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Microbial Communities in Sediments From Different Landform Systems, Svalbard

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Key Points:

- At the sequence level all glaciers and landforms were different in their biodiversity, only 72/1,268 sequences were detected in >2 samples
- Microbial richness was similar across all glacier locations, landforms and glacier types
- Despite differences in community composition, the concentrations and compositions of EPS in sediment and biomass were consistent

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Svalbard is host to a range of glacier types with different thermal regimes, behaviors and complex hydrological networks and landforms. Here, we hypothesize that the (H1) surge-type glaciers will host different microbial community structures, and that (H2) different landform types will also host different microbial community structures. We further hypothesize that (H3) these differences in microbial communities will result in different concentrations and compositions of extracellular polymeric substances (EPS) in response to potentially different stressors associated with surging behavior (H4) or the formation of different landforms. To test these hypotheses, supra- and subglacial sediments from different glacier types and landforms in southwest and west Spitsbergen were collected (26 locations, across 10 glaciers). 16S rRNA gene amplicon sequencing and epifluorescence microscopy was used to determine the microbial community composition, diversity and biomass. At the sequence level all samples were predominantly different across all glaciers and landforms with only 72/1,268 sequences detected in two or more samples. Diversity indices showed samples generally have similar levels of diversity despite these differences in community structure. The EPS concentrations were also similar (0.34 ± 0.71 mg g^{-1}) at all locations excluding a single outlier, suggesting consistent production of EPS. This has implications for understanding the microbial ecosystem response to changing glacial dynamics as the extent and thermal regimes of glaciers shift due to climate change.

Plain Language Summary Glaciers in Svalbard have a diverse range of behaviors and habitats, from channels hosting large volumes of running water to sediments that have been squeezed into the ice. Here, we investigated if differences in biogeography, such as glacier type and landform, affected the community of microbes present and their production of sticky biofilms which protect the microbial cells from extreme cold. To test this, we collected sediment samples from 26 sites across 10 glaciers in Svalbard and analysed the microbial community diversity in these samples using DNA sequencing. We found that every site had a broadly unique community of microbes with some core microbes present throughout. The amount of biofilms in the sediments was consistent regardless of glacier type or landform. This suggests that while the specific microbes vary biogeographically, their overall compositions and biofilm production remain stable.

1. Introduction

Diverse microbial communities have been detected beneath large glaciers and ice sheets around the world from the West Antarctic Ice Sheet (Christner et al., 2014; Mikuki et al., 2016), Taylor Glacier, Antarctica (Mikucki & Priscu, 2007; Mikucki et al., 2004; Montross et al., 2014), to small valley glaciers such as the Haut Glacier d'Arolla in Switzerland (Sharp et al., 1999; Skidmore et al., 2005). The microbial communities that survive and thrive in the harsh conditions of the subglacial zone must endure low temperatures, permanent darkness, and very low nutrient concentrations which exert large controls over the total biomass of these environments (Boetius et al., 2015; Margesin & Collins, 2019; Skidmore et al., 2005). The year-round low temperatures are also associated with lower cellular reaction rates (Boetius et al., 2015), and the freeze-thaw cycling during seasonal shifts in temperature can also lead to cell damage and death (Zong et al., 2023).

Alternatively, supraglacial environments exposed to air typically have higher biomass and different community compositions due to more favorable environmental conditions. The ice surface is exposed to sunlight and atmospheric gases (Zhang et al., 2024) and may also experience extra deposition of airborne bacteria (e.g., Hervas & Casamayor, 2009) or windblown dust (e.g., Irvine-Fynn et al., 2012; Rassner et al., 2024). The supraglacial environment is also host to biomass rich algal patches and cryoconite (e.g., Irvine-Fynn et al., 2021; Marsh

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et al., 2024) which are the most biologically active parts of the glacial system (Säwström et al., 2002). The exposure to sunlight during summer months and supply of oxygenated meltwaters allow cryoconite microbiota to thrive in ways the subglacial environment cannot (Edwards et al., 2011; Hodson et al., 2007).

Adaptations to the harsh environmental conditions of the Arctic, and in particular the subglacial zone, is paramount for cell survival. One such adaptation is the secretion of extracellular polymeric substances (EPS) comprised of polysaccharides, proteins, lipids, nucleic acids and extracellular DNA, which form a large component of multi-species biofilms (Nagar et al., 2021). These biofilms are an important contributor to both cryo- and osmo-protection in extreme environments (Ali et al., 2020; Boetius et al., 2015; Cai et al., 2019; Schaumann et al., 2007). Several studies have shown that the formation of water repellent biofilms could be, in part, due to an adaptive stress response and survival mechanism to prevent sudden extreme amounts of water absorption and cell bursting due to dynamic drying and wetting cycles (e.g., Achtenhagen et al., 2015; Epstein et al., 2011; Seaton et al., 2019; Stewart, 2002; Timoner et al., 2012). The effects of EPS also goes beyond microbial protection, it is a key component of stabilizing sediment surfaces (Gerbersdorf et al., 2009), mineral precipitation and organic matter production to support ecological communities (Nichols et al., 2005; Norman et al., 2015; Or et al., 2007).

Previous works have demonstrated the importance of EPS' and biofilms in supraglacial environments. Langford et al. (2010) found that EPS' present in cryoconite support aggregation and growth as well as increase settling ability of particles driving preferential melt in the cryoconite hole. Biofilm organization in cryoconite can also promote efficient nutrient transport and cycling within the community as well as the accumulation of organic matter (Smith et al., 2016). The presence of microbial biofilms has also been proposed to develop in the subglacial zone at Finsterwalderbreen, Svalbard due to the prevalence of microbially mediated carbonate dissolution processes linked to their presence (Wadham et al., 2010). It is therefore plausible that protective EPS could also be aiding in the survival of microorganisms in the subglacial environment as well as in the supraglacial environment.

1.1. Influence of Glacier Dynamics on Microbial Communities

The dynamics of different glacier types have the potential to exert stress on microbial communities due to shifts in hydrological systems, basal thermal regime changes and disturbance of sediments through basal deformation. Shifts in the flow regime and hydrological system from a relatively stable to a more dynamic and erosive environment through fast water flow or sediment deformation presents a highly variable environment for microbial communities to adapt to (Hodson et al., 2008; Humphrey & Raymond, 1994). An extreme example of shifts in glacier dynamics is seen in glacier surging behavior whereby the glacier experiences cyclical changes in flow; from predominantly slow flow over a prolonged quiescent phase lasting 20–200 years, to short periods of 10–1,000 times faster flow during a surge phase lasting for 1–10 years (Bouchayer et al., 2022; Clarke, 1987; Murray et al., 2000). In Svalbard the number of surging glaciers is believed to range between 13% (Jiskoot et al., 1998), and 90% (Lefauconnier & Hagen, 1991). However, the impacts of these shifts in the subglacial environment during a surge in the context of the microbial community structure and production of protective EPS have not been fully investigated.

