Snow microorganisms colonise Arctic soils following snow melt

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Snow microorganisms colonise Arctic soils following snow melt

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Abstract

Arctic soils are subjected to microbial invasion from either airborne, marine or animal sources. However, in winter, Arctic soils are isolated from outside sources other than snow, which is the sole source of microorganisms. Successful colonisation of soil by snow microorganisms depends on the ability to survive and compete of both, the invading and resident community. Our study monitored snow and soil microbial communities throughout snow melt to investigate the colonisation process of Arctic soils. Microbial colonisation appears to have occurred as all the characteristics of successful colonisation were observed. The colonising microorganisms originating from the snow were already adapted to the local environmental conditions and were subsequently subjected to many similar conditions in the Arctic soil. Furthermore, competition-related genes (e.g., motility, chemotaxis, and virulence) increased in snow samples as the snow melted. Overall, one hundred potentially successful colonisers were identified in the soil and, thus, demonstrated the deposition and growth of snow microorganisms in soils during melt.

Introduction

Microbial colonisation consists of four sequential processes: the introduction of the invader, establishment, growth and spread, and the impact of the coloniser on the resident microbial community [1, 2]. A successful colonisation depends on the adaptability of the incoming taxon to the new habitat and the resistance to invasion from the colonised habitat [1-3]. Successful colonisers first have to adapt and survive local environmental conditions, which may not be favourable compared to their ecosystem of origin; then, they have to compete with the resident community for resources in the new ecosystem [2, 4]. Therefore, their invasive potential depends on effective adaptations increasing the likelihood of survival and competitive advantage. Generally, high growth rates, dispersal ability, phenotypic plasticity and genetic diversity increase the chance of success of the invader [5]. The ability to exploit empty niches would also increases the chance of perennial colonisation. Key genes involved in the adaptation to local environmental conditions include genes associated with the stress response such as oxidative stress or heat shock, but also the cell wall structure or capsule synthesis [6, 7]. Genes increasing competition potential include antibiotic production or resistance, increased motility or better resource utilisation [7, 8]. Being an r-strategist with high growth rates and short generation time is also hypothesised to increase the chances of successful colonisation [5, 9-11] although, in extreme environments such as cold or nutrient limited systems, K-strategists may be better suited to tolerate the biotic/abiotic stressors and thus, more likely to successfully colonise. The chances of successful colonisation also depend on the resident microbial community and resource availability. For instance, high richness, diversity and evenness decrease the chances of colonisation in temperate soils [3, 12-16]. On the other hand, resource pulses, where a

peak in nutrient availability occurs over a short period of time, increases the chances of successful colonisation by increasing resource availability and decreasing competition [4, 14].

Natural communities are constantly subjected to microbial invasions from a wide variety of sources. In the Arctic, these are generally limited to either airborne, marine or animal sources. Aeolian transport of microorganisms, where organisms are aerosolised, transported in the air and deposited in new environments is the most universal dispersal pathway [17]. By characterising and comparing microbial communities living in the air and in the ecosystem of interest, the potential for successful dispersal and colonisation can be inferred by assessing similarities in diversity [18-21]. However, in the case where similarities are identified, whether the airborne microorganisms common to the ecosystem of interest might have travelled from distant habitats or have been aerosolised from the ecosystem of interest in the first place is difficult to determine.

Snow melt is a model that can be used to understand microbial colonisation in general and in the context of airborne dispersal [22]. Snow covers a minimum of 2×10^6 km^2 and a maximum of 45×10^6 km^2 of the Northern Hemisphere every year [23]. Snow influences the global climate through the albedo [24], but also locally through the insulation of soils to ambient environmental and climatic conditions [25]. In addition, the snow cover isolates the underlying soil from contact with microbes from outside sources for up to 10 months a year, as is the case in Svalbard [26]. Pristine snow is primarily seeded from the atmosphere and unique microbial communities develop within the snow over time [27-29] and actively participate in nutrient cycling [30]. Once the snow starts melting, the run-off travels vertically on flat terrain to reach the frozen soil layer and infiltrate the soil when no ice layer exists [28, 31, 32]. Although the percolation rate will vary depending on local conditions, snow microorganisms inevitably encounter soil microbial communities at the end of the snow melt. The snowpack is a source of potential colonisers as it contains between 10^2 and 10^4 microbial cells per mL of melted snow [33-35]. Snow melt also creates a peak in nutrient and solute availability in soils [28, 36-38]. This resource pulse may facilitate the colonisation of soils by snow microorganisms. In the case of soils where the community is rich and diverse and colonisation may generally be difficult [3], the snow melt may be an opportunity for snow microorganisms to establish in a new habitat.

Using snow melt as a model for dispersal and deposition of microorganisms in soil, the aim of this study was to determine the colonisation potential of snow microorganisms by monitoring microbial communities of the melting snow and underlying soil during melt. We used 16S rRNA gene amplicon sequencing to detect potential colonisers and observe shifts in community structure, and shotgun

metagenomics to assess functional potential and identify genes that might favour colonization.

Material and Methods

Sample collection and processing

Samples were collected during the snow melt in spring 2018 in Ny-Ålesund (78°56'N, 11°52'E), Svalbard. The field site consisted of a 100 m^2 perimeter, close to the laboratory of the AWIPEV research station, situated on the far south-end of the Ny-Ålesund village and bordered by Kongsfjorden, a 26-km-long and 3 to 8 km wide fjord on the westcoast of Spitsbergen. Twice a week during the melt season, between May 1st and May 19th, snow pits were dug using a sterilised Teflon shovel [Fig. 1]. To avoid snow contamination, Tyvek full body suits and gloves were worn during sample collection and snow was collected in 3 L sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA). Snow layers were identified and differentiated using standard procedures which included measurement of the snow density, hardness, temperature, liquid water content and grain size and form [39]. Although the snowpack consisted of many snow layers, it was divided in two layers for the purpose of this study. The surface layer, with a depth from 20 cm to 3 cm depending on the sampling date was generally composed of loosely packed snow and was exposed to the constant deposition of airborne microorganisms. All the layers below the surface layer were considered as one layer and characterised as bulk snow. Once at the bottom of the snow pit, 50 g of the underlying soil was collected within the first 15 cm using a sterilised trowel and 750 mL Whirl-Pak bags (Nasco) [Fig. 1, Table 1]. Soil samples were stored at -20°C after collection. Snow samples were processed immediately after collection. Samples were left to melt at room temperature and filtered onto nitrocellulose 0.22 µm filters (Merck Millipore, Germany) using a sterile filtration unit (Nalge Nunc International Corporation). The resulting filters were placed in sterile falcon tubes and stored at -20°C.

