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PREFACE

This document is intended to provide a general overview of the microbial diversity and distribution in snow over glacier surfaces. The aim is to provide a general framework of metrics and methods for the estimation of diversity. The information presented henceforth elaborates on the taxonomic lineages, their quantification, and statistical analyses. The methods are not universal but are recommended among doctoral candidates for the ease of communication and management.

1. Introduction

Microorganisms are ubiquitous and have been thriving on Earth for 3.7 billion years. They are the early forms of life on earth. Through constant evolution under varying climatic and geochemical influences, they have transformed the hot primitive earth into a habitable planet with diverse forms of life (Oró et al., 1990). Their ability to respond and adapt to changes in their habitat is a function of the gradual accumulation of selective genes that favors the viability and genetic fitness of the species (Cooper et al., 2003; Ferea et al., 1999). Such fitness arises from the random event of genetic exchange and mutations among the microbial species (Ferea et al., 1999; Rifkin et al., 2005). Such accumulation of selective genes and mutations confers functional diversity and regulatory mechanisms in microbes which results in the vast diversity of species occupying various habitats on Earth at different levels of the food chain (Fay & Wittkopp, 2007; Le Gall et al., 2005; Rifkin et al., 2005).

Depending on the chemical composition and complexity of the habitat, the microbial diversity varies at different spatiotemporal niches (Eiler et al., 2003; Lutz et al., 2017a; Stibal et al., 2006). For example, the soil is rich in nutrients and supported by the productivity of plants and metazoans are relatively diverse in microbial species (Bastida et al., 2021; Fierer & Jackson, 2006) than the habitats that have limited nutrient pools such as the cryosphere which are subjected to oligotrophy and extreme environmental conditions. However, the cryosphere is still an active biome for the prokaryotic and eukaryotic domains of life (Anesio & Laybourn-Parry, 2012). The microbial abundance of soil can range from $10^{\wedge 8}$ to $10^{\wedge 11}$ cells/g (Lee et al., 2021) but for the snow or glacial ice, it is $10^{\wedge 3}$-$10^{\wedge 4}$ cells/ml (Amato, Hennebelle, et al., 2007; Cameron et al., 2017).

The microbial diversity of the snow over the glacier surface is attributed to the presence of particulates (Mayol et al., 2017a; Smith et al., 2013), dust (Smith et al., 2013), pollen (Sánchez-Parra et al., 2021; Uetake et al., 2006), sea spray (Alsante et al., 2021; Mayol et al., 2014) and bioaerosol (Després et al., 2012) which either gets deposited on the snowpack or provides a nucleation point for cloud condensation and snow formation. For example, the microbial and pollen surface exhibits certain proteins and glycoproteins that promote the ice nucleation of supercooled water and affect the precipitation pattern and snowfall which gets deposited on the glacier surface (B Murray, 2012; Duan et al., 2023; Roeters et al., 2021; Wolber, 1993). Hence, the microbial community of the surface snow shares more similarities with the atmospheric sources (Maccario et al., 2019). The microbial community of atmosphere and surface snow was shown to have an overlap of 75.7% in fall, 56.7% in

winter, and 49.8% in spring among the bacterial communities and 83.9% in winter, 72.9% in fall and 66.3% in spring among the fungal community (Els et al., 2020). The bacterial and archaeal count of the cloud water is estimated to be $10^{\wedge 3}$-$10^{\wedge 4}$ cells/ml and for eukaryotes around $10^{\wedge 2}$-$10^{\wedge 3}$ cells/ml (Amato et al., 2017; Amato, Parazols, et al., 2007) and the pollen emission was estimated at $10^{\wedge 8}$ grains/m^2y (Zhang & Steiner, 2022). A global estimate of microbial load over the tropical and subtropical oceans was estimated to be 2.2×10^{21} and 2.1×10^{21} cells for prokaryotes and eukaryotes respectively (Mayol et al., 2017b). These atmospheric exchanges play a major role in constituting the microbial diversity of the snow. It can originate from a local transport (Harding et al., 2011; Šantl-Temkiv et al., 2018) of a nearby terrestrial or marine habitat or it could travel intercontinental distances as shown by (Smith et al., 2013) which majorly represents sporulating species of Actinobacteria, Firmicutes and Proteobacteria clades.