There are two broad potential effects of a surge cycle on the subglacial microbial communities. First, the more dynamic, extreme and erosive conditions with evolving subglacial environments may make it difficult for microbial communities to become established. The non-surging glaciers therefore would represent a more stable, less dynamic environment with consistent subglacial conditions that may be more conducive to stable microbial communities. Alternatively, the surge may promote an influx of nutrients, oxic waters and a potential transport of biomass from supraglacial zones to subglacial zones through ice fracturing and crevassing. This would therefore have the potential to drive more prolific and diverse microbial communities compared to the stable non-surge type glacier.

On a smaller scale, a factor not often considered in understanding microbial community structure is the geomorphological influence (Viles, 2012). We know that different glacial landforms are formed by different erosional and depositional processes reflective of the glacier dynamics, hydrology and basal thermal regime (e.g., Evans & Evans, 2022). Landforms created in the subglacial environment are formed by a multitude of processes. For example, flutes and streamlined landforms develop where fine saturated sediment fills a basal cavity in the ice that forms on the lee side of boulders or other obstacles (e.g., Evans & Evans, 2022; Glasser & Hambrey, 2005; Ottesen & Dowdeswell, 2006; Roberson et al., 2011). Conversely, meltwater channels and conduits that form outflows for the glacial hydrological systems are usually connected to larger meltwater channels in the subglacial zone, which are flushing potentially large volumes of water into the glacier forefield. Some surging glaciers can also form crevasse squeeze ridges (CSRs), where high basal water pressure and deformable sediments are mobilized and squeezed up into fractures in the ice formed during the fast flow phase of the surge cycle (Christoffersen et al., 2005; Rea & Evans, 2011; Sharp, 1985; Sharp et al., 1988). Alternatively, landforms created in the supraglacial zone have not experienced the same level of reworking and flushing processes but are more exposed to aerobiological input. Here, we propose that the different formation mechanisms of these landform features emerging from the glacial environment, could be influencing the microbial community structure.

In this paper we present four hypotheses to determine the potential roles of glacier types and the landform microhabitats on microbial community structure and production of EPS: (H1) Surge-type glaciers will host different microbial community structures, and (H2) different landform types will host different microbial community structures. We further hypothesize that these differences in microbial communities will result in different concentrations and compositions of extracellular polymeric substances (EPS) in response to potentially different stressors associated with (H3) surging behavior and (H4) the formation of different landforms. Our null hypotheses are that there is no link between the community composition and/or EPS production with the different glacier types and landform microhabitats.

2. Study Sites

Svalbard is host to numerous diverse glaciers ranging from small, vanishing, fragmented glaciers such as Ferdinandbreen, to large, complex surging glaciers such as Paulabreen (McCerery, Davies, et al., 2025). This broad range of glacier behavior makes Svalbard an ideal location to investigate the influence of different glacier dynamics on the microbial community. Sample sites were chosen to cover this high degree of variability in glacier type and microhabitats but then opportunistically based on weather, sea conditions, and land conditions.

Prior to fieldwork, the 10 glaciers identified for sampling were classified as either surge-or non-surge type glaciers using the Randolph Glacier Inventory (RGI) version 6.0 (RGI 6.0 Consortium, 2017), with the exception of Penckbreen, which has recently been observed to have surged and therefore falls within the surging category (Sund et al., 2014). These surge classifications provided a starting point for determining how “dynamic” the glacial system is and the potential conditions the microbial communities may be exposed to beneath the ice. Twenty-six sediment samples were collected from surge-type glaciers Scottbreen, Bakaninbreen, Paulabreen, Skobreen, and Penckbreen, and non-surge type glaciers Renardbreen, Antoniabreen, Charlesbreen, Bullbreen, and Protektorbreen (Figure 1, Table 1).

Intra-glacier variability and the different microhabitats of the glacial system was then targeted by sampling different landform types that can be broadly categorized as being of subglacial, supraglacial or a mixed origin (full descriptions of sample sites and types can be found in Text S1, Figures S1–S6 in Supporting Information S1). Subglacial originating sediments were sampled from (a) flutes and emerging streamlined landforms at the ice margin ice/bed interface, (b) meltwater channels and conduits through access points at the ice margin, and (c) the subglacial zone where access was possible at the ice front due to detachment of ice from the bed. Supraglacial originating sediments were sampled from the ice surface where the glacier front was accessible. Supraglacial samples from Renardbreen are believed to have been of subglacial origin and deposited as cones after being squeezed through a crevasse, whereas the supraglacial sample from Bullbreen and Protektorbreen originate from a debris stripes. Finally, mixed origin sediments were sampled from (a) crevasse squeeze ridges (CSRs) and (b) ice thrusts which have had a subglacial origin and still exist between the ice but with unknown levels of exposure to aerobiological input.

3. Materials and Methods

3.1. Sample Collection

Sediments were collected wearing sterile nitrile gloves and using Fischerbrand 20 ml sterile syringe tubes. The plunger was removed from the syringe and the syringe tube was pushed into the saturated sediments which were then stored in Whirlpak bags. The upper 2–5 cm sediment was removed using single-use Sterileware sampling spatulas. Repeat samples were taken in triplicate from each sampling location within the glacier catchment, taking

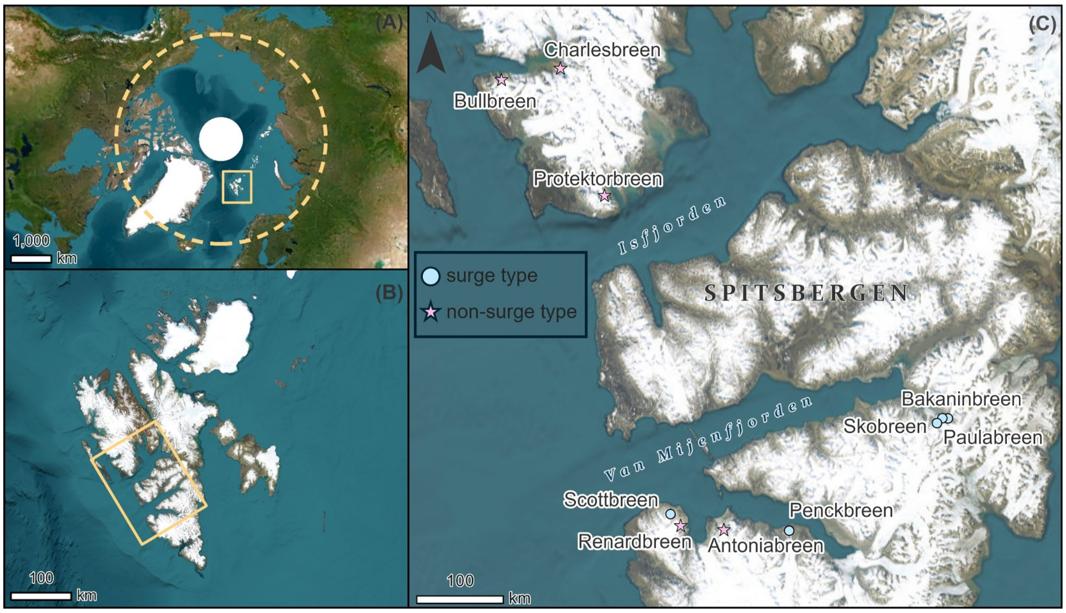

Figure 1. (a) Location of Svalbard in the Arctic Circle (yellow dashed line). (b) Sampling area location on Spitsbergen in the context of the Svalbard archipelago. (c) Location of the glaciers chosen for sediment sampling in southwest and northwest Spitsbergen spanning four fjords; Van Mienfjorden, Van Kuelenfjorden, St. Jonsfjorden and Isfjorden. Google Earth imagery from Dec 2020 Made with Natural Earth. Free vector and raster map data @ naturalearthdata.com. ESRI basemap imagery.

care to obtain samples from distinct sediment types totalling 78 samples from 26 site locations. Sediment samples were collected from as close to the ice-bed interface as possible. Sediments were frozen at −20°C before being transported back to the UK and frozen within 24 hr for long term storage.