DNA extraction, Shotgun metagenomic sequencing, 16S rRNA amplicon sequencing and bioinformatic processing

Soil DNA was extracted using 0.25 g of each sample using the PowerSoil kit (Qiagen, Carlsbad, CA, USA) while snow DNA was extracted from the filters using the PowerWater kit (Qiagen) and following the manufacturers' protocol. The metagenomic library was prepared using the Nextera XT DNA library preparation kit (Illumina, CA, US) and following the manufacturers' protocol. Libraries were cleaned using the AMPure XP beads (NEB), were pooled and loaded on the MiSeq for sequencing. The adaptors were removed from the fastq files using cutadapt [40] and merged in QIIME [41] using FLASH [42]. Merged fastq files were uploaded on MG-RAST [43] and subjected to quality control, which included dereplication, removal of human related sequences, removal

of ambiguous bases (>5) and a quality phred score >15. Once all metagenomes were processed on the MG-RAST server, reads were taxonomically annotated based on the NCBI RefSeq database (Pruitt et al., 2006) with a maximum e-value of 10^{-5}, 80 % identity, 30 bp overlapping length and a minimum abundance of 5 reads. Reads were also functionally annotated by similarity searching against the Rapid Annotation using Subsystem Technology (RAST) database [44], with a maximum e-value of 10^{-5}, 60 % identity, 15 bp overlapping length and a minimum abundance of 5 reads. The RAST subsystem classification assigns genes to functional roles, themselves grouped into subsystems. The taxonomic and gene abundance tables were analysed in R.

16S rRNA gene libraries were constructed using the universal primers 515F and 806R [45] to amplify the V4 region. Amplicons were generated using a high-fidelity Accuprime DNA polymerase (Invitrogen, Carlsbad, CA, USA), purified using AMPure magnetic bead capture kit (Agencourt, Beckman Coulter, MA, USA), and quantified using a QuantIT PicoGreen fluorometric kit (Invitrogen). The purified amplicons were then pooled in equimolar concentrations using a SequalPrep plate normalization kit (Invitrogen), and the final concentration of the library was determined using a SYBR green quantitative PCR (qPCR) assay. Libraries were mixed with Illumina-generated PhiX control libraries and our own genomic libraries and denatured using fresh NaOH and sequenced on the Illumina MiSeq V2 (500 cycles). The resulting amplicons were processed using the DADA2 pipeline [46]. Forward and reverse read pairs were trimmed and filtered, with forward reads truncated at 230 base pairs (bp) and reverse reads at 200 bp, no ambiguous bases allowed, and each read required to have <2 expected errors based on their quality scores. Amplicon sequence variants (ASVs) were independently inferred from the forward and reverse reads of each sample using the run-specific error rates. Reads were dereplicated, pairs were merged, and chimeras were removed. Taxonomic assignment was performed against the SILVA v128 database [47, 48] using the implementation of the RDP (ribosomal database project) naive Bayesian classifier [49]. It resulted in a total of 507,446 reads (18,123 reads/sample on average), assigned against 8,265 ASVs.

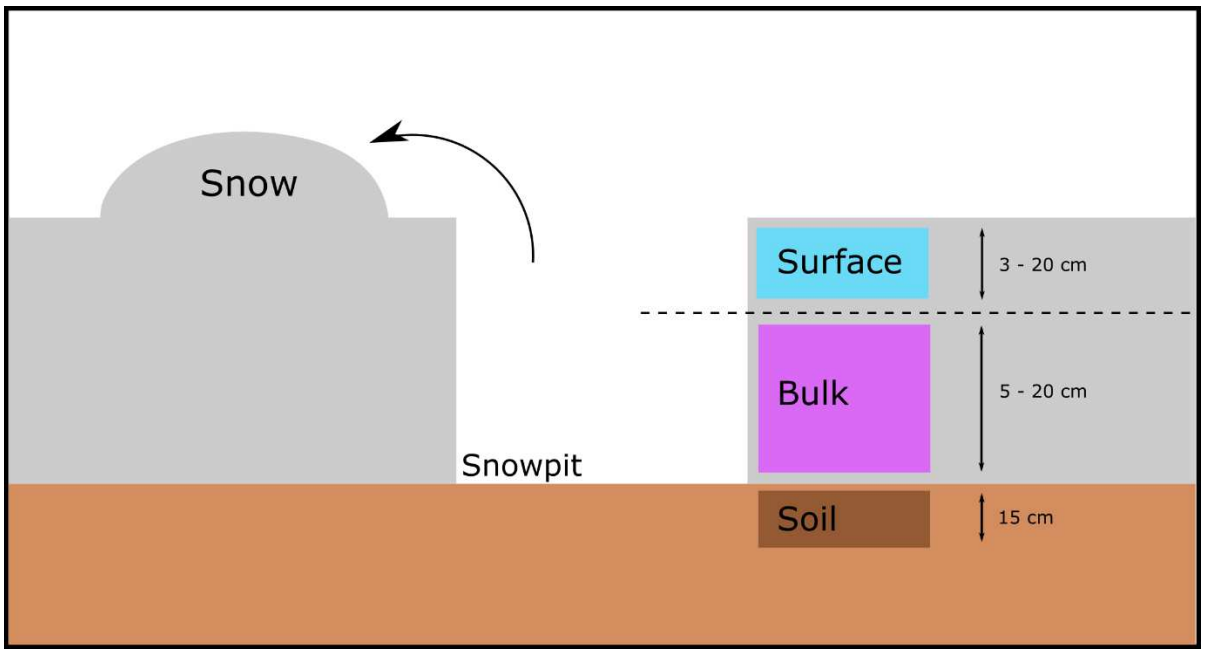


Figure 1: Diagram of the sampling design. A snowpit was dug and surface, bulk and soil samples were collected. At each sampling event, a new pit was dug in a previously undisturbed area. The thickness of the surface and bulk snow layer fluctuated with snow melt and precipitation events.

Statistical analyses

All statistical analyses were performed in the R environment using primarily a combination of the vegan [50] and phyloseq [51] packages. Weather information was extracted from the Norwegian Meteorological Institute eKlima database [52], which records and makes available daily weather information, including temperature, precipitation and snow depth. The diversity of ASVs and functions was calculated on the raw metagenomics tables output from MG-RAST. These tables were then normalised by the number of reads of each sample to account for variation in read number and used to assess differences in composition and function using Principal Coordinate Analysis (PCoA). The relative abundance of functions of interests was evaluated using heatmaps made with ggplot2 [53].

Amplicon sequencing of bacterial communities was conducted to identify potentially colonizing taxa. As it focuses on one group of organisms, the depth provides a more detailed insight into the community. However, due to the low biomass of the snow samples and higher potential for contamination during amplicon sequencing, the prevalence function from the decontam package [54] was used to identify and remove potential contaminants. The ASV table was also manually curated to discard ASVs present in the kits and MiSeq controls in higher abundance

than in other samples. Bacterial diversity was calculated using phyloseq and, as for metagenomics, the table was normalised to investigate community composition. To identify potential colonisers, we removed all ASVs uniquely present in the snow (surface and bulk) and those uniquely present in the soil. We also removed any ASV identified in the soil on the first day of sampling or first identified in the soil. While the absence of taxa may reflect the biases associated with molecular techniques or detection limit, the sequencing depth of each sample should have been sufficient, especially as the first and last soil samples had the deepest sequencing depth [Fig. S1]. The remaining 596 ASVs were considered potential colonisers: absent from the soil on the first day of sampling but identified at later stages and always identified in the snow. From these, colonisers were considered potentially successful colonisers if they were identified in at least two soil samples and were still present on the last day of sampling. Finally, potentially successful colonisers were classified by their most likely life strategy, either copiotrophs (r-strategists), oligotrophs (K-strategists) or unclear [10, 55, 56].