The microbial community transitioning from one habitat to another encounters a physicochemical barrier that naturally filters for species exhibiting habitat-specific functions and resistance which could primarily be related to temperature, desiccation, radiation, and osmotic shock (Aalismail et al., 2019; Lappan et al., 2024; Martiny et al., 2006; Tignat-Perrier et al., 2020). The microbes are also affected by the localized environmental variables (Centurion et al., 2021). For example, the surface snow is affected by strong winds, low temperatures, and desiccation during winter. In summer, it experiences intense radiation, oxidative stress, and osmotic shock that affects microbial growth and their proportion in snow (Larose et al., 2013a). The submerged layers of snow are well insulated and protected from such effects. The meltwater percolates the microbes and nutrients from the surface to the middle and basal layer of the snowpack. Thereby enriching these layers with an active microbial community. Hence the diversity pattern in snow varies spatiotemporally. The cryosphere is commonly populated with algal (*Chloromonas*, *Chlainomonas*, *Sanguina*) and cyanobacterial growth during the summers which enriches the nutritional content of the snow causing algal bloom and bioalbedo. It attracts the growth of Bacteroidetes, Cytophagia, Flavobacteria, Proteobacteria (alpha, beta, and gamma), Firmicutes, and Actinobacteria (Azzoni et al., 2018; Hell et al., 2013; Larose et al., 2013b; Malard et al., 2019a; Schuler & Mikucki, 2023). Such a network of food web leads to competitive or mutualistic growth. Thereby constantly changing the diversity pattern of microbes in snow.

The focus of this deliverable will be to quantify and understand the microbial diversity of snow over glacier surfaces by utilizing a few examples. It will provide a basic introduction to

the metrics used for diversity analyses and the distribution pattern of the algal, bacterial, and fungal communities in snow.

2. Methods and metrics for diversity analyses

Molecular analyses

The metrics required for the diversity analyses of snow can be obtained by culture-dependent or independent methods. However, the culture-dependent method considers viable and culturable cells which is unable to capture the actual proportion and abundance of the microbial community. And it is difficult to replicate the actual environmental conditions in the lab to cultivate all the species. Some microbes prefer to grow in liquid suspension and some in solid whereas some are categorized as viable but non-culturable (VBNC) (Song & Wood, 2021). Hence, the culture-dependent method could lead to biased diversity estimates. The predicted diversity of microbes on earth is around 10^{12} species and an overall production rate of $1.7x10^{30}$ prokaryotic cells/y (Locey & Lennon, 2016; Whitman et al., 1998). Such a number is quite difficult to manage and quantify in a lab. Hence, the advent of amplicon and metagenomic sequencing has provided an advantage in diversity analyses to a deeper taxonomic level. Especially the amplicon sequencing gives robust access to the evolutionary conserved regions of the genetic material.

For example, the 16S rRNA gene spanning approximately 1500 bps is represented by 9 hypervariable regions interspersed by highly conserved evolutionary regions **(Fig. 1)**. The combination of these variable and conserved regions are used to study the phylogeny of the prokaryotes (Johnson et al., 2019; Woese & Fox, 1977), Similarly, the 18S rRNA gene **(Fig. 2)** consists of 9 hypervariable regions representing the evolutionary record of the eukaryotic domain (Field et al., 1988; Hugerth et al., 2014). The Internal Transcribed Spacer (ITS1) and ITS2 region (Schoch et al., 2012; Usyk et al., 2017) are found as spacers in the eukaryotic ribosomal rRNA cistron and are specifically used for the phylogenetic study of the fungal population. Targeting these conserved and hypervariable regions with specific primers captures the diversity at a molecular level and the choice of primer can affect the distribution profile of the microbial diversity. It is a highly sensitive approach.