3.2. Sequencing and Microbial Community Analysis

To determine the community composition and assign taxonomy to the microorganisms found in the sample, all samples were sequenced on an Illumina MiSeq. First, the total genomic DNA was extracted from 0.25 g of sediment from all 26 samples using a Qiagen PowerSoil Pro kit following the manufacturer's instructions. Extraction kit controls were also used for quality assurance. The concentration of DNA was measured using Qubit hsDNA kit (Invitrogen), which showed all samples had low extracted DNA concentrations below the detectable limit (≤ 0.5 ng ml^{-1}), apart from sample Renardbreen S3 (0.6 ng ml^{-1}).

Bacterial profiling of the variable region 4 (V4) of the 16S rRNA gene was carried out by NU-OMICS in singleton (Northumbria University). Bacterial community sequencing was carried out as described by the Earth Microbiome Project Protocol (Caporaso et al., 2012) with modified primers (Apprill et al., 2015; Parada et al., 2016). PCR was carried out using 1x KAPA Hotstart Robust MasterMix, 0.2 μM each primer and 1 μl of template DNA in a total volume of 25 μl under the following conditions; 94°C 3 min, 35 cycles 94°C 45 s, 50°C 60 s, 72°C 90 s with a final extension 72°C 10 min. One positive and one negative control sample were included in each 96 well plate and carried through to sequencing. PCR products were normalized using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen) as described in the manufacturer instructions and pooled per 96 well plate. Each pool was cleaned using 1:1 Ampure XP:Pooled amplicon, quantified using fragment size determined by BioAnalyzer (Agilent Technologies) and concentration by Qubit™ 1X dsDNA High Sensitivity assay kit (Invitrogen). Pools were combined in equimolar amounts to create a single 4 nM library then denatured using 0.2N NaOH for 5 min and diluted to a final concentration of 4.5 pM, supplemented with 20% PhiX and loaded onto a MiSeq V2 500 cycle cartridge.

Quality filtering and trimming, clustering, removal of chimeras, and analysis of the construction of the amplicon sequence variation (ASV) table were performed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline in RStudio (Callahan et al., 2016). Forward reads were trimmed to 200-bp and reverse reads to 100-bp

Table 1
Summary of the Sampling Sites and Sample Types Taken and the MiSeq Amplicon Reads per Sample

Surge category	Glacier	Sample	Sample type	Reads per sample	Included (I) or excluded (E)
Surge-type	Scottbreen	S1	Subglacial	3,349	I
		S2	Flute	257	E
		S3	Subglacial	2,966	I
	Bakaninbreen	S1	CSR	5,099	I
		S2	MWC	222	E
	Paulabreen	S1	Thrust	332	E
		S2	Subglacial	7,375	I
		S3	Thrust	2,453	I
		S4	Subglacial	3	E
	Skobreen	S1	Thrust	4,118	I
		S2	Thrust	7,366	I
	Penckbreen	S1	CSR	2,164	I
		S2	CSR	9,851	I
Non-surge type	Antoniabreen	S1	MWC	3,862	I
		S2	MWC	31,596	I
	Bullbreen	S1	Flute	3,338	I
		S2	Flute	6,308	I
		S3	Surface	1,292	I
	Charlesbreen	S1	Subglacial	4,023	I
		S2	MWC	22,196	I
	Protektorbreen	S1	Surface	14,177	I
		S2	MWC	3,035	I
	Renardbreen	S1	MWC	2,540	I
		S2	Surface sediment	1,189	I
		S3	Surface sediment	232,167	E
		S4	Subglacial	3,442	I

Note. CSR—crevasse squeeze ridge, MWC—meltwater channel. Following rarefaction curve analysis, the samples with anomalously high (>100,000) or anomalously low (<1,000) reads were excluded from parts of the analysis (see Section 3.2). All other samples were checked for representative levels of sequencing by the rarefaction curves (Figure S7 in Supporting Information S1).

based on an analysis of the quality scores. The number of reads per sample varied by orders of magnitude after denoising and chimera removal, with reads between 3 and 232,167 (Table S1 in Supporting Information S1).

Taxonomy was assigned using the SILVA v138 database (Quast et al., 2013; Yilmaz et al., 2014) and the naïve Bayesian classifier method (Wang et al., 2007). One thousand three hundred twenty-four ASVs were assigned across all samples. The Decontam package (Davis et al., 2017) was then used to remove those ASVs also found in the negative control samples, leaving 1,268 ASVs that were taken forward for analysis. Analysis of rarefaction curves (Figure S1 in Supporting Information S1) and reads per sample was used to identify anomalously high or low read samples or samples that did not achieve a suitable level of sequencing allowing for representative analysis of diversity indices (Table 1).

3.3. Total Biomass

Total biomass was estimated by counting the number of cells in a subset of sediment samples using epifluorescence microscopy and a 4′, 6-diamidino-2-phenylindole (DAPI) stain. Laboratory consumables were sterilized as per McCerery, Hall, et al. (2025) to remove potential contaminants and autofluorescing compounds. 1–2 g of sediment was saturated using 1 ml of Gibco pH 7.4 phosphate buffered saline (PBS) in a 1.5 ml Eppendorf tube. Four surging samples and four non-surging samples were selected for enumeration of cells with three subsamples taken from each sample to ensure replicability and representation of the total sample (see, Kirchman et al., 1982). Samples were vortexed for 2 min to separate cells from the sediment and into the solution. The samples were then centrifuged at 12,000 RPM for 1 min to separate the suspended sediment from the solution containing the cells. The supernatant, avoiding the sediment pellet, was pipetted onto a Whatman Cyclopore Track-Etched Membrane (0.2 μm pore size). Excess liquid was vacuumed through the filter paper using a gentle vacuum ($\leq$0.20 mbar pressure) before staining with 1 μL of DAPI (Thermo Fisher Scientific) stain (5 mg mL^{-1}) and incubated in the dark for 5 min. Samples were then washed twice with 0.5 ml of PBS and dried by vacuum before mounting on a microscope slide with 1 drop of immersion oil and a cover slip. Each sample was analysed at 20x magnification on an epifluorescence microscope (Leica DM5000B). Approximately 100 fields of view were counted to cover the full area and account for the low biomass of the samples.

