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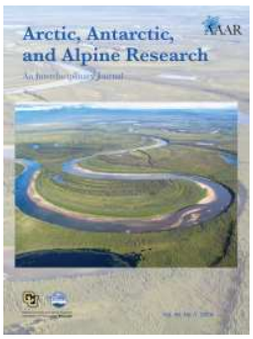

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Seasonal changes in the viability and abundance of bacterial cells in the snowpack ecosystem of a High Arctic ice cap

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ABSTRACT

Microbes play an essential role in nutrient turnover within Arctic environments, and their contribution to biogeochemical cycles can depend on several factors, including but not limited to cell viability. In this study, we employed the SYBR-PI dual cell stain to epifluorescence microscopy to enumerate proportions of potentially viable and non-viable bacterial cell populations within a melting snowpack on an ice cap, Foxfonna in Svalbard. Non-viable cells dominated on Foxfonna ($2.5 \pm 0.36 \times 10^7$ cells m^{-2}) during the June to early July period, when biological production was usually at its peak. Furthermore, non-viable cells also dominated the total cell abundance within superimposed ice (223 ± 242 cells mL^{-1}) and glacial ice (695 ± 717 cells mL^{-1}) beneath the snow. We propose that the rapid, early loss of cell viability was caused by a number of abiotic and biotic factors. Hence, necromass (dead cell residue) contributed to the export of organic matter to downstream ecosystems.

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Snowpack; viable cells; non-viable cells; Svalbard; High Arctic

Introduction

As the Arctic heats up 5–7 times faster than the global average (Isaksen et al. 2022), a rapid and significant increase in snowmelt runoff is expected toward the end of the 21st century (Hanssen-Bauer et al. 2018; Schmidt et al. 2023). Additionally, with increasing rain-on-snow events, even in the midst of winter (Førland and Hanssen-Bauer 2000), snowpacks are becoming warmer, wetter, and less persistent. This equates to a rapidly changing environment for the rich consortium of microorganisms that reside in snowpacks, and an increase in the release of microorganisms to downstream environments with meltwater runoff (Stevens et al. 2022). However, the contribution of snow melt versus glacier ice melt to these microbial cell fluxes is unknown. Furthermore, it is not known what proportion of these microbes remain viable through the seasonal cycle of accumulation and ablation in glacial snowpacks, i.e., *before* their release to downstream ecosystems. In the context of nutrient and carbon cycling within supraglacial snowpacks, this is especially important, as viable microbes have been known to sequester 50–70 percent of the nutrient reservoir within snowpacks before its release to runoff (Hodson 2006).

Svalbard, in the High Arctic, is a region more than half-covered by glaciers and ice caps, which translates into approximately an area of 3.4×10^4 km^2 (Nuth et al. 2013). Snow cover can persist for up to 7–8 months of the year in the ablation areas of these glaciers, and even longer in higher elevation firn accumulation areas. The dynamic and often extreme conditions encountered within such snowpacks raise questions about the ability of microorganisms to maintain their membrane integrity and carry out metabolic activity. For these reasons an intact cell membrane is considered an indirect indicator of a viable cell with the capacity to grow and proliferate under suitable conditions in the snow (Davey et al. 2004).

Various techniques have been used to examine the integrity of a cell membrane and in the context of snow and ice environments, epifluorescence microscopy has had a good history of application to show cell densities in deep ice cores (e.g., Price and Bay 2012; Santibáñez et al. 2018), surface glacial ice on the Greenland ice sheet

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(e.g., Stibal et al. 2015), Antarctic snow (e.g., Michaud et al. 2014), Svalbard snow (e.g., Amato et al. 2007), mountain snow covers in Japan (e.g., Segawa et al. 2005), and snow/air in the Canadian High Arctic (Harding et al. 2011), as well as cell fluxes from a supraglacial catchment on a glacier in Svalbard (Irvine-Fynn et al. 2012). However, not all these studies examined the associated viability of the microbes, and those that did relied on techniques other than microscopy, such as isolating and culturing viable microbes (Segawa et al. 2005; Harding et al. 2011), or ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy to check for biodegradation of organic compounds (Amato et al. 2007). Irvine-Fynn et al. (2012) did not incorporate viability within their study, but assumed viable cells in order to estimate total cell carbon and nutrient export.

There exists a relatively small body of literature on cell viability studies related to supraglacial snowpacks. For example, Bradley et al. (2022) used a suite of analyses such as metabarcoding, metatranscriptomic, and the bioorthogonal non-canonical amino acid tagging (BONCAT) technique, to differentiate between active and inactive cell fractions in snow and glacial ice from an Icelandic ice cap and a glacier in Greenland. Otherwise, Malard et al. (2019) evaluated cell viability in Antarctic snow, but in the context of relic deoxyribonucleic acid (DNA) (i.e., remains after cell death) and its impacts on microbial diversity analyses. An example from a different yet comparable environment to the snowpack is that of lake ice, where the resident microbial communities experience the same seasonal freeze and thaw cycle which characterize melting snowpacks. In their study of such an environment in the McMurdo Dry Valley lakes, Dieser et al. (2010) used a combination of techniques such as Propidium Monoazide with quantitative Polymerase Chain Reaction (PCR) and electrophoresis to examine membrane integrity.