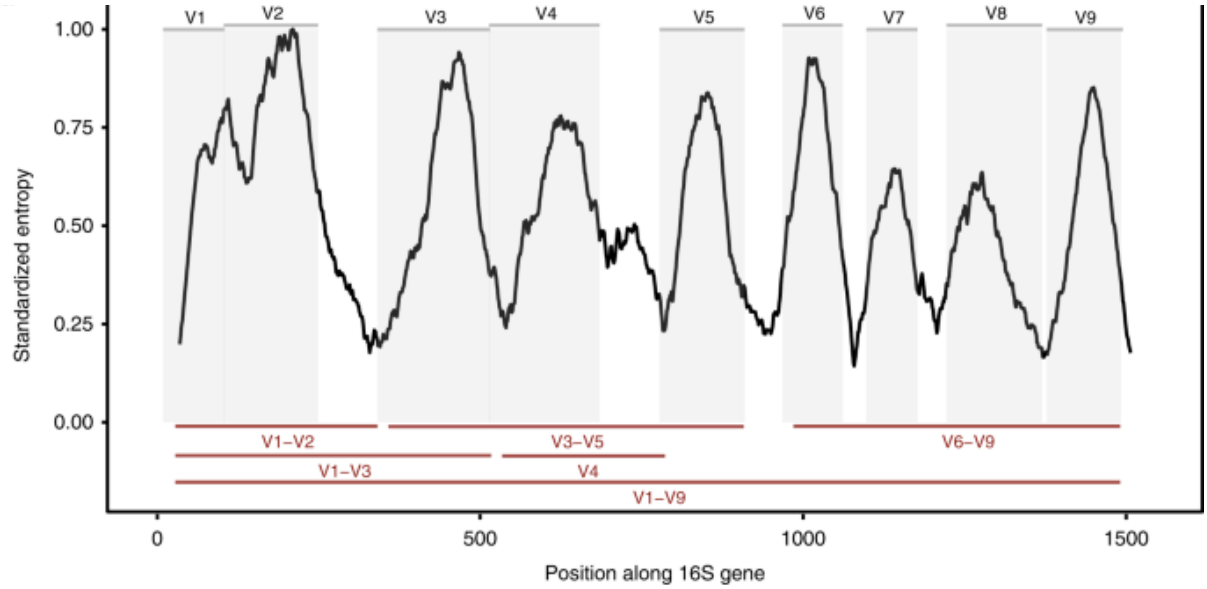

Fig. 1 Shannon entropy of 16S rRNA gene based on alignment of all unique representatives from Greengenes database against a reference of 16S gene of *Escherichia coli* K-12 MG1655. Adapted from Johnson et al., 2019.

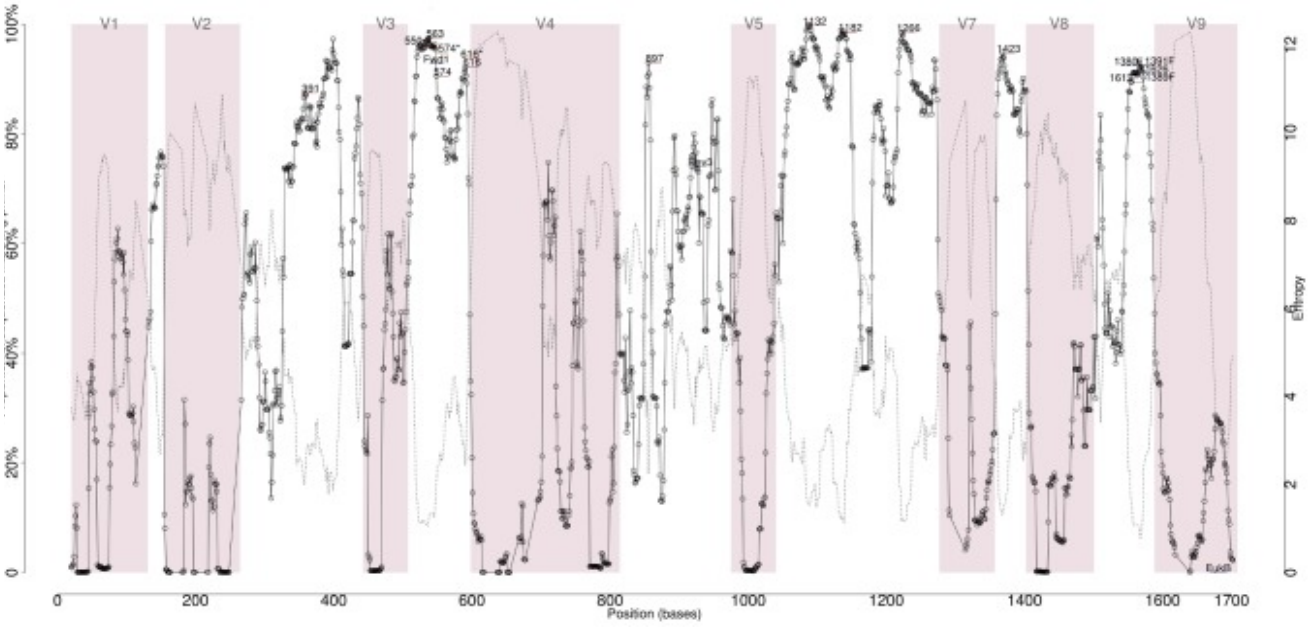

Fig. 2 The entropy of each position is depicted by a dotted grey line. The position numbering refers to the *Saccharomyces cerevisiae* strain FM-sc-08 18S ribosomal RNA gene. Adapted from Hugerth et al., 2014.

