over- and under-represented in 16S rRNA (cDNA) compared to 16S rRNA genes (Figure 4). All detected phyla/proteobacterial classes with the exception of *Cyanobacteria* were under-represented in 16S rRNA (cDNA) for cryoconite (Figure 4A, 5A), whereas Alphaproteobacteria, Acidobacteria, Firmicutes and WPS2 were over-represented in snow (Figure 4B) and Alphaproteobacteria, Verrucomicrobia, OD1 and Firmicutes were over-represented in stream water communities (Figure 4C).

OTUs present at ≤ 1% relative abundance constituted a large percentage of the bulk community within rank abundance curves (Figure 5A-C). Applying the ≤0.1 % of relative threshold commonly used to delimit the "rare" biosphere (Pedrós-Alió, 2012), 57 % of cryoconite OTUs, 62 % of snow OTUs and 63 % of stream OTUs would be considered "rare" taxa within the Dark Zone bulk community. On the basis of the rarefied dataset size, taxa would need to exhibit a minimum mean relative abundance of ≥0.005% to be represented in datasets of this size. Rank abundance curves show that 48 % of rare cryoconite-habitat OTUs, 40.45 % of rare snow-habitat OTUs and 42.36 % of rare stream-habitat OTUs exhibit positive protein synthesis potentials (PSP, the ratio between 16S rRNA gene and 16S rRNA [cDNA] relative abundance) over the course of 7 weeks. In each community (Figure 5 A-C), taxon

PSP is negatively correlated with mean taxon relative abundance (Spearman correlation; cryoconite: r = -0.63, p < 0.0001, snow: r = -0.65, p < 0.0001, stream r = -0.55, p < 0.0001). The trends exhibited are congruent with the notion that certain rare taxa in surface habitats in Greenland's Dark Zone exhibit disproportionately high protein synthesis potential.

Dynamics of high-PSP OTUs.

To establish the contribution of high PSP OTUs over time, OTUs exhibiting weekly PSP averages ≥1 in the dataset were plotted over time and compared to their relative abundance within the 16S rRNA gene dataset (Figures 6-8). For the cryoconite community (Figure 6), 30 of 34 OTUs meeting this criterion were members of Cyanobacteria, with taxa assigned to *Leptolyngbya* representing the majority, including both the highest PSP OTU (*Leptolyngbya*-76) and highest relative abundance OTU (*Leptolyngbya*-3). In the snow community, Alphaproteobacteria represented 22 of 30 taxa with weekly average PSP ≥ 1 (Figure 7). Notably, *Methylobacterium*-1 is highly abundant in the first week of the study with a corresponding mean PSP of 6.8. However, for the next three weeks, while *Methylobacterium*-1 shows much lower relative abundance, its PSP is strikingly high (ranging 185- to 304-fold). *Methylobacterium*-1 is not detected in snow community after week four. In all, six OTUs assigned to *Methylobacterium* are prominent in the high PSP taxa of the snow community. This is echoed within the stream community (Figure 8). Here, *Methylobacterium*-1 again shows high PSP values, in the range of 49 - 111 between weeks two and seven, however its relative abundance is low, amounting to <2 % of the community overall. Four OTUs assigned to *Methylobacterium* are present among 28 Alphaproteobacteria OTUs, with Sphingomonadaceae taxa well represented. In all, 45 OTUs show weekly average PSP ≥1. Seven *Cyanobacteria* affiliated with *Leptolyngbya* (including the *Leptolyngbya*-3 and *Leptolyngbya*-76 prominent in the cryoconite community) are present with four members of Acidobacteria. The prominence of high PSP taxa in stream water from lineages conspicuous

within the snowpack and cryoconite community is consistent with the runoff export of potentially active taxa from surface habitats of the Greenland Ice Sheet's Dark Zone.