In the present study, we expected meltwater and nutrient availability (see Dayal et al. 2023) to influence the temporal and spatial distribution of viable and non-viable cells within the snowpack throughout the summer on Foxfonna, an ice cap in Svalbard. We also compared the viability of microorganisms in the winter snow accumulation to the underlying glacial ice in order to deduce whether the predominant role of glacial runoff is the delivery of viable or non-viable cells to downstream ecosystems (Mindl et al. 2007; Hood et al. 2009). Next, we considered the role of the transient refrozen snowmelt layer (or "superimposed ice") that forms upon the cold glacier surface after the onset of snowmelt in summer because, while superimposed ice is often an important ecosystem in its own right (e.g., Hodson et al. 2021), the refreezing process might contribute to cell mortality. Furthermore, by better understanding whether cells remain alive and viable prior to export, we can begin to quantify their role in the transformation and turnover of nutrients and the linkages between glacial and downstream ecosystems better understood.

Using epifluorescence microscopy and dual cell-staining, this study assessed: 1) the proportions of potentially viable and potentially non-viable cell populations in a melting snowpack through the summer, and 2) spatial and vertical heterogeneity in these cell populations.

Materials and methods

Study site

Foxfonna (78°07′-78°09′N; 16°06′-16°11′E) is a small (4 km^2) mountain ice cap in Central Spitsbergen, Svalbard (Figure 1) approximately 12 km east of the settlement at Longyearbyen. It lies between 550 and 808 m.a.s.l. and is up to 80 m thick (Rutter et al. 2011). The site has a network of seven stakes that have been used to assess the mass balance since 2007, and have reported sustained mass balance decline since 2008 (Hodson, Unpublished Data). The study described here was conducted during 2016, after a strong winter accumulation that was followed by a high ablation rate that resulted in near-complete snow cover removal from the entire ice cap by the end of the summer (Dayal et al. 2023).

Snow pit sampling

Field sampling was undertaken to coincide with key periods in the seasonal evolution of the snow cover (Dayal et al. 2023). These periods are summarized in Table 1. Hence, pre-melt surveys were carried out once in April, followed by June and then twice in July (period of primary summer melt). Based on the directional aspect of Foxfonna, seven stakes (NW, SW, S, AWS, SE, NE and N) were chosen for snow pit sampling (Figure 1). At each of the seven stakes, the following samples were collected monthly into sterile Whirl-pak (*Nasco*) bags using a sterile graduated cylinder: surface snow (0–20 cm depth), mid snow (from 20 cm depth to the base of the snowpack; sampled every 30 cm or so), refrozen snowmelt superimposed upon the glacier ice at the base of the snowpack. These samples are hereafter referred to as "TOP," "MID" and "SUP ICE" respectively, whilst "GL ICE" refers to the uppermost 25 cm of glacier ice extracted at each stake during the early and late July surveys only (refer to Dayal et al. 2023 for further sampling details).

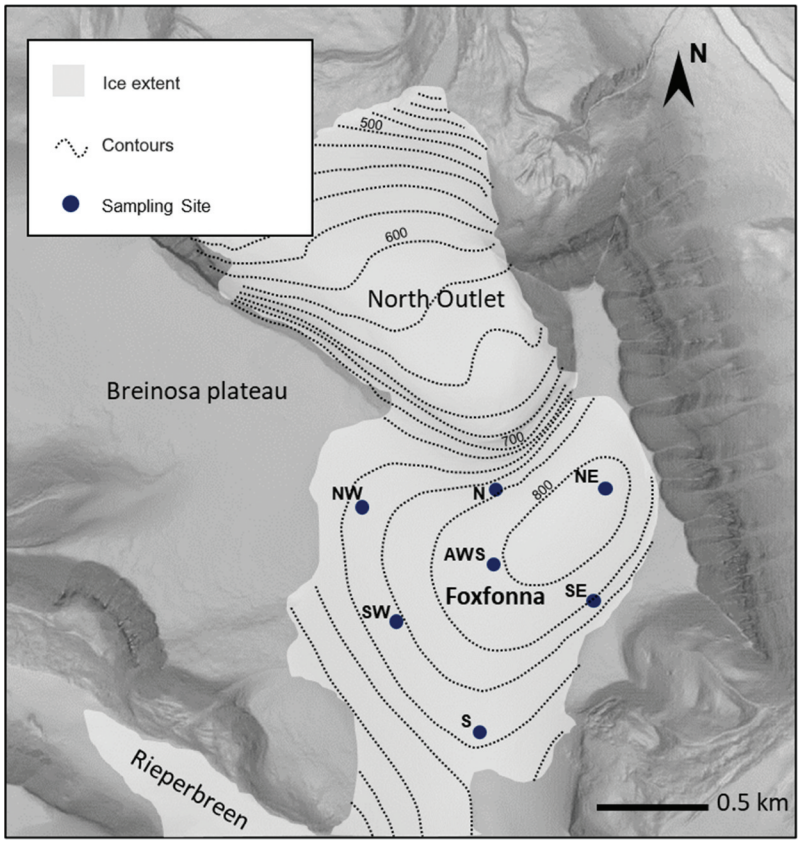

Figure 1. Foxfonna ice cap and its North Outlet glacier in Svalbard, with sampling sites marked.