Total biomass calculated in these samples was then compared with those published in the literature from polar environments using a strict search criteria. That criteria required studies to have used the same technique of epifluorescence microscopy and the same cell stain (DAPI) to be comparable. We focused on reports of glacial sediments and glacial ice, this included surface ice sediments but excluded cryoconite.

3.4. EPS Extractions and Quantification

As EPS is predominantly composed of polysaccharides and proteins, their concentrations provide an estimated proxy EPS concentration in the sediment samples as per Gerbersdorf et al. (2009), Basuvaraj et al. (2015), and Chen et al. (2017). A gentle heat extraction method was employed to separate EPS from the sediment particles. 10 g of sediment was extracted using 30 ml of preheated (70°C) 0.05% NaCl solution, then agitated on a Platform Rocker STR6 at 50 revolutions min^{-1}. The sediment was pelleted by centrifuging at 4,000 g for 15 min and the supernatant, loosely bound EPS (LB-EPS), was decanted into a clean universal tube ready for analysis. The remaining pellet composed of the tightly bound EPS (TB-EPS) was resuspended at room temperature in 0.05% NaCl solution and heated in a water bath at 60°C for 30 min. Samples were then centrifuged at 4,000 g for 15 min and the supernatant containing the TB-EPS was decanted into a clean universal tube ready for analysis.

The extracted polysaccharides and proteins were concentrated using a freeze-drier at −80°C for 48 hr and resuspended in 2 ml of PBS. A modified resorcinol-sulfuric acid method outlined in Monsigny et al. (1988) was used to quantify the polysaccharide content of LB- and TB-EPS extracts. 200 μl of sample, 200 μl of 6 mg ml^{-1} resorcinol (5-methylresorcinol anhydrous CAS: 504-15-4), and 1 ml of 99% sulfuric acid was added to a 1.5 ml Eppendorf tube and briefly vortexed. The tubes were then heated at 90°C for 30 min before being cooled to room temperature for a further 30 min. 1 ml of solution was added to a cuvette and analysed on a Colourwave CO7000 Medical Colorimeter at 490 nm wavelength. Samples were analysed in triplicate to obtain error values. The concentration of polysaccharides present in the sample was then determined using a glucose dilution series calibration curve. The composition of the polysaccharide portion of the EPS was confirmed using 2M tri-fluoroacetic acid acidification of a subset of samples and analysed using high-performance liquid chromatography (HPLC) Dionex ICS-5000^{+} DC (Thermo Fisher) attached to a Pulse Amerometric Detector (APD) using a Dionex CarboPacTM PA200 guard and analytical column. Standards of monosaccharides galactose, glucose, mannose, arabinose, xylose and rhamnose were used to identify the different compounds of the EPS.

To quantify the protein concentrations in the EPS extracts a Pierce™ BCA Protein Assay Kit was used. 100 μl of sample and 1.9 ml of working solution was added to a 2 ml Eppendorf tube and briefly vortexed. The tubes were then heated at 60°C for 30 min then cooled to room temperature for a further 30 min. 1 ml of solution was added to a cuvette and analysed on a 6850 UV/Vis spectrophotometer at 526 nm wavelength. Samples were analysed in triplicate to obtain error values. The concentration of proteins present in the sample was then determined using an albumin dilution series calibration curve.

3.5. Environmental Variables

The environmental variables, water content and total organic carbon (TOC) content were used to detect any potential links between the environmental conditions of the sediment with the community composition and/or the production of EPS'. For both water content and TOC, approximately 7–10 g of sediment added to a ceramic crucible and placed in an oven at 105°C for 24 hr. The water content was then determined using the following equation,

$$\%\text{ Water Content} = 100 \times \frac{\text{sample }(g) - \text{LOI}_{105}\ (g)}{\text{sample }(g)}$$

Where "sample" is the wet weight before alteration and LOI_{105} is the sample weight after oven drying for 24 hr at 105°C. For TOC, the crucibles were then placed back in the furnace at 550°C for a further 6 hr and allowed to cool down before reweighing. TOC was determined using the following equation,

$$\%\text{ TOC} = 100 \times \frac{\text{LOI}_{105}\ (g) - \text{LOI}_{550}}{\text{LOI}_{105}}$$

Where LOI_{550} is the sample weight after oven drying for 6 hr at 550°C.

3.6. Statistical Analyses

Statistical analyses were applied to the microbiological data set in the R environment using phyloseq and vegan packages. Alpha diversity indices were calculated using the richness function (Shannon and Simpson indices). Statistical differences in alpha diversity indices between surge-type categories and landform categories was determined using two sample *t*-tests and non-parametric Kruskall-Wallis tests performed using the core R package (R Core Team, 2022) and phyloseq (McMurdie & Holmes, 2013). Dissimilarity was determined using ordination, principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity matrix, with microViz (Barnett et al., 2021) and phyloseq R packages. Permutation tests to determine differences between microbial community structures were performed using Permutational Multivariate Analysis of Variance - PERMANOVA (adonis) using the vegan package (Dixon, 2003). Non-parametric Spearman's rank correlation was used to identify any statistically significant relationships between microbial community structures and EPS concentrations. Visualization was performed using the Microbiome (Lahti & Shetty, 2018), MicEco (Russell, 2021) and ggplot2 packages (Wickham, 2016).

4. Results

4.1. Microbial Biomass and Diversity

Biomass estimates using epifluorescence microscopy showed cells counts of 122 ± 42 cells g^{-1} of sediment (Figure 2). The highest number of cells was detected in two samples from Renardbreen, one from the ice-bed interface (S1) and the other from a cone of exposed subglacial sediment on the glacier surface (S3). The highest cell count in Renardbreen S3 was significantly higher than all other samples with 1.3×10^5 cells g^{-1} of sediment, whereas all other counts were in the range of 5.8×10^1–2.0×10^2 (Table S3 in Supporting Information S1).

The 16S rRNA gene amplicon sequencing produced a total of 1,324 ASVs assigned across the 26-sediment samples (1,268 after removal of those also found in negative controls) after pre-processing, totalling 33 phyla and 72 species. Analysis of the rarefaction curves and number of reads sequenced revealed outlier samples with anomalously high (<100,000 reads) and low (<1,000) reads in comparison to the tens of thousands in the majority of the samples (Figure S7 and Tables S1, S2 in Supporting Information S1). In order to perform true like for like comparisons, particularly when analysing the diversity indices, we excluded those samples from further analysis (Table 1). Of the 26 samples collected, 21 were analysed (with 299 ASVs across a library size of 1,199–37,854) for their microbial community composition and diversity indices.