Data availability

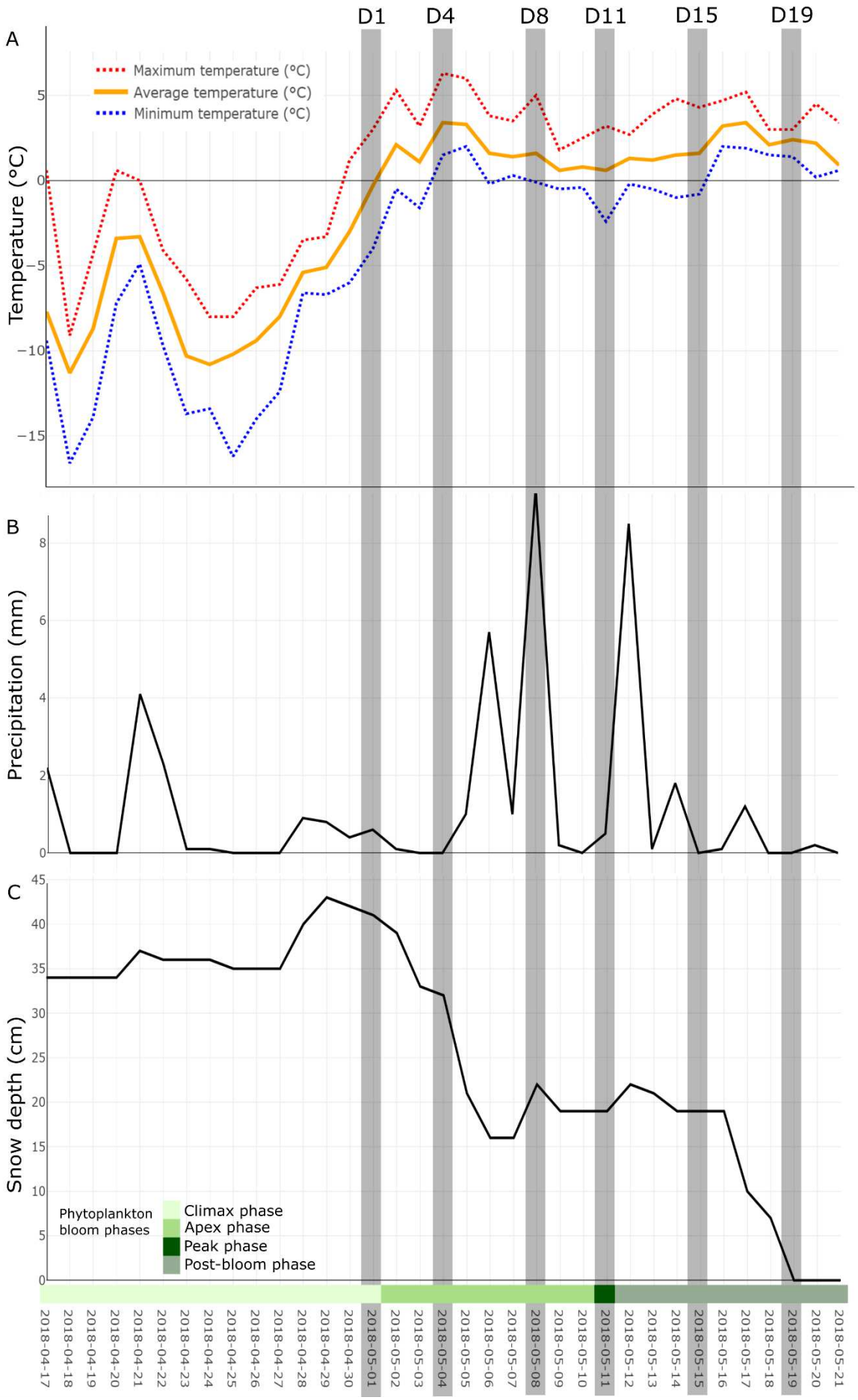
The metagenomes used for this manuscript are deposited on MG-RAST under the accession project number MPG89221 [Table S1]. The amplicon sequences used in this manuscript are deposited at the European Nucleotide Archive under the BioProject accession number PRJNA564220.

Results

Weather conditions during snow melt

The temperature [Fig. 2A], precipitation [Fig. 2B] and snow depth [Fig. 2C] variation was extracted from eKlima for the sampling period and 14 days prior. We sampled at six different times during the snowmelt: May 1st also known as day 1 (D1) was the baseline community where no major changes in snow conditions had occurred yet. Temperatures were below 0°C, precipitation was low, and the snowpack was stable at 34 cm depth and up to 43 cm on April 29th. April 30th was the first day with temperatures above 0°C, but the snow melt began on May 2nd when average temperatures reached 2.1°C. Thus, at day 1 (D1), we sampled a stable snowpack. We also sampled on May 4th (D4), May 8th (D8), May 11th (D11), May 15th (D15) and May 19th (D19), which was the last day of the snow melt. We observed a peak of snow melt between days 4 and day 6, where 50 % of the snowpack melted in 48 hours and the snow depth went from 32 cm to 16 cm. This coincided with a peak in temperatures to over 6°C. The snowpack stabilised between day 8 and day 16, with an increase in precipitation and

203 average temperatures around 1°C. The second peak in snow melt occurred between day 16 and day
204 19, with maximum temperatures reaching over 5°C. This led to the complete melt of the remaining
205 snowpack. May 19th was the last sampling day (D19) when the remaining snowpack measured 7cm
206 deep and melted by the end of the day. As the snowpack melted, the sampled layer thickness
207 decreased over time. However, because of the changing density, the volume of melted snow (melt
208 water) filtered remained relatively stable, around 1500 mL per sample, and therefore, should have a
209 minimal impact on the presented results. We should note that a study monitoring airborne microbial
210 communities during the same time period recorded an algal bloom in Kongsfjorden [57], with the
211 peak of the bloom on May 11th [Fig. 2].


D1
D4
D8
D11
D15
D19
A
Maximum temperature (°C)
Average temperature (°C)
Minimum temperature (°C)
Temperature (°C)
B
Precipitation (mm)
C
Snow depth (cm)
Phytoplankton bloom phases
Climax phase
Apex phase
Peak phase
Post-bloom phase

212

Figure 2: Ny-Alesund weather information before and during the sampling period for (A) air temperature (°C), (B) precipitation (mm) and (C) snow depth (cm). Grey areas indicate sampling days. The different phases of the phytoplankton bloom in Kongsfjorden are based on observations from Feltracco et al. [57].