Table 1. Description of the seasonal evolution of snow cover prior to each sampling survey.

Survey (2016)	Description of snow cover on Foxfonna
23 April	Snowpack was dry with no melt water production. Possible snow redistribution after wind erosion of the surface likely affected surface layer.
8 June	Increase in water content with the rapid development of snow into wet or larger coarse-grained ice crystals occurred, but no runoff. As a result, ice lenses and superimposed ice started to form.
9 July	Intense melt water production through snowmelt on the ice cap occurred. Growth of superimposed ice ceased. As the snowpack slowly disappeared, slush or basal melt water formed, and the underlying layer of superimposed ice became exposed in places.
31 July	Superimposed ice and glacier ice ablation dominate runoff when almost no snow cover remained.

Sample processing

The collection of samples was carried out under varied temperature conditions, ranging from −15°C to 10°C, from 23 April until 31 July 2016. Post sample collection, samples were stored frozen in sterile 1 L Whirl-paks (*Nasco*) at −20°C until their pre-processing at the University Center in Svalbard (UNIS). At UNIS, to minimize biogeochemical changes, all the samples were melted in the dark at ambient room temperature (~ 21°C), mixed thoroughly by shaking, before transfer from Whirl-pak bags to sterile 15 mL Corning® centrifuge tubes and refrozen (at −20°C) for transport and analysis back in the UK. Given that cells in snow and ice environments are accustomed to freeze-thaw cycles (Maccario et al. 2015), it is reasonable to assume that the cell populations collected are adapted to such temperature changes and thus the storage and transport of the samples mirrors their environmental conditions. On the other hand, we acknowledge that it is difficult to assess the impact of time and storage conditions on cell viability within the samples, and therefore the results presented are to be considered as best estimates. Simultaneously, due to the ambiguous nature associated with the definition of a "viable cell," the terms "live" and "dead" cells are avoided in favor of the terms "potentially viable" and "potentially non-viable" instead.

Epifluorescence microscopy for potentially viable and potentially non-viable cell counts

Details of epifluorescence microscopy using a Widefield Nikon Live Cell System for total cell counts have been

previously reported in Dayal et al. (2023). For cell viability, the present study utilized a protocol developed based on the work of Barbesti et al. (2000) and Grégori et al. (2001) which was conducted on cultured bacteria and bacteria from aquatic and marine waters. These studies employed a nucleic acid double-staining assay, SYBR Green II (*Molecular Probes*) and propidium iodide (PI, *Invitrogen*) wherein the quenching or energy transfer properties of the stains were utilized in order to assess bacterial viability. Therefore, after both the stains bound to the nucleic acids inside the cell, one of three scenarios played out 1) A complete quenching of the permeant stain (SYBR Green II) occurred, indicating cells with compromised membranes because they allowed PI to enter and produce a red fluorescence signal. In such cases, the cells were identified as non-viable. Otherwise, 2) a lack of quenching indicated cells with intact membranes and therefore deemed viable because they excluded PI and displayed only SYBR II–induced green fluorescence. And finally, 3) a partial quenching or lowering of green fluorescence, because of energy transfer to PI resulting in an increase in red fluorescence indicated cells with slightly damaged membranes.

Post imaging, images were converted to 8-bit grayscale on the software ImageJ. Cells were counted using the *Analyze Particles* function with a size range of 0.2 to 2 μm^2 and a circularity of 0 to 1. This was done to exclude the counting of mineral debris and remove noise. Filamentous bacteria or snow algae were measured manually on the software, as they were larger (10–20 µm). The cell counts (cells mL^{-1}) were calculated as a product of the counts per image and the microscope's field of view (FOV), divided by the volume of the sample filtered. For ease of data analysis, damaged cell counts were included within the non-viable category.

Statistical analyses

A parametric test of difference was employed for statistical analyses on log transformed data (paired *t*-test). However, for stake-wise comparison (non-log-transformed data), the Wilcoxon test was used.

Results

Seasonal changes in cell loading

Figure 2a shows the seasonal evolution of the snow cover on Foxfonna and of the cell loading. Here, potentially viable and non-viable cell loadings (cells m^{-2}) were calculated as the product of the concentration (cells mL^{-1}), the thickness of the snow layers (cm) and the density of the snow layers (g cm^{-3}). The sum of the separate TOP, MID, and SUP ICE cell loadings was then used to represent the total cell loading at each stake. These were then averaged to provide an estimate representative of the entire ice cap. The "Total" was calculated from the sum of the average potentially viable and non-viable cell loadings. GL ICE is excluded here because the focus of the study was upon the snowpack ecosystem. However, its cell concentrations in the upper 25 cm will be used for comparative purposes later.