Keystone species-high PSP rare OTU relationship

Taxa that exert a disproportionate influence on the structure of the microbial community, despite low or moderate abundances can be termed keystone species (Power & Mills, 1995). High betweenness centrality, measured as the shortest number of paths between any two other OTUs passing through that OTU, is interpreted as a hallmark of a keystone species since it reflects a disproportionate level of association between multiple taxa and the putative keystone species(Peura *et al.*, 2015). Consequently taxa with high betweenness centrality scores can be thought of as "hubs" within the network structure of the community. Co-occurrence analysis identified sixteen OTUs with betweenness-centrality scores (in the range 7.16 to 0.2; Table 1). All have cumulative positive PSP ratios in at least one habitat over the course of the study, and with the exception of one *Comamonadaceae* OTU with a cumulative mean PSP of 1.98, the remainder show high to very high maxima in their cumulative PSP ratios, in the range 10.5 - 405.8. Again, *Methylobacterium*-1 is represented, with the highest (snow: 405.8) and second highest (stream: 368.2) cumulative mean PSPs. Two other OTUs assigned to *Methylobacterium* show the next highest cumulative mean PSPs in stream and snow. For cryoconite, *Leptolyngbya* assigned OTUs are prevalent as keystone taxa with high cumulative mean PSP. Of the four *Leptolyngbya* assigned OTUs, *Leptolyngbya*-76, *Leptolyngbya*-106 and *Leptolyngbya*-3 show the highest cryoconite PSPs, but have modest betweenness scores. The considerable overlap between putative keystone species and high PSP taxa, including those present at low abundance, presents the possibility that taxa with high levels of protein synthesis potential are influential in the dynamics of their communities irrespective of their relative abundance. Thirteen of the sixteen OTUs are most closely related to taxa distributed across the global cryosphere (Table 1).

DISCUSSION

Snow bacterial communities

Our results provide the first insights into the dynamics of bulk and potentially active communities of decaying snowpacks in the ablating zone of the ice sheet during the transition to bare ice. At the start of the study bacterial PSP is positive (Figure 2), however this rapidly declines for the remainder of the sampling period. In contrast, 16S rRNA gene copy numbers increase by 3-4 orders of magnitude for the remainder of the sampling period. This is likely due to the accumulation of biomass within decaying snow due to physical processes rather than biological growth (Björkman *et al.*, 2014). Melting snowpacks are physically and chemically dynamic environments, and it appears only a few lineages are able to maintain their populations in supraglacial snow as it decomposes to slush (Hell *et al.*, 2013), with other taxa being washed out. Indeed, 16S rRNA gene and 16S rRNA (cDNA) OTU profiles were highly significantly different over time (Supplementary Table 2).

Here, snowpack 16S rRNA gene copies greatly exceed 16S rRNA copy numbers, indicating the bulk community is likely to be exported as cells with low PSP. For example, the relative under-representation of Bacteroidetes in 16S rRNA (cDNA) raises the possibility that cellulose-degrading taxa become quiescent when dissociated from sources of complex organic carbon, for example supraglacial phototrophs (Smith *et al.*, 2016). It is therefore likely that the abundant groups of bacteria in decaying snow serve as sources of cellular carbon and nutrients rather than viable taxa capable of inoculating downstream habitats. The rare, high PSP *Methylobacterium* sp. OTUs detected represent an exception which will be discussed below.

Although most of the Greenland Ice Sheet is perennially covered with snow, few studies have examined the snowpack microbiology of the Greenland Ice Sheet (Cameron *et al.,* 2014). Moreover, the highly isolated setting of field sites coupled with the potential for contamination of low-biomass samples make such studies challenging. By establishing a field camp for the duration of the study, careful handling of samples and the sequencing of negative controls we were able to mitigate these limitations. Negative controls returned very small numbers of reads (Supplementary Table 3). Prominent groups of bacteria in our study were not represented in negative controls with the exception of seven reads matching *Phormidesmis priestleyi*, likely indicating post amplification carry-over of a dominant amplicon type at negligible levels compared to its abundance in field samples.

Cryoconite bacterial communities

Lower copy numbers of 16S rRNA genes were amplified by qPCR compared to previous studies employing 16S rRNA gene qPCR based upon larger, wet-weight samples (Stibal *et al.,* 2015). However, the overall trends are consistent between both studies. Considering potential limitations in extraction efficiency and biases inherent in all PCR based analyses, we avoid treatment of qPCR data as absolute quantities of 16S rRNA genes or 16S rRNA in our samples and limit our comparison to trends within the dataset.

Exceptionally high ratios of 16S rRNA to rRNA genes were measured in cryoconite (Figure 2). Combined with amplicon sequencing data revealing cyanobacteria were overwhelmingly dominant within the 16S rRNA (cDNA) population (Figure 3, Figure 6) we interpret this as evidence of the high PSP of cyanobacteria within the cryoconite granules. Since filamentous cyanobacterial phototrophs such as *Phormidesmis priestleyi* are well known as ecosystem

engineers (Edwards *et al.*, 2014, Cook *et al.*, 2015) of cryoconite granules through their primary production and granule-building (Langford *et al.*, 2010), this is highly plausible.