Microbial communities change with snow melt

Overall, bacteria and eukarya dominated all the communities examined while archaea, viruses and other sequences were virtually absent from the metagenomic dataset [Fig. S2]. Putative eukaryotic sequences represented approximately 30% of the sequences across sampling dates, with a peak in relative abundance in surface snow samples on day 8 [Fig. S2]. The eukaryotic diversity of surface snow generally decreased over time [Fig. 3A] and the PCoA showed the variability of community composition with communities on day 8 and day 15 clustering away from other days [Fig. 3B]. Sequences annotated as Ascomycota (fungi), Basidiomycota (fungi), Streptophyta (green algae and plants) and Chlorophyta (green algae) largely dominated the dataset. A peak in Chlorophyta (Chlorophyceae) on May 8th and a peak in Streptophyta (Charophyceae) on day 15 were observed and both were associated with a decrease in Ascomycota relative abundance [Fig. 3C]. The eukaryotic diversity of bulk snow also decreased over time [Fig. 3A], however, the PCoA showed that the communities formed two distinct clusters: the first three and the last three sampling days [Fig. 3B]. These were characterised by the increase in Streptophyta (Charophyceae) from day 11, the day of the phytoplankton peak in Kongsfjorden. The relative abundance of Ascomycota remained relatively stable over time while Basidiomycota and Chlorophyta relative abundances decreased over time [Fig. 3C]. In comparison, the soil eukaryotic community was more stable in terms of diversity and community composition [Fig. 3A, 3B]. Ascomycota, Basidiomycota, Chlorophyta and Streptophyta were well represented, and their relative abundance was stable [Fig. 3C].

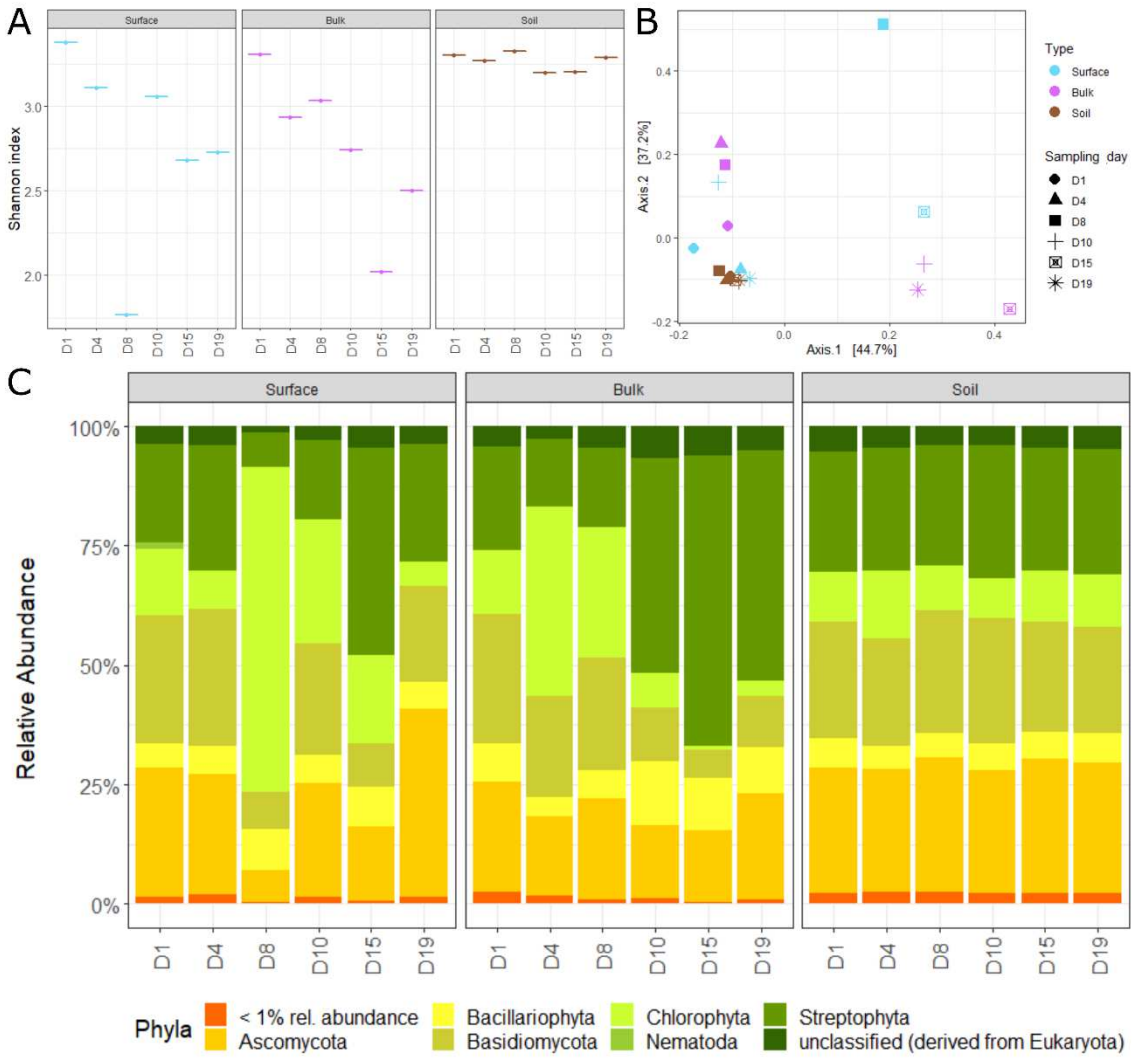


Figure 3: Eukaryotic communities assessed via shotgun metagenomic sequencing. (A) Eukaryotic diversity (Shannon index) at each sampling day. (B) PCoA of the eukaryotic communities (Bray-Curtis dissimilarity) showing the stability of soil communities, the two clusters of bulk snow and the variability of surface snow communities. (C) Eukaryotic community composition at the phylum level with phyla representing less than 1% of the community clustered together.

Bacterial sequences represented approximately 70% of the sequences [Fig. S2] of the metagenomic dataset. The bacterial diversity of surface snow also decreased over time, similarly to the eukaryotic diversity [Fig. 4A]. The PCoA showed the variability of community composition [Fig. 4B]. In surface snow, a peak in the relative abundance of Proteobacteria occurred on day 15, driven by the sharp rise in Betaproteobacteria, in a community previously dominated by Alphaproteobacteria. Actinobacteria decreased over time while Bacteroidetes increased over time, driven by the increase in Flavobacteriia [Fig. 4C].

In bulk snow, the diversity also decreased [Fig. 4A] and the community composition formed the same two clusters [Fig. 4B]. A shift in the community composition was observed from day 11 onward, driven by the sudden increase in the relative abundance of Betaproteobacteria and Bacteroidetes (Flavobacteria) [Fig. 4C]. All other taxa decreased after this shift (such as Actinobacteria, Chloroflexi and Firmicutes), and some almost disappeared from the taxonomic profile (such as Cyanobacteria, Planctomycetes and Verrucomicrobia). In the soil, no major shifts in diversity nor in community composition were observed [Fig. 4].