The average autotrophic cell abundance on the ice cap through the melt season was 0.5 ± 2.7 cells mL^{-1} (see Dayal et al. 2023). This was considered insignificant when compared to the bacterial cell numbers, and hence the bacterial community form the basis for discussion in this study.

Figure 2a shows that the average Snow Water Equivalent (SWE; product of layer thickness and density) and total cell loading across the ice cap were relatively unchanged between April and June, although there was a marked increase in the proportion of the cell population that was potentially viable (Figure 2b). However, between June and early July, the total bacterial cell loading increased significantly (p-value = 0.05 where $\alpha = 0.05$) by almost an order of magnitude from $5.3 \times 10^6 \pm 2.7 \times 10^5$ cells m^{-2} in June, to $3.8 \times 10^7 \pm 4.3 \times 10^6$ cells m^{-2}, early July. Surprisingly, this was due to an increase in potentially non-viable cell loadings (also statistically significant; p-value = 0.02 where $\alpha = 0.05$; Figure 2c) in early July, compared to June ($2.5 \times 10^7 \pm 3.6 \times 10^6$ and $7.3 \times 10^5 \pm 3.6 \times 10^4$ cells m^{-2}, respectively). Next, the period from early July to late July showed a reduction in total and potentially non-viable cell loadings, although the change was not statistically significant for total cell loading (p-value = 0.07), which decreased from ($3.8 \times 10^7 \pm 4 \times 10^6$ to $6.1 \times 10^6 \pm 1.4 \times 10^6$ cells m^{-2}). An increase in potentially viable cells occurred at this time (from $2.5 \times 10^6 \pm 2.1 \times 10^5$ to $3.8 \times 10^6 \pm 6.7 \times 10^5$), although this was not statistically significant either (p-value = 0.08 where $\alpha = 0.05$; Figure 2c). By contrast, the decline in potentially non-viable cell loading (from $2.5 \times 10^7 \pm 3.6 \times 10^6$ to $1.5 \times 10^6 \pm 4.1 \times 10^5$ cells m^{-2}) was significant (p-value = 0.03 where $\alpha = 0.05$; Figure 2c). Figure 2a shows that this period was also marked by the rapid depletion of the snowpack water equivalent, showing that the loss of cells was most likely caused by meltwater runoff.

Figures 3a and 3b show that the marked increase in the total cell loading between June and early July was also associated with an increase in the spatial variability from one stake site to another, resulting in

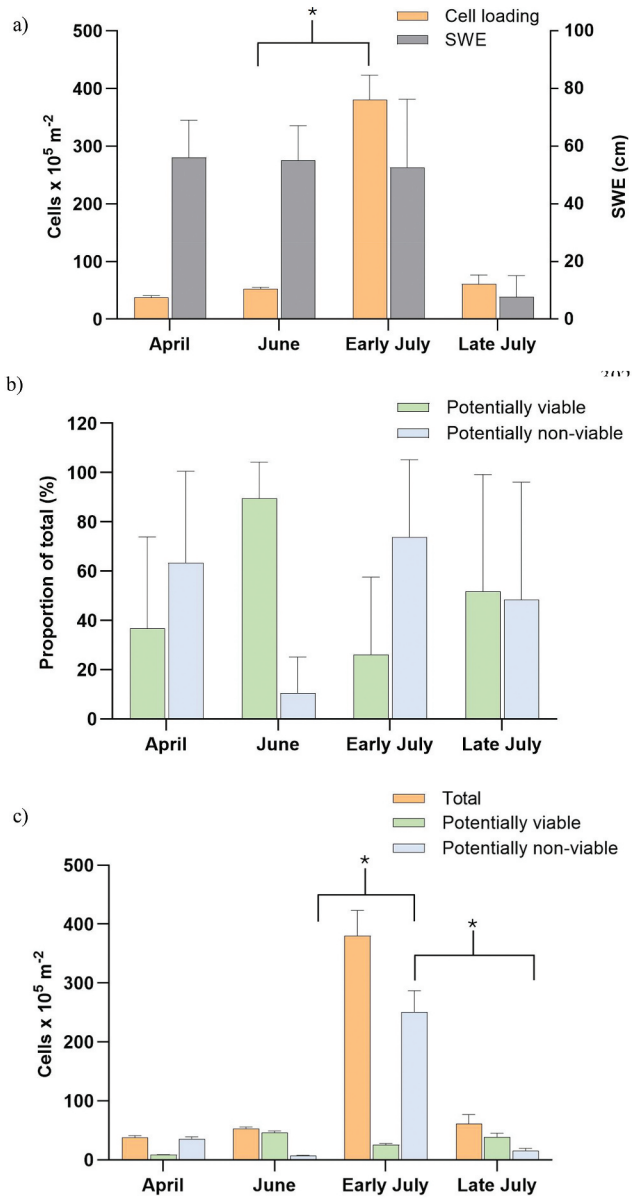

Figure 2. Seasonal change in a) average SWE (cm) and total cell loading (cells m^{-2}) b) proportions of potentially viable and potentially non-viable cells c) Total, potentially viable and potentially non-viable cell loading (cells m^{-2}) within a glacial snowpack on Foxfonna. Error bars are standard deviation.