Other work within the same field season at the same site lends support to our findings. Firstly, *Phormidesmis priestleyi* was isolated in culture and genome sequenced (Chrismas *et al.*, 2016) and secondly perturbation of cryoconite hole structure and microbial activity revealed *Phormidesmis* sp. employ sensitive photoadaptive mechanisms to optimize carbon sequestration in cryoconite holes (Cook *et al.*, 2016). Correspondingly, the prominence of OTUs extremely closely related to *Phormidesmis priestleyi* (Table 1), albeit assigned to *Leptolyngbya* (-3 and -76) within the high PSP (Figure 5) and keystone taxa (Table 1) of cryoconite granules extends insights from previous studies by linking specific *Phormidesmis* lineages with metabolic activities within Arctic cryoconite.

Importantly, previous analysis of 16S rRNA genes has resolved a single *Phormidesmis* OTU is cosmopolitan (Segawa *et al.*, 2017) within diverse Arctic glacial settings (Edwards *et al.*, 2011, Cook *et al.*, 2016, Gokul *et al.*, 2016, Uetake *et al.*, 2016). However, in our study, while one *Phormidesmis* OTU (*Leptolyngbya*-3) is likely to play a structural role, two other lineages (*Leptolyngbya*-76, *Leptolyngbya*-106) have PSP in gross excess to their biomass, indicated by contrasting trends in PSP and 16S rRNA gene relative abundance (Figure 6). The bulk bacterial community structure of cryoconite granules was stable over the course of the study, consistent with prior studies (Musilova *et al.*, 2015). However the *Phormidesmis* dominated 16S rRNA (cDNA) pool of the bacterial community of cryoconite changed over time, with highly significant changes revealed by PERMANOVA (Supplementary Table 2). Therefore, the potential for metabolic and structural niche differentiation among cryoconite *Phormidesmis* merits further investigation.

Supraglacial stream water bacterial communities

Meltwater runoff from the Greenland Ice Sheet surface is thought to be a major contributor to sea level rise (Smith *et al.*, 2017). Although this meltwater is an important source of organic carbon and nutrients to downstream ecosystems (Bhatia *et al.*, 2013, Bhatia *et al.*, 2013, Hawkings *et al.*, 2016, Musilova *et al.*, 2017) and the microbial fluxes in outflows from the Greenland Ice Sheet have been studied (Cameron *et al.*, 2017, Dubnick *et al.*, 2017), the absence of data on microbial export from the Greenland Ice Sheet surface represents a critical lacuna in our understanding of the Greenland Ice Sheet ecosystem. Within this study, this is addressed by 16S rRNA gene and 16S rRNA (cDNA) qPCR and sequence data which reveal the export of microbial groups prevalent in snow and cryoconite in meltwater from three ephemeral supraglacial streams.

Quantitative PCR reveals the stream water bacterial community in the first week of the study contains approximately equal copy numbers of bacterial 16S rRNA genes and 16S rRNA (cDNA) resulting in a bacterial PSP of 0.97. By the second week, both genes and rRNA have increased their average copy number by two orders of magnitude. Highly significant differences were observed in the community structure of bulk, but not active stream bacterial communities over time (Supplementary Table 2). Only one profile each of the bulk and active communities could be analysed from week one, but both were strongly dominated by Alphaproteobacteria. Subsequent weeks are marked by a more diverse bulk bacterial community, although *Alphaproteobacteria* were highly dominant in the potentially active bacterial community throughout. Sphingomonadaceae, Acetobacteraceae, and Rickettsiaceae are prevalent in the high PSP *Alphaproteobacteria* detected in stream water, but the highest PSP taxon is *Methylobacterium*-1, which is also a high-betweenness putative keystone species.

The presence of cyanobacterial taxa associated with cryoconite, including *Leptolyngbya-3* and *Leptolyngbya*-76 in stream water indicates the export of potentially active primary

producers. It is likely these cyanobacteria originate from biomass sheared from cryoconite granules either present in cryoconite holes, as distributed cryoconite on the ice surface or in stream cryoconite (so-called "hydroconite"; (Hodson *et al.*, 2007). It is likely these phototrophs represent sources of highly bioavailable dissolved organic carbon exported from the glacier surface (Musilova *et al.*, 2017).