The non-surging glaciers had a higher species richness with a total of 48 phyla and 37 species in comparison to the surging glaciers with 32 phyla and 30 species respectively. A Welch *t*-test was performed on the Shannon and Simpson Indices of all unique taxa between surging and non-surging categories and revealed no statistically significant differences between the Shannon Indices ($t = 1.13$, $df = 18.86$, $p = 0.27$) nor the Simpsons Indices ($t = 0.60$, $df = 14.15$, $p = 0.56$) (Figure 3a). A Kruskal-Wallis chi-squared test also showed no statistically significant differences between the Shannon and Simpson indices by landform category (*chi-squared* = 1.21, $df = 5$, $p = 0.94$ and *chi-squared* = 2.13, $df = 5$, $p = 0.83$). The highest variation in diversity indices is seen in the ice-surface sediment samples, whereas consistent values were calculated in the subglacial and thrust samples across different glaciers and surge categories (Figure 3b). Similarly, when grouping samples by their primary

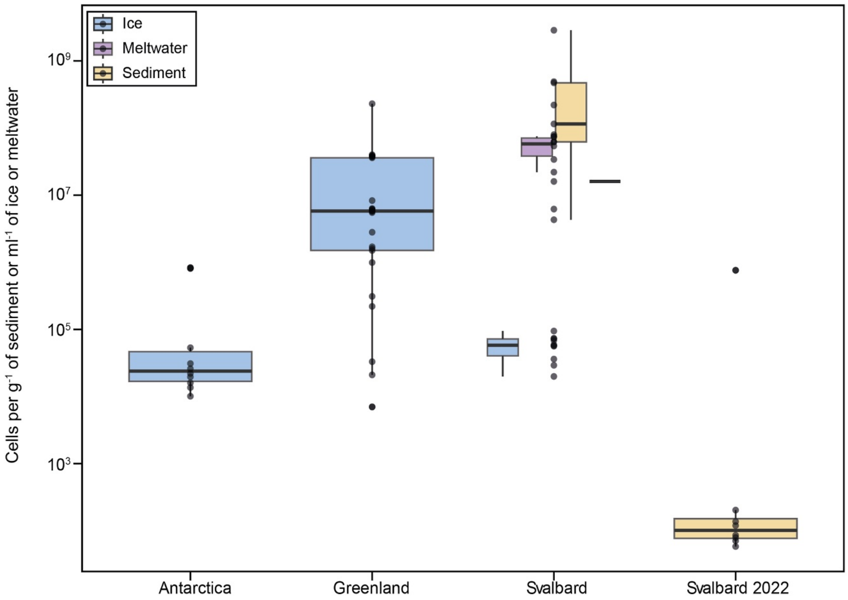

Figure 2. Cell counts per g^{-1} or ml^{-1} of glacial ice, meltwater or sediment from Antarctica, Greenland and Svalbard using epifluorescence microscopy and DAPI staining techniques. Svalbard 2022 represents the samples counted as part of this study. Cell counts were obtained from Kaštovská et al. (2007), Yung (2008), Yde et al. (2010), Grzesiak, Zdanowski, et al. (2015), Grzesiak, Górniak, et al. (2015), Musilova et al. (2015), Górniak et al. (2017), and Thomas et al. (2020, 2021).

origins that is, ice surface sediment, subglacial sediments or mixed, all sample mean values fall within similar ranges (Figure 3c).

4.2. Microbial Community Composition

Community composition was determined by assigning taxonomy to the 16S rRNA gene amplicon sequencing. Of 1,268 detected ASVs, 71 occurred across two or more samples and 11 ASVs across five or more samples. After removing outlier samples (Table 1) and assigning taxonomy, a single core phyla was identified across all samples; *Proteobacteria* accounting for an average of 55% of detected ASVs per sample. This was closely followed by *Bacillota* (25%), and at smaller quantities samples also contained *Bacteroidota*, *Cyanobacteria*, *Actinomycetia*, and *Acidobacteria* (Figure 4a).

Across all samples, regardless of surge category or landform, the genus *Pseudomonas* was almost ubiquitous (Figure 4a). At landform level, community composition showed flutes were particularly low in diversity and were dominated almost exclusively by *Proteobacteria* and *Bacillota* (Figures 3b and 4a). Whereas more richness could be seen in the CSR samples, which contained more *Acidobacteria* (16%), subglacial and meltwater channels with more *Bacteroidota* (14% and 18% respectively) and ice surface sediments with more *Actinomycetia* (6%) (Figure 4a).

At the class level, most samples are dominated by *Gammaproteobacteria* (37%) followed by *Alphaproteobacteria* (17%), *Bacilli* (16%), and *Bacteroidia* (9%). This trend continues when breaking down samples into surge versus non-surge categories however, again, more distinction is seen when investigating samples at the landform and microhabitat level. CSRs were more typically dominated by *Alphaproteobacteria* (37%) and meltwater channels by *Gammaproteobacteria* (56%), whereas ice surface sediment samples are dominated by either *Bacilli* or *Gammaproteobacteria* (Figure 4b). One of the three flute samples collected here was excluded from analysis due to low read counts, with the remaining two samples also having the lowest diversity of taxa amongst all landform type, composed primarily of either *Gammaproteobacteria* (77% in one sample) or *Clostridia* (64% in the other sample).

A PCA was then used to determine any community grouping based on the landform types and surge categorization (Figure 5). The PCA showed that the supraglacial samples cover the lower two quadrants whereas all other landform types cover three or four of the quadrants. The surge and non-surge type categories also cover all four