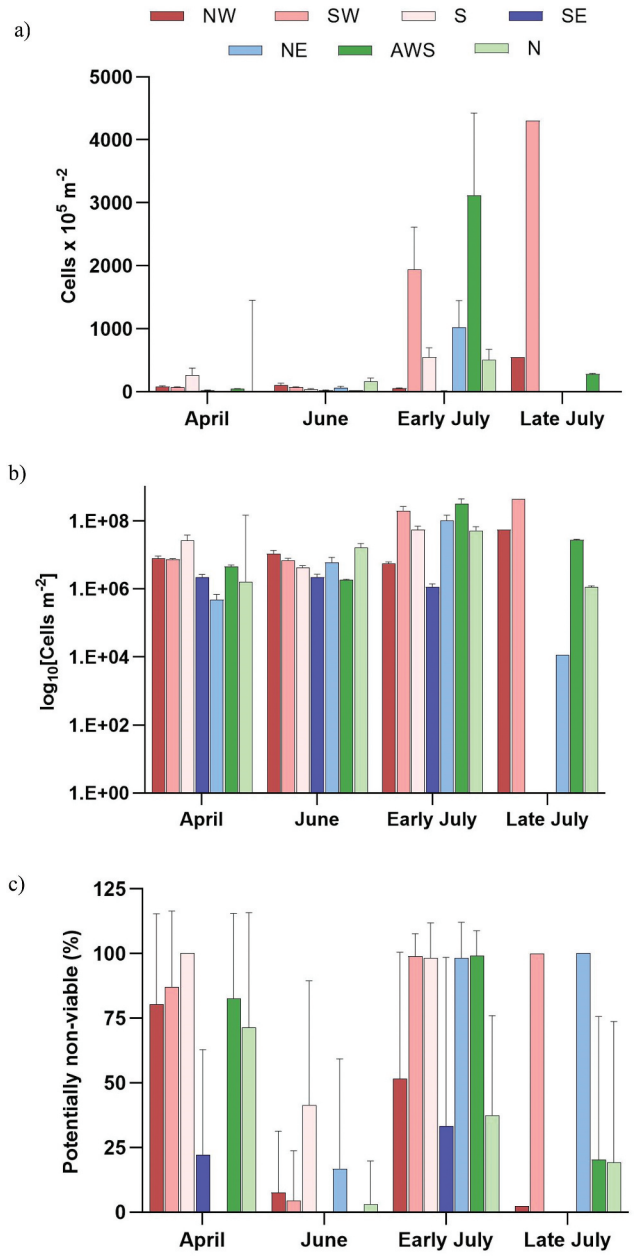

Figure 3. Seasonal change in a) total cell loading (cells m^{-2}) b) log-transformed total cell loading (cells m^{-2}) c) proportions of potentially non-viable cells, within a glacial snowpack on Foxfonna (n = 94).

large standard deviations. This was enhanced further by the complete removal of snow from several (but not all) stakes when the final survey was conducted (late July). Figure 3c shows that the proportion (%) of the total cells at each stake that were potentially non-viable also demonstrated significant spatial variability, but this was not limited to late season. Otherwise, the data clearly show that potentially non-viable cells dominated throughout the summer, except in the period prior to the June survey (Figure 2b).

Vertical differences in cell concentrations within the snowpack

To understand whether changes in the vertical distribution of potentially viable and potentially non-viable cells changed as energy and liquid water became available at the start of the ablation season, the vertical distribution of cell concentrations (not loadings) at each site during April and June were compared (Figure 4). We found no consistent patterns in the distribution of the potentially non-viable cells. However, Figure 4 shows that during