High Protein Synthesis Potential Rare Taxa in the Dark Zone of Greenland

A consistent pattern for all three habitats sampled was that bulk and potentially active communities of snow, cryoconite and stream habitats were highly significantly different (Supplementary Table 2, Figure 3). Furthermore, a small subset of taxa were consistently over-represented in 16S rRNA (cDNA) compared to their corresponding 16S rRNA gene relative abundances, most notably the Cyanobacteria in cryoconite and Alphaproteobacteria in snow and stream habitats. Other taxa were typically under-represented. The majority of taxa present within the surface of the Dark Zone appear to exhibit low protein synthesis potential. This may be due to resource limitation, dormancy or the detection of DNA associated with dead cells (Blazewicz *et al.*, 2013). Each of the above scenarios has important ecological implications. Firstly in terms of maintaining a pool of organisms which may respond to stimuli such as allochthonous resources, and secondly, the maintenance of a "seedbank" of conditionally viable cells (Lennon & Jones, 2011), or at the very least, the contribution of carbon and nutrients in otherwise oligotrophic environments in the form of necromass. Differentiation between these scenarios is beyond the scope of 16S rRNA gene analyses (Blazewicz *et al.*, 2013) and the potential for "active" taxa to be mis-classified as "dormant" by 16S rRNA (cDNA) / 16S rRNA gene comparison has been described in computational simulations (Steven *et al.*, 2017).

Nevertheless, a further notable trend which was consistent across all three habitats was a marked prominence of rare taxa among those with high PSP. Such patterns have been observed in other, comparable contexts, for example within proglacial streams in the European Alps (Wilhelm *et al.*, 2014). In those systems, such trends have been considered hallmarks of habitats exhibiting severe fluctuations in their environmental conditions such as temperature, discharge or solar radiation at timescales briefer than the doubling time of the resident community (Lennon & Jones, 2011, Wilhelm *et al.*, 2014). Here, no overall trends were apparent in terms of the influence of meteorological parameters from week to week, however when monitored continuously (Figure 1) profound oscillations in temperature, incoming short- and long- wave radiation, air temperature, energy flux and melt intensity are apparent at diurnal to sub-weekly periodicity. Considering the sluggish growth of organisms at temperatures at or near freezing (Anesio *et al.*, 2010), it is likely that such oscillations create rapidly changing niche spaces at timescales shorter than community doubling times. It is therefore likely that organisms exhibit high PSP relative to their biomass when they are able to maintain activity but not achieve net population growth in the face of unstable fitness.

Consequently, through their metabolic activities, high PSP rare taxa may be disproportionately influential within their communities, fitting the definition of keystone taxa. This is coherent with the corresponding prevalence of high PSP rare taxa among putative keystone taxa identified by betweenness (Table 1). Gokul *et al.* (2016) previously identified supraglacial keystone taxa in the cryoconite communities of a High Arctic ice cap, and the present study extends the case that specific rare taxa exert disproportionate influence on the bacterial communities of glacier surfaces through maintaining high levels of metabolic potential. A recent synthesis (Jousset *et al.*, 2017) has highlighted that rare taxa may represent overlooked keystone species through a variety of mechanisms. Here, the tendency of high PSP rare taxa to also represent “hubs” or “bottlenecks” in the network structure of the

bacterial communities, as intimated by their high betweenness centrality score (Peura *et al.*, 2015), suggests a potential mechanism for rare taxa to act as keystone species. High PSP may indicate either the abundance of ribosomes to support protein synthesis, or at least the potential to form ribosomes rapidly (i.e. the presence of 16S rRNA). Consequently, it is tempting to speculate high PSP rare taxa may be at an advanced state of readiness to synthesize proteins (e.g. enzymes catalysing the breakdown of otherwise recalcitrant carbon or nutrient sources,) which enhance the fitness of co-occurring taxa. In this manner the highly active rare biosphere may prove disproportionately influential for the structure and function of the surface communities of the Greenland Ice Sheet. It should be noted that rare taxa may be especially vulnerable to seasonal changes(Debroas *et al.*, 2015, Hill *et al.*, 2015) or indeed local extinction(Jousset *et al.*, 2017), but may also deliver important ecosystem functions (Hausmann *et al.*, 2016). Therefore, the potential impact upon community stability in the harsh conditions of the climate-sensitive Greenland Ice Sheet is emphasized.