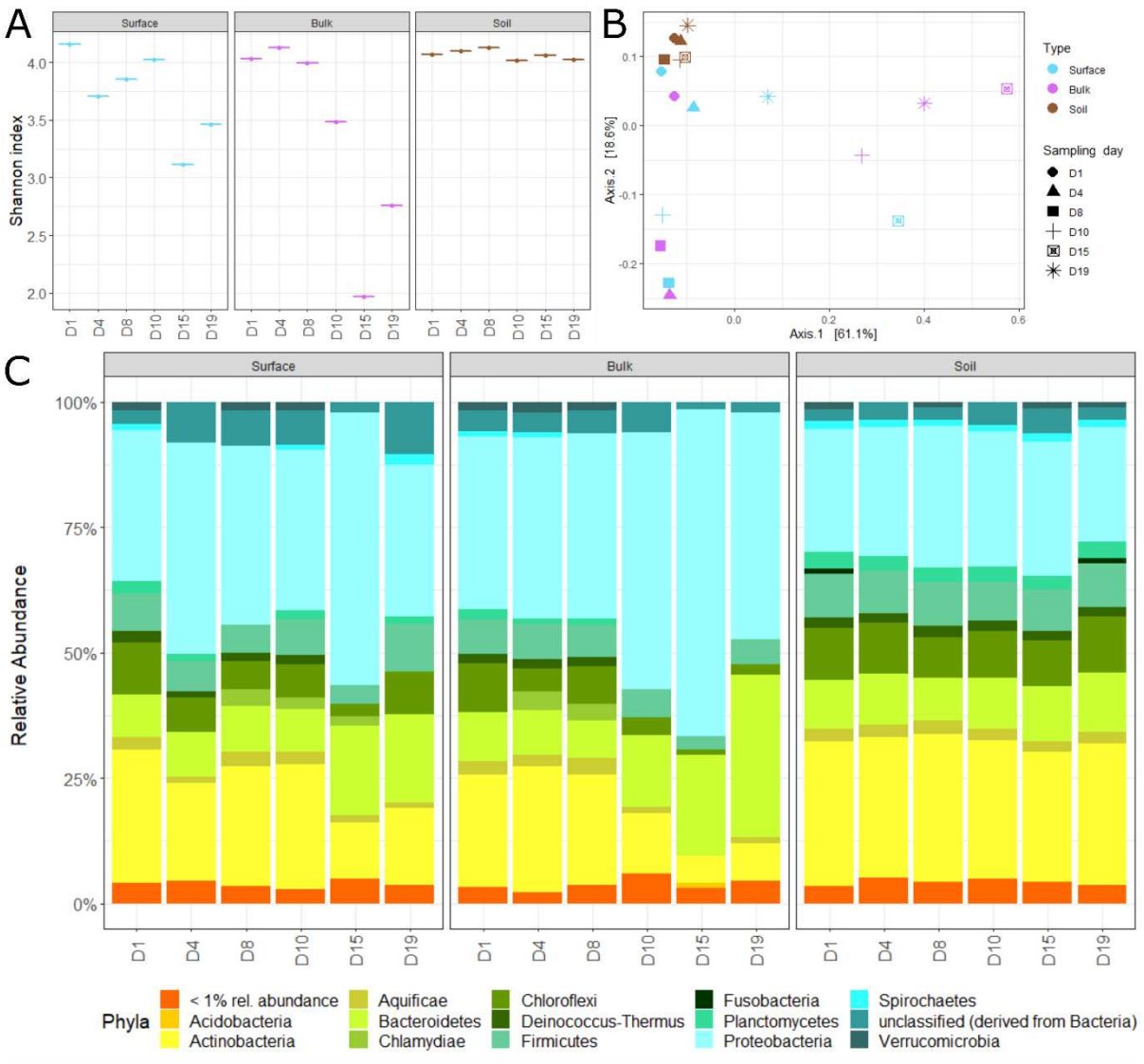


Figure 4: Bacterial communities assessed via shotgun metagenomic sequencing (A) Bacterial diversity (Shannon index) at each sampling day. (B) PCoA of the bacterial communities (Bray-Curtis dissimilarity) showing the stability of soil communities, the two clusters of bulk snow and the variability of surface snow communities. (C) Bacterial community composition at the phylum level with phyla representing less than 1% of the community clustered together.

Changes in the functional profile of microbial communities with snow melt

The number of functional annotations was variable in the snow but stable in soil samples [Fig. 5A]. The PCoA of the functional profile using the RAST subsystems highlighted some variations from one sample to the next without any clear clustering of the snow samples. On the other hand, soil samples and some of the snow samples clustered closely together, illustrating a similar functional profile over time [Fig. 5B]. Overall, the most abundant subsystems identified across all sample types and sampling days were related to cell function and metabolism, such as amino acid, carbohydrate, fatty acid or protein metabolism, and to proteosomes and ribosome function within the 'clustering-based subsystem' [Fig. 5C]. The relative stability of housekeeping genes over time was a good indicator that any functional changes observed were likely the result of ecological changes, such as variations in environmental conditions, rather than the influence of the number of sequencing reads. In both, surface and bulk snow, we observed changes in genes associated with membrane transport, iron acquisition, motility, virulence and stress response associated genes. Some of these genes are likely to influence the colonisation potential by playing a role in competition, adaptation and survival.