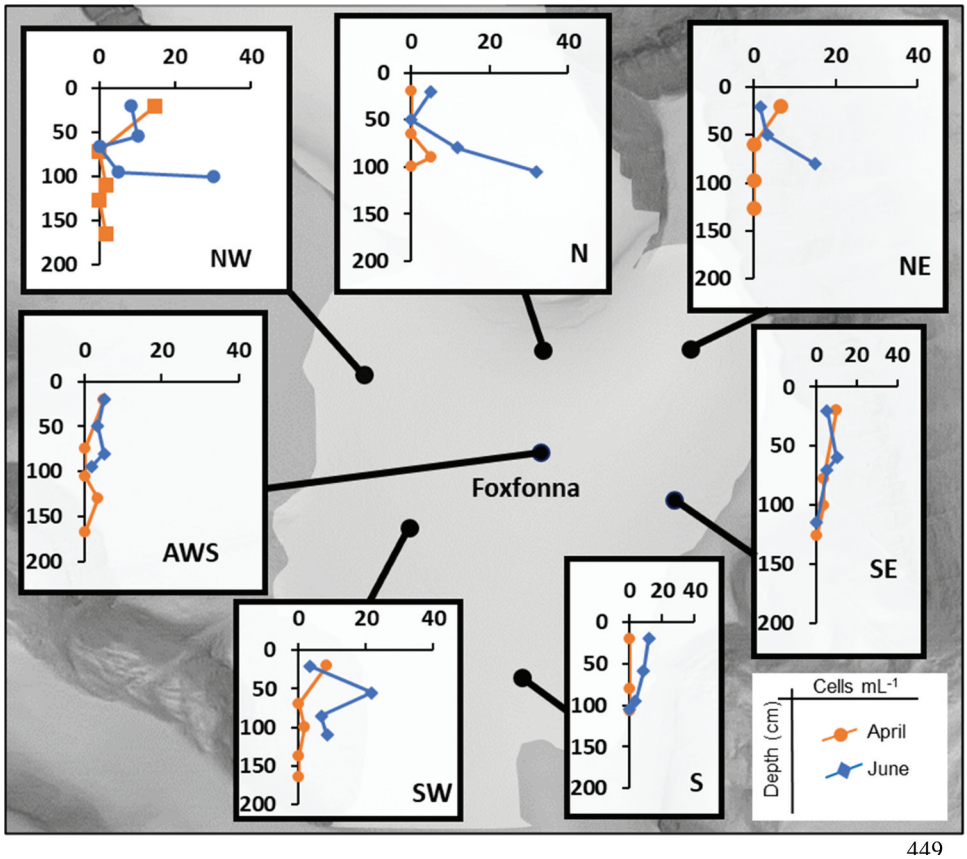

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Figure 4. Vertical distribution of potentially viable cell abundance (cells mL^{-1}) through the snowpack in April and June, 2016 on Foxfonna (n = 1 at each depth).

this interval, the potentially viable cell population often increased markedly at depths approaching 100 cm in the northern part of Foxfonna, and depths nearer ca. 50 cm elsewhere, with the exception of stake S, which demonstrated the greatest increase in potentially viable cell concentrations at the surface.

After June, snowmelt percolation and re-freezing formed ice lenses within the snow and superimposed ice at its interface with the underlying glacier. The latent heat release and other energy sources at this time of year bring the entire snowpack to the melting point, such that water becomes increasingly mobile and abundant within the snowpack (Dayal et al. 2023). Cell concentrations in early July snow cover (averaged at each stake) were therefore compared with those in superimposed ice and surface glacier ice. Table S1 shows that the heterogeneity remained significant, and so differences were not very clear. However, potentially viable cells were only dominant in the superimposed ice (445 ± 0 cells mL^{-1}) at stake N (Table S1) and so most cells that relocated to the superimposed ice layer were potentially non-viable. Potentially viable cells also stayed below 30 cells mL^{-1} in the snow during this interval. Therefore, potentially non-viable cells formed the highest proportion of the total cell abundance in both snow and superimposed ice. Potentially non-viable cells also dominated the surface glacier ice at Foxfonna (Table S1), where average cell concentrations were greater than in superimposed ice. However, once more, the concentrations were so spatially variable that the difference was not statistically significant at the 95 percent confidence level. The location of the potentially non-viable cells was typically dominated by a single sample at each site. Samples with significant concentrations of non-viable cells (i.e., > 10^2 cells mL-1) were all located beneath the upper snow surface at depths of 50 cm or more (Figure 5).

Discussion

The results presented above demonstrate great variability in the distribution of potentially viable and non-viable cells across the ice cap. However, in spite of this variability, two drastic changes in the seasonal development of Foxfonna's snowpack bacterial community demand further discussion. First, in the early summer, total cell loading changed little, yet there was a marked, statistically significant switch toward a bacterial community dominated by potentially viable cells (from an