Furthermore, Table 1 reports these taxa typically possess very close relatives (either as environmental sequences or named isolates) in a diverse range of habitats within the global cryosphere, with two implications. Firstly, this lends pragmatic support to their likely authenticity within the communities of the Greenland Ice Sheet Dark Zone, but secondly, the inference is that adaptations resulting in disproportionately high PSP and high betweenness centrality may be common among cosmopolitan species in the polar and alpine regions.

Implications for biogeochemical cycling

The prominence of OTUs assigned to *Methylobacterium* in the high PSP taxa of snow and stream water communities (Figure 7, Figure 8) is very apparent. In particular, the exceptionally high PSP shown by the *Methylobacterium*-1 OTU is striking for both habitat types, with other related OTUs (*Methylobacterium*-6342 and *Methylobacterium*-1508)

showing very high PSP. *Methylobacterium*-1 is well represented within the snowpack 16S rRNA gene profiles of week 1, but then shows disproportionately high PSP in following weeks before its loss from the snowpack community by the fifth week. This would suggest continued protein synthesis potential is maintained for some time in spite of its rapidly diminished population size within the snowpack.

Combined, this pattern of high PSP taxa affiliated to the genus *Methylobacterium* merits further consideration. *Methylobacterium* are well known facultative methylotrophs (Chistoserdova *et al.*, 2003, Chistoserdova, 2011), raising the prospect of methyl metabolism as a hitherto unappreciated metabolic strategy on the Greenland Ice Sheet. Redeker et al (Redeker *et al.*, 2017) provided direct evidence of trace gas metabolism in polar snowpacks through the cycling of methyl halides and dimethyl sulphides. Although Redeker et al (2017) did not explore the diversity of microbes associated with methyl cycling, it is possible that *Methylobacterium* in the decaying snowpacks of the Dark Zone are involved in cycling of climate-relevant trace gases.

The relevance of disproportionately high PSP *Methylobacterium* to biogeochemical cycling in the Dark Zone is further amplified when their status as the dominant 16S rRNA (cDNA) taxon in meltwater exports is considered. Supraglacial meltwater from the Dark Zone is typically routed via surface streams into moulins to the bed of the Greenland Ice Sheet, an environment conducive for methane cycling(Yang & Smith, 2016). In catchments fed by meltwater from the Dark Zone, variable rates of methanogenesis and methane oxidation have been observed, with potential impacts for the global methane cycle (Dieser *et al.*, 2014, Lamarche-Gagnon *et al.*, 2019). The discharge of highly oxygenated supraglacial meltwater into the subglacial environment is strongly associated with the cessation of methanogenesis and consumption of methane via aerobic oxidation (Dieser *et al.*, 2014). The export of high PSP *Methylobacterium* from the Dark Zone surface in this meltwater, as detected here, raises

the prospect that surface-derived taxa inoculating the bed of the Greenland Ice Sheet play a role within a subglacial consortium nourished by the oxidation of methane. Further investigations focused on the fate of supraglacial microbiota transferred to the subglacial ecosystem could reveal whether this process is sufficient to mitigate the subglacial synthesis of this potent greenhouse gas.

Conclusions

In summary, 16S rRNA gene and rRNA (cDNA) quantification and sequencing of snow, cryoconite and stream water bacterial communities from the Dark Zone of the Greenland Ice Sheet was conducted at weekly intervals during the melt season of 2014. Recently, attention has been focused on the albedo-reducing properties of microbial consortia within the Dark Zone (Williamson *et al.*, 2019) highlighting the importance of microbial interactions in the future of the Greenland Ice Sheet. Our study addresses the related question of microbial community dynamics, and reveals that rare taxa appear to be disproportionately active. Notably, these taxa appear central to the structure of their communities and may play under-appreciated roles within the carbon cycle of the Greenland Ice Sheet. The presence of high-PSP rare taxa within *Methylobacterium* in melting snow and stream-water raises the prospect of supraglacial methyl compound cycling and export to the subglacial ecosystem. Our study represents a targeted locus amplicon sequencing approach, which in future could be complemented with genome-resolved metagenomics and direct process measurements of carbon cycling and export in both Dark Zone surface and connected downstream habitats. This would further elucidate the connections between these communities, climate change and impacts on downstream riverine and marine ecosystems from the most expansive supraglacial bare ice habitat in the Northern Hemisphere.

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Table 1 provided as a separate file

Supplementary Information accompanies this paper online

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