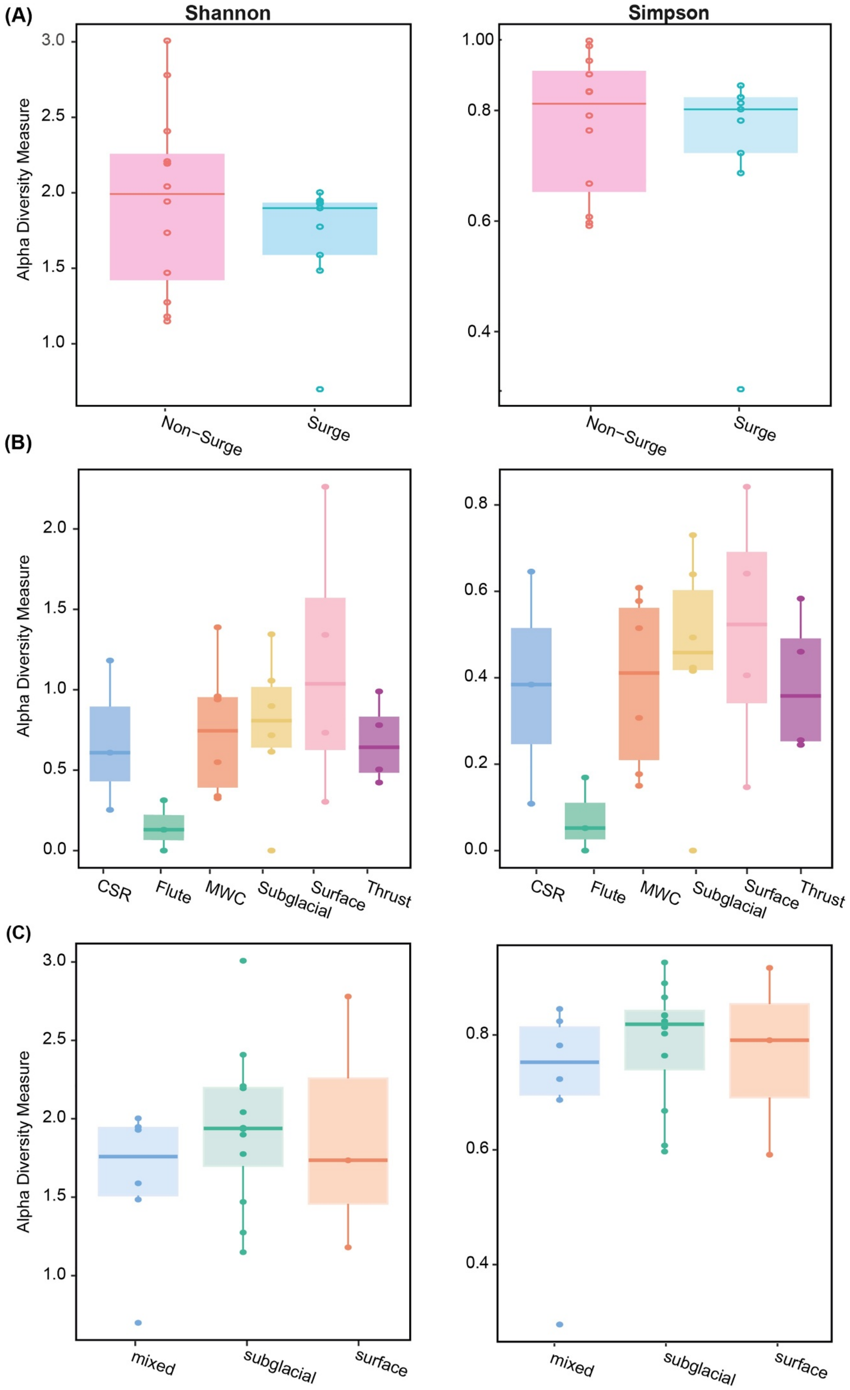

Figure 3. (a) Shannon (left column) and Simpson (right column) alpha diversity indices between (a) surging and non-surging glaciers using unique taxa, (b) different landforms using unique taxa and the (c) broad landform origins that is, mixed origin (CSR and thrust) subglacial (subglacial, flute, MWC) and surface (supraglacial and ice surface sediment) using unique taxa. CSR—crevasse squeeze ridge, MWC—meltwater channel.

quadrants of the PCA plot. A PERMANOVA was performed using the Bray-Curtis method to determine dissimilarities between landform types and community compositions, this also showed no statistically significant effect ($R^2 = 0.25$, $p = 1$) suggesting all samples were similarly different.

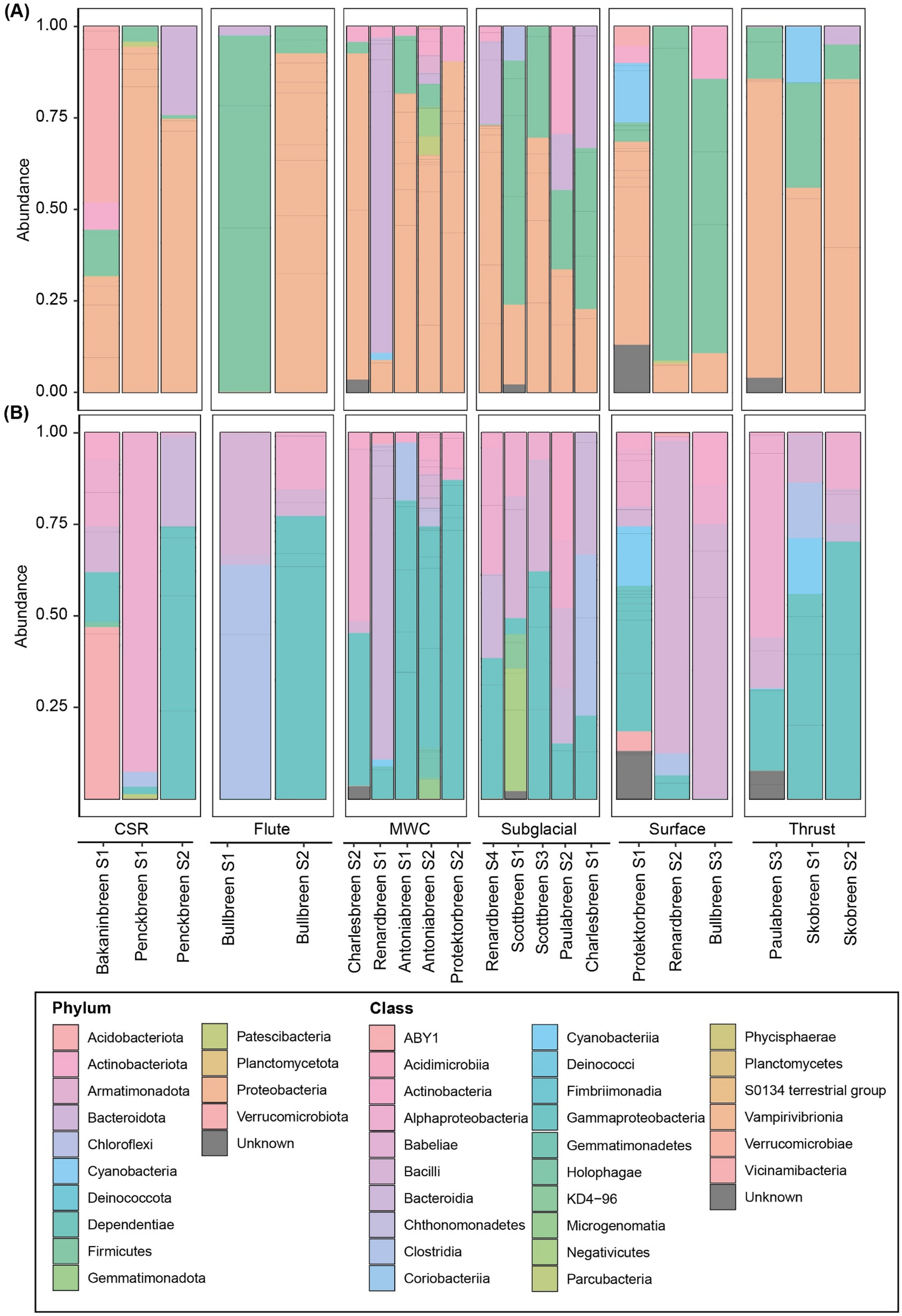

Figure 4. Composition of the microbial community at each sampling location and glacier grouped by landform type. (a) Community composition at the phylum level. (b) Community composition at the class level. CSR—crevasse squeeze ridge, MWC—meltwater channel.