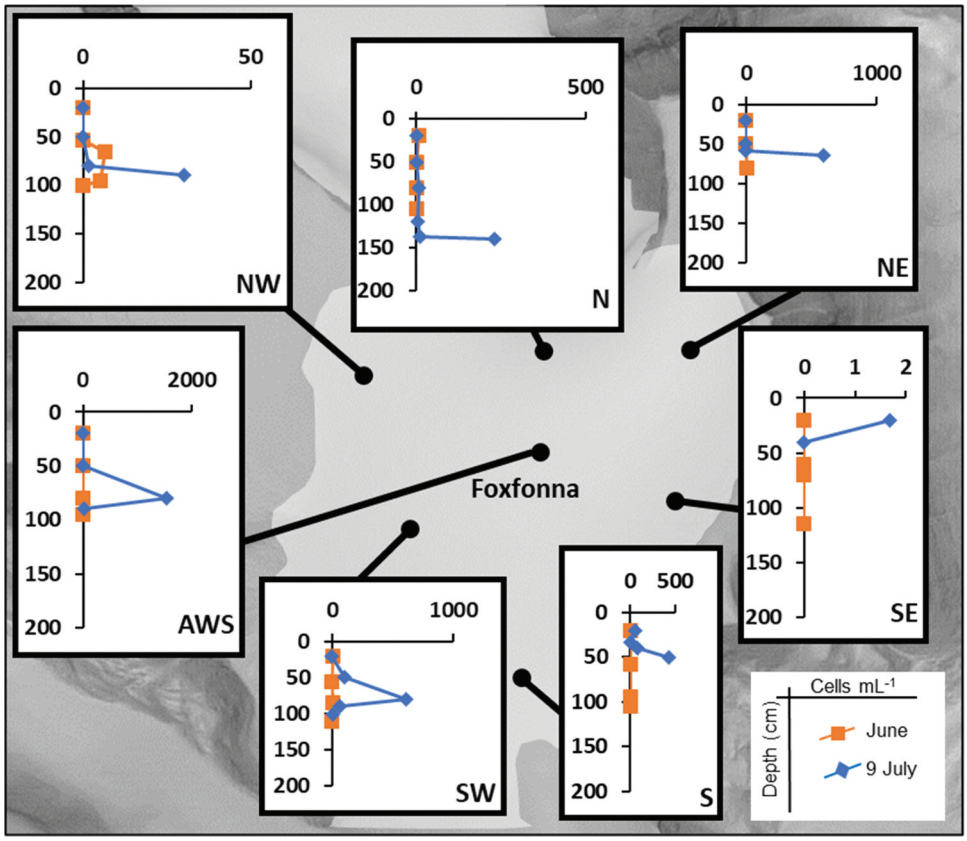

Figure 5. Vertical distribution of potentially non-viable cell abundance (cells mL^{-1}) through the snowpack in June and early July, 2016 on Foxfonna (n = 1 at each depth).

ice cap average of ca. 2 percent in April to ca. 90 percent in June). Second, a near-order of magnitude increase in cell loading occurred throughout the rest of June, but resulted in the dominance of potentially non-viable cells across the ice cap by early July. The situation changed little thereafter, although the rapid loss of snow cover and spatial variability made patterns difficult to find. The above events give the impression of a "boom and bust" scenario that is initiated with the arrival of water and energy for microbial metabolism within Foxfonna's snow. These two dynamic shifts are therefore discussed further below, before we consider their implications for organic matter release to downstream ecosystems.

Seasonal changes in cell viability I: Early summer boom

Figure 4 showed that the increase in potentially viable cells in June occurred below the surface at most sites, but with significant variability and with no important overall gain in biomass across the ice cap. Furthermore, the potentially viable cell concentrations in surface snows decreased at almost all sites (except Stake S, where it increased and Stake AWS, where no changes occurred). Spatio-temporal variability in the availability of essential nutrients such as N and P (see Dayal et al. 2023) did not show any clear correlations with these distribution patterns of the various cells, and so other factors such as water availability are thought to be more influential. It seems intuitive that those cells close to, but not immediately at, the snow surface would respond best to the arrival of liquid water in early summer. This would include Stake S, but its southerly aspect and lower elevation perhaps caused the loss of the surface snow layer due to ablation just prior to sampling. Otherwise, at this point of the season, percolation of liquid water to the very base of the snowpack was unlikely due to refreezing, which first occurs beneath the surface of the snow, releasing latent heat that helps bring the snow to the melting point here (Dayal et al. 2023). Lower temperature variability and more liquid water availability were therefore likely to be important for stimulating the biological productivity in the upper part of the snowpack when the sampling survey was undertaken. However, cells within snow at the immediate surface would have been subjected to a history of physical stress such as more intense ultraviolet (UV) irradiation, freezing, and desiccation cycles than those immediately below. The

surface snow also experienced the most wind redistribution prior to the onset of surface melting in June, and it is expected that this is also less conducive for maintenance of cell viability (e.g., Terzeiva et al. 1996).

Seasonal changes in viability II: Mid-summer bust

June to early July was identified as the key period for biological productivity due to an increase in average bacterial cell loading from $5.3 \times 10^6 \pm 2.7 \times 10^5$ cells m^{-2} in June to $3.8 \times 10^7 \pm 4.3 \times 10^6$ cells m^{-2} in early July (Figure 4). This bacterial growth was the highest of the melt season on the ice cap. However, a community dominated by potentially viable cells in June ($4.7 \times 10^6 \pm 2.5 \times 10^5$ cells m^{-2}) switched to a predominantly non-viable population by early July ($2.5 \times 10^7 \pm 3.6 \times 10^6$ cells m^{-2}). Therefore, one or more stressors seemed to limit the bacterial life cycle within the snowpack. Several abiotic and biotic processes are therefore examined below.

An initial hypothesis that was quickly rejected was that the community was principally derived from the ocean surface in concert with marine aerosol deposition, and then subject to osmotic shock after immersion in dilute snowmelt. This proposal was considered on account of the proposed dominance of marine picocyanobacteria (10^{-12} µm) in Arctic/Antarctic ice cores (Price and Bay 2012). Marine aerosol was described in Foxfonna snow melt by Dayal et al. (2023), and so some transport of marine bacteria to the site is indeed likely. However, the microscopy conducted in the present study allow us to discount picocyanobacterial on account of their insufficient size. The dominance of marine microorganisms in most glacial snowpacks does seem unlikely, but their resilience to osmotic stress (e.g., Maccario et al. 2015 and references therein) during the melt season is an interesting problem that might bear relevance to organic carbon cycling (Smith et al. 2017).