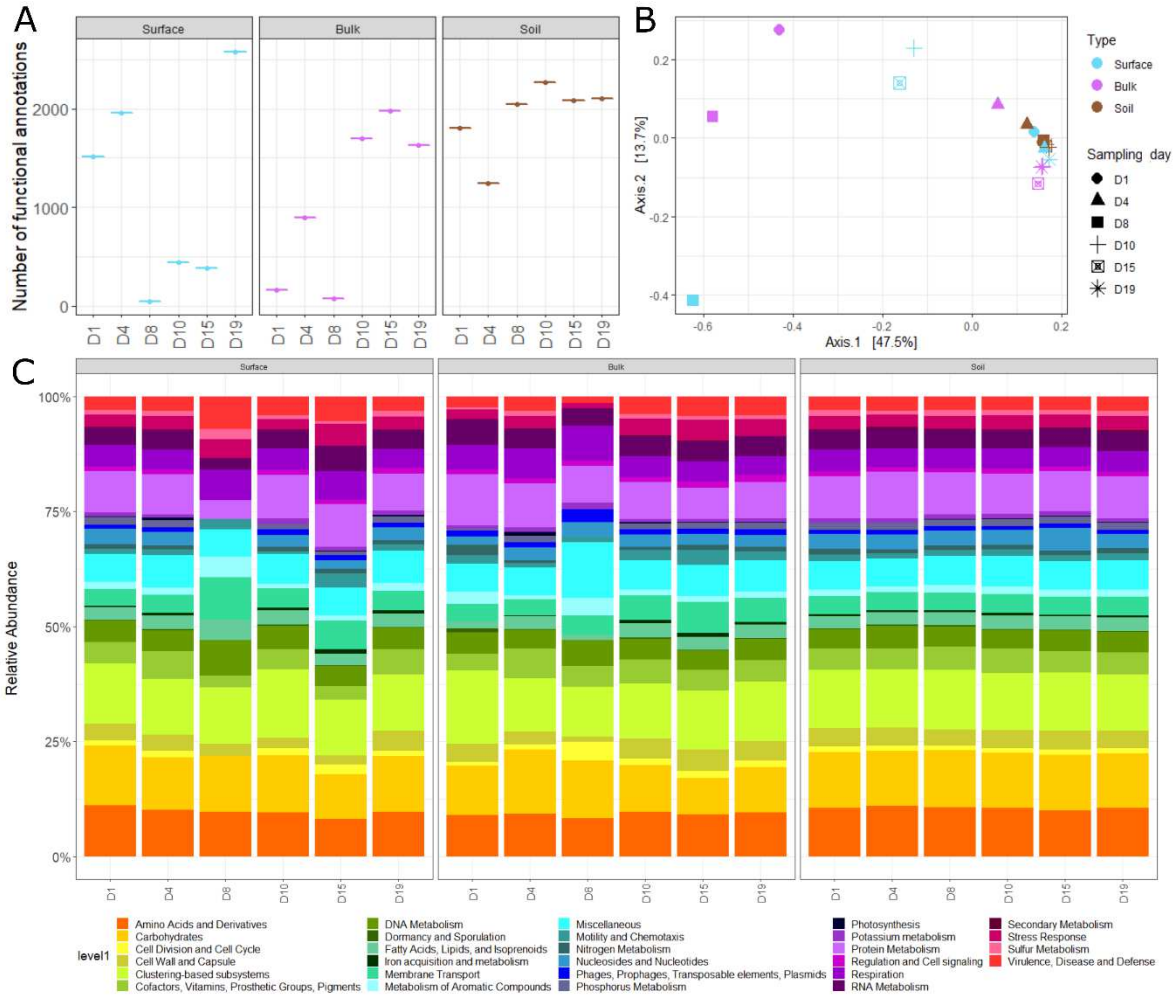


Figure 5: Functional profile of communities assessed via shotgun metagenomic sequencing. (A) The number of functional annotations at each sampling day. (B) PCoA of the functional profile (Bray-Curtis dissimilarity) showing the stability of soil communities, and the variation of the snow profiles. (C) Functional profile of each sample at level 1 of the RAST subsystems.

For example, the relative abundance of genes associated with the stress response represented approximately 5 % of the functional profile and increased over time in snow samples [Fig. 5C]. Genes associated with cold shock were virtually absent while heat shock genes were identified. In surface snow, heat shock genes were especially abundant at the beginning and end of snow melt. In bulk snow, heat shock genes were detected on May 4th, the warmest day, which was also reflected in soil with a peak in relative abundance of heat shock genes [Fig. 6]. We also identified ectoine and betaine biosynthesis genes, both compounds are used to protect against drought, salinity, temperature or osmotic stress [58]. The relative abundance of these genes increased in snow microorganisms at the end of snow melt. Oxidative stress genes were also commonly identified, especially in snow microorganisms [Fig. 6].

Genes associated with motility and chemotaxis represented approximately 2 % of the functional profile and generally increased over time [Fig. 5C]. This was reflected by the increase in flagellar motility associated genes from May 11th onward, especially in the bulk snow and the higher relative abundance of bacterial chemotaxis genes in snow samples [Fig. 6].

At the level 1 of subsystem classification, genes associated with virulence, disease and defence accounted for approximately 5 % of the profile and were primarily classified as 'resistance to antibiotics and toxic compounds' [Fig. 5C]. Genes associated with cobalt-zinc-cadmium resistance were the most abundant and were identified in all samples [Fig. 6]. Besides environmental resistance genes, antibiotic resistance genes were also common and the most abundant was the multidrug resistance efflux pump, which was present in all samples. Genes associated with the resistance to fluoroquinolones and beta-lactamase were primarily observed in soils and were stable over time while, in snow samples, their relative abundance fluctuated over time [Fig. 6].

Other genes relevant to colonisation included genes associated with dormancy and sporulation although they represented <1 % of the functional profile and changes over time were not observed. Similarly, genes associated with the regulation and cell signalling represented approximately 1 % of the functional profile while quorum sensing and biofilm formation related genes were virtually absent from the profile [Fig. 5C].