Statistical analyses

Given the variation in microbial species from habitat to habitat we need a general approach to compare and quantify the microbial diversity. We use the metrics of abundance, richness, and evenness to quantify the microbial population. Where abundance refers to the absolute count of species, richness defines the count of different types of species and evenness explains the equal distribution of different types of species in a habitat. Utilizing these metrics, statistical methods like Shannon index (richness and evenness)(Ludwig & Reynolds, 1988), Chao index (richness or rare species)(Anne Chao, 1984), Simpson index (evenness or relative abundance)(Simpson, 1949) and, Gini index (Gini, 1912)(evenness) provides the diversity estimates of microbes in a habitat. Such metrics are useful in comparing the diversity within (alpha-diversity) and between the habitat (beta-diversity) and also the overall region (gamma diversity)(Whittaker, 1972).

Shannon index:

It is used to measure the diversity of species in a community by estimating the richness and evenness among the species. The negative value H` is converted to positive and the higher values indicate higher diversity in a community or sample and vice versa. The maximum diversity of a sample or habitat is expressed as ln(N), where N is the total number of species or OTUs.

$$H' = -\sum_{i=1}^{S} p_i \ln (p_i)$$

where:

- H′: Shannon diversity index
- S: total number of species in the community (species richness)
- p_i: proportion of individuals belonging to species i (i.e., $P_i = n_i / N$, where n_i is the number of individuals of species i and N is the total number of individuals of all species)
- ln: natural logarithm

Simpson's index:

It is used to measure the dominance of species in a community by estimating the total number of species and their relative abundance in a sample. The value of D ranges from 0 to 1 with the highest diversity (0) to no diversity (1) respectively. The use of 1-D simplifies the explanation where the higher value indicates high diversity and vice versa. The reciprocal of

1/D gives the diversity values from low diversity of 1 to high diversity of max value equating to the richness of the sample (number of different types of species).

$$D = \sum_{i=1}^{S} \frac{n_i(n_i-1)}{N(N-1)}$$

where:

- D: Simpson's diversity index
- n_i: number of individuals of species i
- N: total number of individuals of all species

Chao index:

It is used to estimate the total richness of the species in a community by considering the distribution of rare species (singletons and doubletons). Higher values of the Chao index suggest a higher number of species and diversity.

$$S = S_{obs} + \frac{f_1^2}{2f_2}$$

where:

- S: species richness
- S_{obs}: observed number of species in the sample (total count)
- f_1: number of species observed exactly once (singletons)
- f_2: number of species observed exactly twice (doubletons)

Gini index:

It is used to estimate the unequal distribution of species in a community, It considers the species richness and evenness. Where G=0 specifies even distribution and G=1 specifies highly uneven distribution of species in a community in terms of evenness.

$$G = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}|x_i - x_j|}{2n^2\mu}$$

where:

- G: gini index
- N: number of species
- x_i: abundance of species i
- μ: the mean abundance of species, given by; $\mu = \frac{1}{n}\sum_{i=1}^{n} x_i$

3. Microbial diversity of snow over glacier surfaces

Several studies have been conducted to describe the microbial processes and structure in snow from different habitats of the Arctic (Bradley et al., 2023; Lutz et al., 2015, 2017b; Winkel et al., 2022), alpine (Hotaling et al., 2022; Yang et al., 2016) and Antarctic region (Lopatina et al., 2013; Luo et al., 2020; Malard et al., 2019b; Stoppiello et al., 2023). Given below are some studies on the microbial diversity from the Arctic, alpine, and Antarctic regions.

Microbial diversity of the Arctic samples

For the arctic habitat, we will utilize one example to explain the diversity pattern of microbes in Iceland. The study conducted by (Lutz et al., 2015) describes the microbial composition of the red snow obtained from the glaciers and ice caps of Iceland. The sampling site is shown in **Fig 3**. The sampling covered the western (Snaefellsjökull), northern (Drangajökull), southern (Vatnajökull, Eyafjallajökull, Mýrdalsjökull), and the central glacier (Hofsjökull) and highland (Laugafell). One snow sample was collected in 2014 from the layer of freshly cooled lava (Eyafjallajokull).