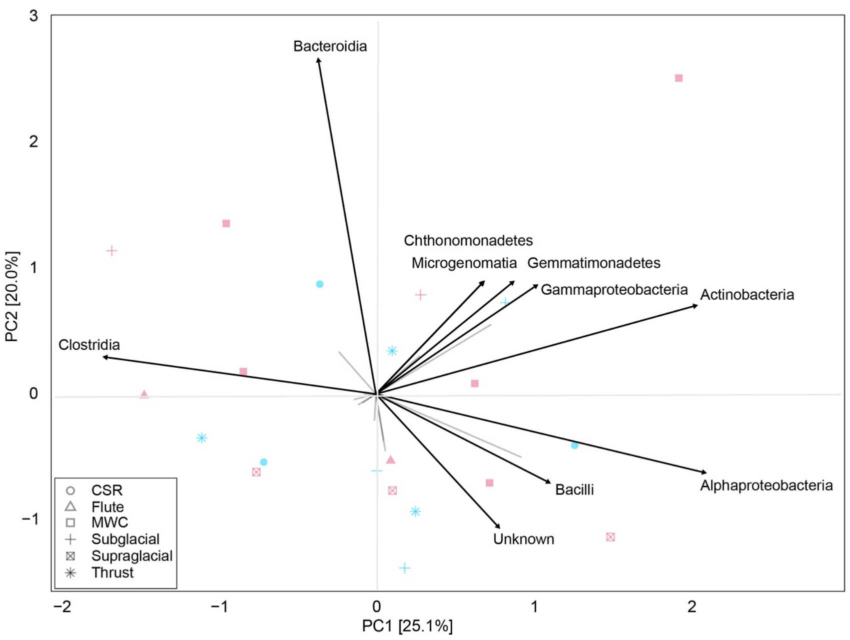

Figure 5. Principal component analysis (PCA) of 21 samples, excluding outlier samples described in Table 1, grouped by landform and surge category using Bray-Curtis dissimilarity with a $\log^{10}$ transformation. CSR—crevasse squeeze ridge, MWC—meltwater channel. Shapes denote the landform type and color the surge classification, pink—non-surge and blue-surge type. Arrow annotations denote the microbial variables influence on sample differences.

4.3. EPS Production and Environmental Variables

Polysaccharide and protein concentrations were calculated using colorimetry and combined to get an estimated proxy EPS concentration per sample. The components of the polysaccharides were also assessed using a subset of samples analysed on a HPLC (Figure S8 in Supporting Information S1). This showed a similar polysaccharide composition across the subset of samples with a dominance of glucose and at lesser concentrations of galactose, mannose and rhamnose.

The mean calculated concentration of EPS in the surging glacier samples was 0.20 mg g^{-1} compared to non-surging glaciers with a slightly higher concentration of 0.49 mg g^{-1} (Table 2). The surging glacier EPS concentrations were generally more stable between sites with a low standard deviation (±0.09) whereas there was much higher variation between the non-surging glacier sites (±0.98), driven primarily by the anomalously high

Table 2
Summary of the Environmental Variables Measured in the Sediment Samples Grouped by Surge Category and Landform Types

		Water content (%)		Total organic carbon (%)		Protein (mg g^{-1})		Polysaccharide (mg g^{-1})		Total EPS (mg $g^{-1)}$	
Category		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Surge-type	Surge	15.69	5.68	2.60	1.07	0.08	0.062	0.12	0.044	0.20	0.0087
	Non-surge	17.31	9.78	1.18	0.71	0.12	0.17	0.36	0.83	0.49	0.98
Landform	CSR	12.26	1.95	2.94	0.61	0.06	0.010	0.10	0.012	0.17	0.010
	Flute	9.37	3.36	0.98	0.37	0.07	0.056	0.11	0.011	0.18	0.051
	MWC	18.23	8.06	1.33	1.05	0.04	0.036	0.06	0.027	0.12	0.042
	SubG	17	10.44	1.53	1.15	0.09	0.10	0.12	0.026	0.21	0.12
	SupraG	16.05	6.02	1.88	0.66	0.23	0.25	0.97	1.31	1.20	1.53
	Thrust	22.10	4.79	3.20	0.59	0.12	0.08	0.15	0.06	0.28	0.10

Note. CSR—crevasse squeeze ridge, MWC—meltwater channel, SubG—subglacial, SupraG—supraglacial.

Renardbreen S3 value of 3.85 mg g^{-1}. All other EPS concentrations were consistently below 1.0 mg g^{-1}. Meltwater channels had the lowest EPS concentrations of all samples (0.12 mg g^{-1}). Discounting all outlier samples, Spearman and Pearson correlation tests were performed to test for any potential relationship between EPS concentrations, community composition and alpha diversity indices. This revealed no correlation and a weak non-significant relationship between EPS concentration and community composition (Spearman—$R_s = -0.05$, $p = 0.83$ and Pearson—$R_s = 0.06$, $p = 0.79$), and between EPS concentration and Shannon diversity indices (Spearman—$R_s = -0.09$, $p = 0.70$).

Environmental variables such as water content and TOC were also measured to determine potential linkages between environmental conditions and EPS'. The highest TOC was measured in thrust samples (3.2%) and the lowest in flute samples (0.98%). A Pearson's Correlation Coefficient analysis was also performed on the environmental variables to determine if there was any relationship between TOC or water content and the concentration of EPS' which demonstrated a very weak or weak correlation between all the variables.

5. Discussion

5.1. Impact of Glacier Behaviour and Landform Types on Microbial Community Structure

All of the landforms and glacier types here can fall within the categorization of "environmentally harsh." In Wilhelm et al. (2013) and Jacobsen and Dangles (2011), environmental harshness is defined as an environment that experiences any number of combinations of; mechanical disturbance, extreme cold, extreme darkness and low nutrients. Here, different samples will have experienced different degrees and types of harshness in terms of water washing and the mechanical disturbances which may be driving some of the drastically different community compositions in these samples.

At all sample sites, a number of core phyla were identified comprising *Proteobacteria*, *Bacillota*, *Cyanobacteria*, *Actinomycetia*, and *Acidobacteria*, all of which have been detected previously in polythermal glaciers in Svalbard (e.g., Perini et al., 2019), John Evans Glacier, Ellesmere Island (e.g., Skidmore et al., 2005), a subglacial aquifer in Taylor Glacier, Antarctica (e.g., Campen et al., 2019), ice and subglacial meltwater from Pico Bolìver, Venezuelan Andean Mountains (e.g., Rondón et al., 2016), and Subglacial Lake Hodgson, Antarctica (e.g., Pearce et al., 2013). *Proteobacteria* in particular dominates subglacial environments globally (Shu & Huang, 2022). At this level it was expected to see some similarities in the community composition between glacier types and landforms.