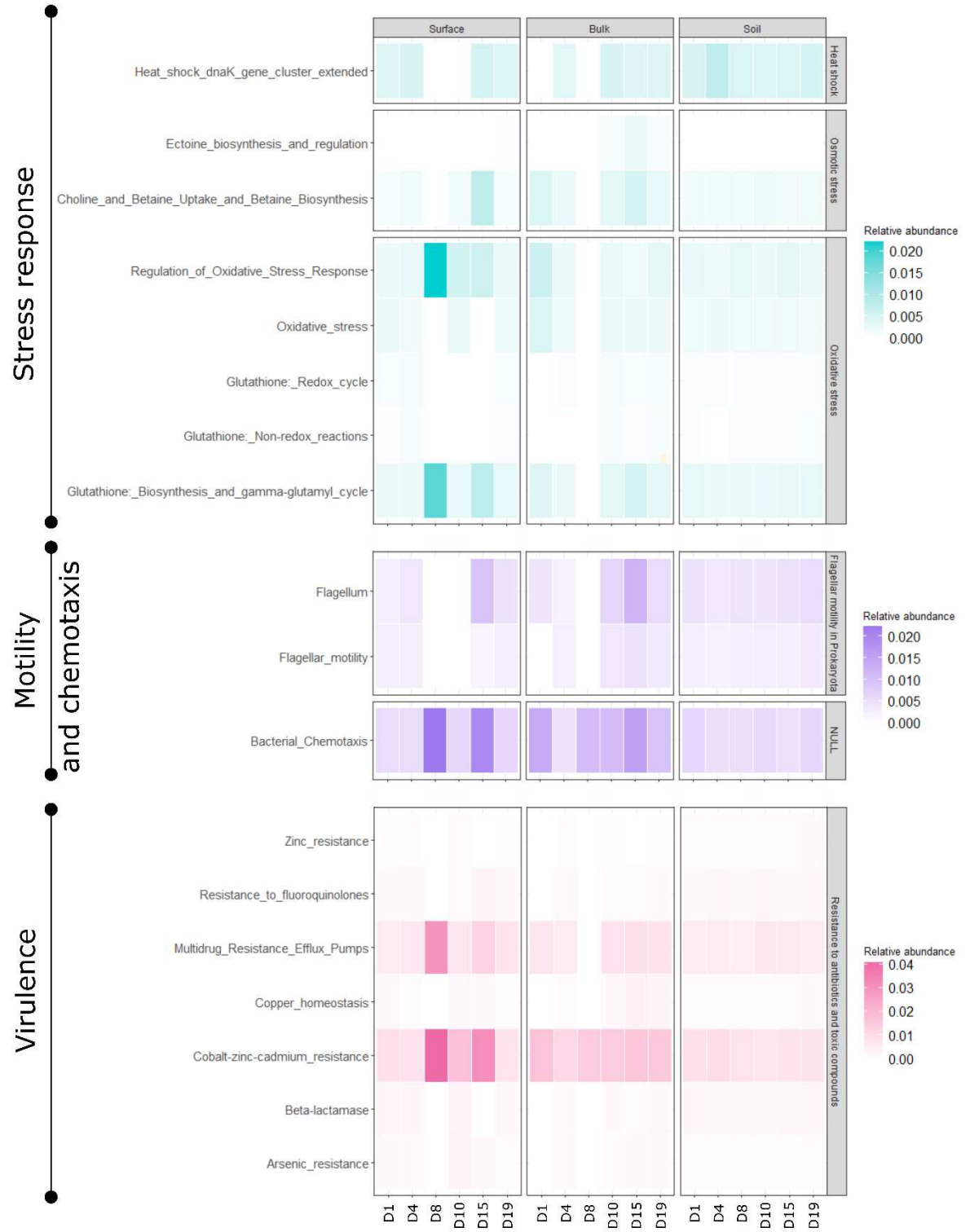
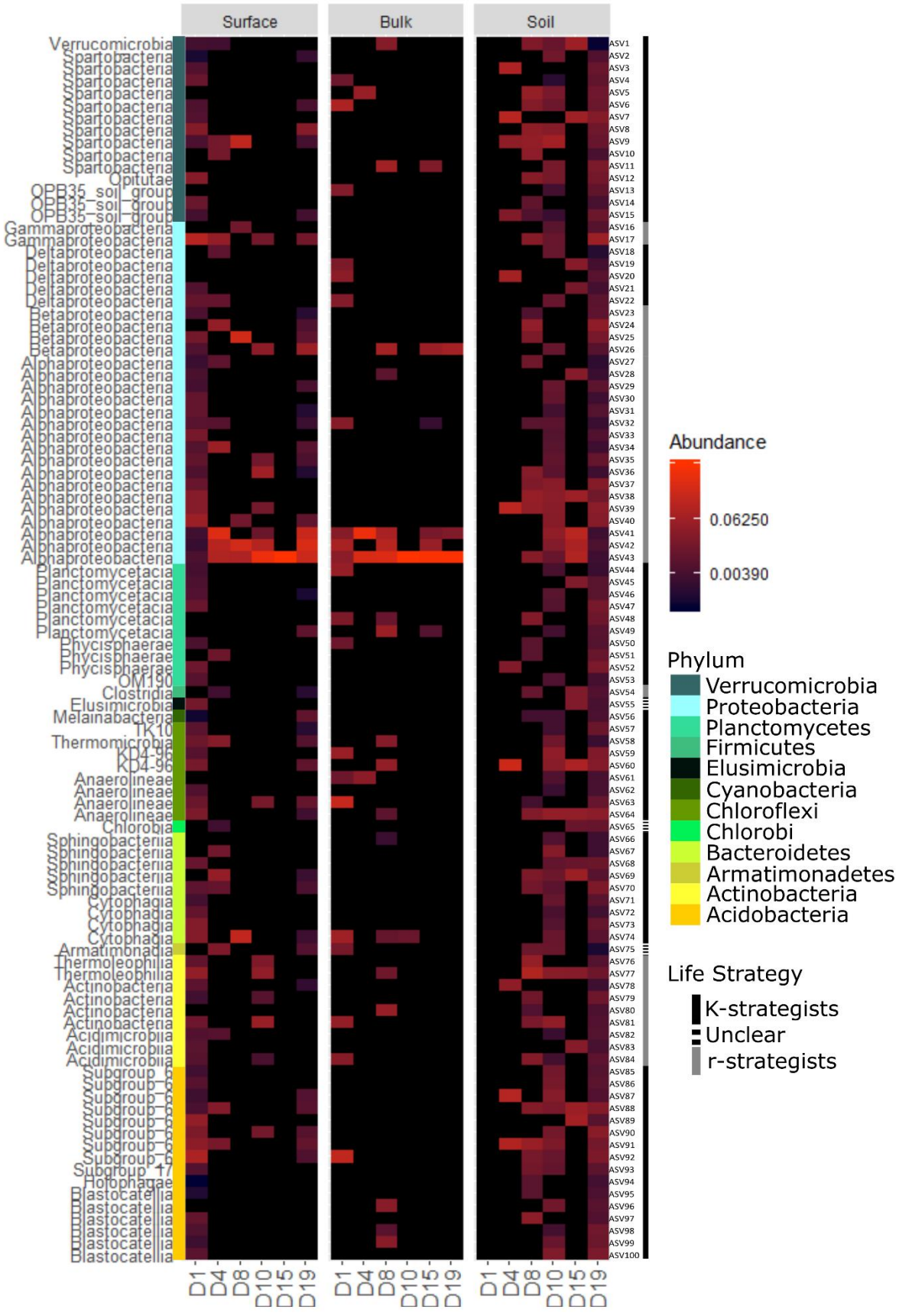


Figure 6: Heatmap of the relative abundance key functional genes likely playing a role in colonisation and illustrating changes in abundance with sample type and sampling day.

Bacterial colonisation of soils during snow melt

In addition to shotgun metagenomics, we assessed the bacterial communities using 16S rRNA gene amplicon sequencing to identify potential bacterial colonisers. The patterns of bacterial diversity and community composition using 16S were similar to the metagenomics results [Fig. S3]. Overall bacterial diversity decreased over time in snow samples. We did observe increased variability in diversity for the soil samples [Fig. S3A]. In terms of community composition, the same clusters were identified [Fig. S3B]. The notable differences observed [Fig. S3C] were the high relative abundance of Acidobacteria, which were at low relative abundance in the metagenomic survey, and the associated decrease in Actinobacteria relative abundance. We also observed an increase in Proteobacteria in the amplicon survey. Using the amplicon data, we identified 596 ASVs considered as potential colonisers. They were first identified in the snow and subsequently observed in the soil. Of these, 100 ASVs were considered potentially successful colonisers as they were identified in at least two soil samples and were still present on the last day of sampling [Fig. 7]. We classified these ASV by their most likely life strategy [55, 56] and found that 65 of the 100 potentially successful colonisers were considered oligotrophs (K-strategists) [Fig. 7].


Surface
Bulk
Soil
Abundance
0.06250
0.00390
Phylum
Verrucomicrobia
Proteobacteria
Planctomycetes
Firmicutes
Elusimicrobia
Cyanobacteria
Chloroflexi
Chlorobi
Bacteroidetes
Armatimonadetes
Actinobacteria
Acidobacteria
Life Strategy
K-strategists
Unclear
r-strategists
D1
D4
D8
D10
D15
D19

333

Figure 7: Heatmap of the relative abundance of potentially successful colonisers where each row corresponds to an ASV ordered by class. The coloured bar indicates the taxonomy at the phylum level. The full list of taxonomy and relative abundance of each ASV is in the supplementary.