Next, we re-considered the processes of refreezing and UV light penetration that were thought to limit the proliferation of viable cells in surface snows earlier in the season. With regard to refreezing, we expected to see a greater proportion of potentially non-viable cells within the superimposed ice when compared to the parent snowpack. Since we saw no such significant difference (not shown: see Tables S1 and S2), we assumed this process had little influence. However, snow metamorphosis, resulting in grain growth as the snowpack becomes isothermal (at the melting point), has a marked effect upon the penetration of UV light into snow (e.g., Hodson et al. 2017), facilitated by the low absorption of light at UV wavelengths by water in any phase (Warren 2019). An increase in the penetration of harmful UV rays deeper into the snowpack and for longer time periods was therefore possible during the June to early July period, and so the potential for damage to the cellular structure is also likely to have increased. However, it is unlikely that harmful UV light propagated all the way through the snow at this stage of the summer due to scattering, and we saw no clear bias in the proliferation of non-viable cells at the surface. Therefore, if UV light was influential, the non-viable cells would have to be relocated by the percolating snowmelt. This indeed might explain the tendency for most non-viable cells to accumulate in single samples at depths of 50 cm or more (Figure 5), but since the superimposed ice layer represented the most important layer formed by such percolation, the fact that non-viable cells were not dominant here suggests that either other factors were influential, or the relocation failed to transport the cells to the base of the snowpack, perhaps due to obstruction by ice lenses.

Potential biotic factors for causing the loss of cell viability include predator-induced mortality of bacteria (Laybourn-Parry and Pearce 2016). For example, heterotrophic nanoflagellates graze on bacteria, but no such microbes were seen during microscopic or sequencing analysis of the samples (Dayal 2021). Similarly, high viral infection with high bacterial mortality rates is prevalent in supraglacial habitats. However, there are few available studies on bacteriophages (viruses that infect bacteria) or virus-like-particles (VLPs) in the polar regions and relevant studies instead focus mostly on cryoconite holes (e.g., Anesio et al. 2007; Bellas et al. 2013; Bellas, Anesio, and Barker 2015), polar lakes (e.g., Kepner, Wharton, and Suttle 1998; Säwström et al. 2007, 2008) or supraglacial melt water (Rassner et al. 2016).

The above studies show that despite several reasons for strong viral-host relationships to be expected in the polar regions (Anesio and Bellas 2011), there exists a paucity of datasets for low temperature habitats, including snow. But if viruses can be considered "the most abundant biological entity on the planet" (Anesio and Bellas 2011), then their role in snowpack microbiota needs to be investigated, especially as viruses are known to shuttle between marine and terrestrial reservoirs and are important players in global biogeochemical cycling (Suttle 2005).

One of the factors that can be considered important in the context of this study is the lytic cycle of a virus wherein the virus overtakes the cell's machinery to instruct it to produce more viruses, with the eventual destruction and death of the cell as it bursts to release new viruses. Currently, factors that can trigger this lytic cycle in supraglacial habitats are unknown, although there is an indication from sea ice/pack ice studies that ultraviolet light might be

one such trigger (Gowing et al. 2002, 2004). Since marked changes in the penetration of UV light would have occurred in conjunction with snow grain metamorphosis during June, then this trigger is plausible.

Considering this past research of viruses in aquatic and marine environments, Anesio and Bellas (2011) hypothesized that pseudolysogeny could be the strategy of survival for viruses in ultraoligotrophic environments and one of the questions asked in their paper that holds relevance to this study, concerned the relationship between nutrient gradients and their effect on bacteria-virus interactions. In this research, the loss of cell viability followed a key period of biological productivity (due to increased nutrient and liquid water availability). It is possible, then, that the cells in June were likely in a starved and pseudolysogenic state (i.e., harbored dormant viruses) and upon an increase in nutrient availability began to grow, prompting the viruses to make use of this now available metabolic energy to initiate replication and force the bacterial community to enter the lytic cycle.

Conclusions

This is the first seasonal study of changing viable and non-viable cell populations within a snowpack ecosystem upon a High Arctic ice cap. One of the more significant findings was that non-viable cells soon dominate the ice cap after a biologically productive early summer period (June to early July). Subsurface snows (up to 100 cm) were found to be more conducive to the proliferation of cells compared to surface snow (top 20 cm) during the pre-melt period (April–June), likely owing to better protection from harmful UV irradiation, freeze-thaw cycles and wind erosion. However, a striking result of our work was that the rapid loss of viability can occur shortly afterward. Its causes remained elusive, but most likely involved a combination of biotic and abiotic factors. When considering the most likely causes, it was easier to discount abiotic factors than it was to confirm biotic factors. The latter includes viral lysis, whose impact upon snow ecosystem functioning constitutes a promising future research avenue for understanding the drastic changes our study has revealed.

It is not known whether the rapid loss of viability in the snowpack ecosystem of Foxfonna during this study is a recurring phenomenon. However, its reported occurrence shows that the transfer of non-viable cells by runoff can likely contribute to the export of labile organic matter to downstream ecosystems.

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AD and AJH conceptualized the project. The responsibility of data curation was handled by AD and ALS. AD and AJH undertook the formal analysis and investigation. Funding was acquired by AD and AJH. Original draft was written by AD with review and editing of the manuscript performed by AD, AJH, MS and ALS.

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