At the more detailed sequence level however there is broadly different microbial communities both inter- and intra-glacially. At each glacier, a number of different landforms and microhabitats were sampled, all with drastically different hydrodynamics, which could play an important role in determining the overall community composition in the glacial system as proposed in Tranter et al. (2005). For example, where there is fast running and potentially well oxygenated water through channelized systems, there is an oxic environment with efficient gas exchange and a supply of nutrients (Tranter et al., 2005). However, due to the higher water velocities in these systems, it is more difficult for communities to establish and not be washed out to a lower energy part of the system (Tranter et al., 2005). This is likely the dominant environment for the highly dynamic meltwater channel samples here and may be contributing to the particularly low EPS concentrations in these samples. Being close to meltwater channels also has the added benefit of a replenished nutrient supply and oxygenation of the environment which may be influencing the microbial communities establishing in these environments (e.g., Kohler et al., 2020; Varliero et al., 2023). In contrast, low velocity and potentially anoxic environments such as the subglacial zone and ice thrusts may have more stable microbial communities due to their lower erosive power, though in some respects they may have harsher environmental conditions that is, lower nutrient supply and oxygenation. These communities however are less exposed to mechanical disturbance from water washing which is key for the EPS to remain in situ (Miller & Lane, 2019; Roncoroni et al., 2019). The high biomass, diversity and EPS concentrations in the ice surface sediments may be due to the deposition of airborne bacteria (e.g., Hervas & Casamayor, 2009), windblown dust (e.g., Irvine-Fynn et al., 2012; Rassner et al., 2024), and/or biomass rich sources of microbiota such as the algal patches or cryoconite (e.g., Irvine-Fynn et al., 2021; Marsh et al., 2024). This could be the primary driver of the anomalously high biomass, diversity and EPS concentrations in the Renardbreen S3 sample. Despite all these differences though, the diversity, production and potential stability of EPS appears to be mostly stable across all samples.

5.2. Total Microbial Biomass and EPS in Different Glacier Microhabitats

Fluorescence microscopy showed similar cell counts between all samples regardless of landform type or surge category, falling within the lower range of cell counts previously reported in the subglacial microbiology literature of 10^1–10^9 (e.g., Boetius et al., 2015; Davis et al., 2023; Doyle & Christner, 2022; Foght et al., 2004; Gaidos et al., 2004; Kaštovská et al., 2007; Lanoil et al., 2009; Montross et al., 2014; Pearce et al., 2013; Sharp et al., 1999; Skidmore et al., 2005; Sonjak et al., 2006; Stibal et al., 2012). This could be because of water washing and cell dilution, as sampling locations were often close to meltwater channels, or it could be a factor of the cell extraction techniques used to separate cells from the sediment particles. The similarity in biomass between samples, excluding the single outlier sample, suggests that despite the hydrodynamic differences occurring between these glacier and landform types, a stable low biomass community size is still established. Importantly, the higher biomass sample Renardbreen S3 still falls within the normal reported range of cells from these environments and thus does not represent a significantly high biomass sample in the context of other works. The 4–5 orders of magnitude higher biomass in Renardbreen S3 may be a factor of the supraglacial environment and colonization from air and dust however, this is not seen in the other supraglacial samples analysed here. This raises an important question as to how many samples are required to obtain a representative overview of a glacial system and to potentially capture these outliers.

In all samples, low TOC was ubiquitous, which is typical for subglacial sediments (<3%) and is likely a limiting factor for microbial community establishment and productivity (e.g., Foght et al., 2004, Kaštovská et al., 2007). Although significant differences were detected between surge type and non-surge type detected TOC as well as between landforms, this did not appear to correlate with any differences in EPS.

The EPS produced by these communities are heteropolysaccharides with multiple sugar monomer units. The calculated concentrations and compositions were also low; however, the community composition of these samples indicates a potential for EPS production, with microbial mats in Antarctic and Arctic water bodies being primarily composed of *Cyanobacteria*, *Proteobacteria*, and *Bacteriodota* (e.g., Doytchinov & Dimov, 2022; Valdespino-Castillo et al., 2018; Varin et al., 2012), all of which were detected here. Despite works on EPS production in extreme environments such as deep sea hydrothermal vents (Arena et al., 2009; Panosyan et al., 2018) and polar marine environments (Aslam et al., 2012; Casillo et al., 2017; Liu et al., 2013; Mancuso Nichols et al., 2004), there has been little work exploring the concentrations and composition of EPS' in the glacial system (Ali et al., 2020). Similar environments such as biocrusts from Arctic soils have EPS concentrations on average of 1.5–3.5 mg g^{-1} (Mugnai et al., 2020), the samples collected here fall an order of magnitude lower. Although Renardbreen S3 falls within the same order of magnitude as those detected in the developed biocrusts. This is likely an effect of the slightly higher biomass and diversity observed in supraglacial environments.

Although the EPS concentration and composition was not significantly different between the different glacier or landform types, it is likely the case that the microbes are productive and producing EPS' in the subglacial zone. The similarity in biomass, diversity and EPS concentration between samples within glaciers and between glaciers, despite the different community structures indicates that the specific taxonomy is not necessarily the driving factor in some of the biological processes occurring in the glacial environment. This suggests there is a potential critical level of diversity and EPS production that can be reached in communities of different compositions.

5.3. Implications for a Changing Glacial Landscape

Svalbard is particularly sensitive to the effects of climate change with most glaciers on the archipelago rapidly thinning (Morris et al., 2020; Nuth et al., 2013). Previous works have focused on how the wider ecological zones will be impacted by glacier retreat (e.g., Bourquin et al., 2025; Hotaling et al., 2017; Jia et al., 2024). Here we see that the microhabitats of the glacier environment may reveal potential changes and pathways for microbial community shifts which may not affect overall diversity and production of compounds such as EPS'. We show also that under current conditions, the microbial community, in terms of biomass and EPS, is relatively stable despite the different types of glacial microhabitat. This stability may be limited by the degree of environment harshness. Thus, there is potential for these two indicators to drastically change as the environment becomes less harsh and colonization of more productive species may begin to dominate. Such changes will have important implications for wider ecosystem functioning as predominant biogeochemical processes change in a deglaciating landscape.

6. Summary

Overall, in common with other studies, all glaciers sampled here had similar phylum compositions, but broadly different and unique class communities and sequences. The differences in community detected at these levels does not appear to be linked to the type of glacier or the landform or microhabitat from which the sediment was sampled. There also appeared to be some distinction in community composition at the class level potentially driven by environmental differences in glacial landforms and microhabitats. In more dilute and erosive microhabitats such as meltwater channels microbial communities were dominated by *Gammaproteobacteria*, whereas flute sediments emerging from the subglacial zone had a much lower diversity of taxa at all levels. Interestingly, despite all of these differences in landform environment and community composition the overall diversity, total biomass (122 ± 42 cells g^{-1}), EPS concentrations (0.34 ± 0.71 mg g^{-1}) and EPS compositions were consistent across all samples. An outlier sample with orders of magnitude higher biomass and EPS concentrations (8.1×10^5 cells g^{-1} and 3.85 mg g^{-1} respectively) was also detected and raises important questions around how many samples are required to obtain representative overviews of whole glacial systems. Overall, this demonstrates a potential critical biomass and EPS concentration required to stabilize microbial communities in glacial environments.

Inclusion in Global Research Statement

All permits for this research were granted by The Governor of Svalbard via the Research in Svalbard (RiS) portal.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The amplified sequence data, cell counts, protein and polysaccharide concentrations data and supplementary information used for microbial community composition analysis and EPS analysis in this study are attached as supplementary files. The raw DNA sequences from this project are deposited on NCBI's SRA under the BioProject PRJNA1298205, accession numbers SAMN50260318 to SAMN50260337.

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