Discussion

In this study, microbial communities in the snowpack and the soil were monitored to evaluate the colonisation potential. This was the first study to characterise microbial communities during snow melt and to monitor changes in the underlying soil during snow melt in the field. As the snowpack melts, it is an important source of microorganisms, and therefore, of invaders and potential colonisers for the underlying soil. As such, the snow microbial communities (surface and bulk) were considered as the invading community with the need to adapt to the new habitat and the underlying soil was considered as the resident community resisting to the invasion [1-3]

Based on the weather data, the snow melt lasted 19 days and represented a period of intense change for the microbial communities living in those ecosystems and was likely reflected in the taxonomic and functional profiles. Disturbances to the ecosystem have been shown to facilitate microbial colonisation [59], and therefore, the snow melt may be an opportunity for invading microorganisms to colonise the soil system. Furthermore, the snow melt has been previously characterised as a pulse in nutrient availability [28, 36-38] and although nutrient fluxes were not measured, resource pulses have been shown to facilitate colonisation in temperate soils [14, 60, 61].

Invading community

Due to the important role of snow cover on the climate by reflecting solar radiation (albedo) and the negative role of snow algae darkening the snow and decreasing the albedo [62, 63], a number studies have investigated and described eukaryotic communities in Arctic snow with a focus on algal communities [64-67]. Overall, Chlorophyta and Streptophyta dominated the algal community with variable sequence abundances over time. These taxa are commonly identified in Arctic snow [65, 66] and the abundance peaks after the onset of snow melt are characteristic of algal blooms [63]. Snow algae are essential to the ecosystem as they actively fix carbon and become sources of organic carbon available for heterotrophic organisms [63, 68]. This increase in available organic carbon was likely reflected by the increase of bacterial r-strategists (which grow faster in presence of available carbon) in the communities following the putative algal bloom [9, 55]. Many Betaproteobacteria are often considered r-strategists with fast growth rates [55] and have been associated with algal blooms [66, 69]. The genus Massilia, from the family Oxalobacteraceae, was responsible for the sudden increase in Betaproteobacteria. Members of the Massilia genus are heterotrophic, aerobic, motile, mesophile with a lower growth limit of 2° C and representative isolates have some resistance to antibiotics [70].

Therefore, they may be well adapted to compete and grow in the melting snowpack, especially considering their faster growth rates [71]. Bacteroidetes were also part of the shift in bacterial community composition and while they have often been considered r-strategists [10, 55], it has recently been suggested that they should actually be classified as K-strategists [56]. Often associated with algal blooms [69], their relative abundance first decreased before a subsequent increase, a pattern similar to that identified by Lutz et al. [66] and positively correlated with the increased concentration of dissolved organic carbon due to algal blooms[66]; providing further evidence to suggest that an algal bloom occurred during snow melt. While the shift in snow community composition might result from atmospheric deposition from the phytoplankton bloom occurring in Kongsfjorden at the same time and reflected by the increase in Oxalobacteraceae in the atmosphere [57], we cannot exclude the possibility of an algal bloom also occurring as the snow melted. A simultaneous decrease in Cyanobacteria relative abundance was observed (primarily classified as Nostoc) as the abundance of Betaproteobacteria, Bacteroidetes, Chlorophyta and Streptophyta increased. The decrease of Cyanobacteria may be due to the competition with other organisms, and in this case, heterotrophic bacteria, as has been previously reported in laboratory experiments [72, 73]. As available organic matter increases, heterotrophic bacteria grow faster and use the limited phosphate resources, leaving the Cyanobacteria phosphate limited [72].

The large taxonomic shifts in bacterial populations observed on day 15 in the surface snow and from day 11 onward in bulk snow may have resulted from changes in environmental conditions. Increased temperatures, liquid water and nutrient availability may have promoted the growth of certain taxa such as Betaproteobacteria (Oxalobacteraceae) and Bacteroidetes (Cytophagia, Flavobacteriia and Sphingobacteria). The shift in taxonomy was reflected by some changes in the functional profile, essentially by an increase in iron acquisition, membrane transport, virulence, motility and stress associated genes, likely to influence the colonisation potential by playing a role in competition, adaptation and survival.

Resident community

The changes occurring in the snow eukaryotic communities over time were not reflected in the soil eukaryotic community. The soil bacterial community was dominated by Acidobacteria and Proteobacteria (mainly Alphaproteobacteria and Betaproteobacteria). An increase in Verrucomicrobia was observed over time while they decreased in snow samples. The abundance of Firmicutes peaked on day 4, 11 and 15, driven by the increase of Clostridia, commonly identified in Arctic soils [74, 75] and considered r-strategists with fast growth rates [10, 55], which may be favoured by the changing environmental conditions and out-compete some taxa. The functional profile was relatively stable over time compared to the snow. The main difference observed occurred on day 4 with an increase in

heat shock genes, potentially reflecting the changes in environmental conditions as the snow melt was initiated on day 1 with a peak in temperature on day 4.

Colonisation potential

A successful colonisation depends on the alpha diversity, ability to survive and compete of both, the invading and resident community [2]. The invaders need to be well adapted to the environmental conditions of the new ecosystem they are attempting to colonise. In this case, the snow microbial communities already lived in similar abiotic conditions, although with its share of differences (e.g. carbon availability or photochemistry), they should have already been better adapted to the soil ecosystem than microorganisms coming from elsewhere, increasing their chances of successful colonisation [1, 2]. The invaders also need to be good competitors, which is particularly difficult to evaluate in natural, complex ecosystems such as soils. Genes involved with competition such as increased motility, chemotaxis and virulence were observed. The increase of these genes in the snow communities may provide a competitive advantage by increasing the potential for a successful colonisation of the soil. The functional profile of the soil remained relatively stable over time with no apparent increase in competition genes.

While 596 ASVs were initially present in the snow only and later identified in the soil, 100 ASVs were considered potentially successful colonisers. Interestingly, the majority of these colonisers were considered K-strategists [10, 55] and is against the hypothesis that r-strategists are more likely to colonise due to faster growth rates [5]. K-strategists may have the advantage in the Arctic as they may be better adapted to survive stressful environmental condition and resource limitations [9, 10]. However, we did not assess whether these potentially successful colonisers did survive in the long run and the impact they may have had on the soil community. Laboratory microcosm experiments suggested that 30 days after the end of melt, only few ASVs successfully colonised the soil, primarily classified as r-strategists [22]. Therefore, whether these 100 ASVs were successful remains undetermined and while K-strategists may be favoured after the melt, r-strategists may be more successful in the long-run. Monitoring communities throughout the Arctic summer season should be conducted to assess whether colonisers can permanently colonise, grow into the soil community and the impact it may have on resident communities.

Declarations

Ethics approval

This is an observational study and therefore, no ethical approval was required.

Consent to participate and consent for publication

No approval was required to accomplish the goals of this study because the experimental work was conducted with environmental microbial communities.

Data availability

The 16S rRNA dataset is deposited at the European Nucleotide Archive under the BioProject accession PRJNA564220. The shotgun metagenomics dataset is deposited on MG-RAST under the accession mgp89221.

Code availability

All codes for analysis can be obtained by contacting the corresponding author.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LAM, DAP, TV and CL designed the project. BBP, RL and CL conducted the field work. LAM did the laboratory work, bioinformatic processing, statistical analyses and drafted the manuscript. All authors

commented on previous versions of the manuscript and all authors read and approved the final manuscript.

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