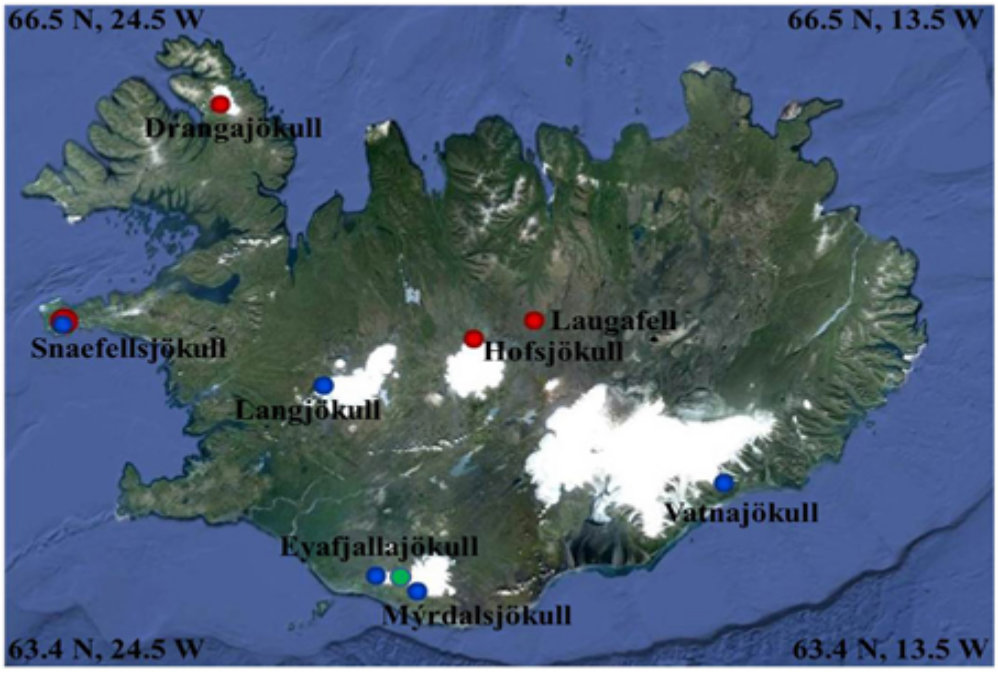

Fig. 3 Map of Iceland indicating the sampling site in the red, blue and green dots. Adapted from Lutz et al., 2015.

The samples collected were processed for amplicon sequencing. For the eubacteria, the samples were amplified with 16S rRNA gene primers of 27F (5′-

AGAGTTTGATCMTGGCTCAG) and 375R (5′-CTGCTGCCTYCCGTA) spanning the V1-V2 hypervariable region. For the eukaryotes, 18S rRNA gene primers of 528F (5′-GCGGTAATTCCAGCTCCAA) and 706R (5′-AATCCRAGAATTTCACCTCT) were used to amplify the V4-V5 hypervariable region. The PCR cycle is followed, by initial denaturation at 95°C for 5 min and then 30 cycles of "denaturation at 95°C for 30 s, annealing at 60°C for 30 s and elongation at 72°C for 30 s" followed by final elongation at 72°C for 10 mins. For archaebacteria, the 16S rRNA gene was subjected to a nested PCR with 20F and 915R primers with a PCR cycle following initial denaturation at 95°C for 5 min and then 35 cycles of "denaturation at 95°C for 30 s, annealing at 62°C for 30 s and elongation at 72°C for 180 s" followed by a final elongation at 72°C for 10 mins. The amplified fragment was used as a template for the 2nd PCR with 21F (5′-TCCGGTTGATCCYGCCGG) and 519R (5′- GWATTACCGCGGCKGCTG) primers spanning the V1-V2 hypervariable region. The PCR cycle was performed with initial denaturation at 95°C for 5 min followed by 30 cycles of "denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s" followed by final elongation at 72°C for 7 min.

Algal and Fungal diversity

The sequencing of 18S rRNA amplicons yielded 108,790 sequences clustered at 97% similarity with QIIME. The OTUs aligned against the Silva database revealed that Chloroplastida (green algae) and Fungi were dominant in all the samples followed by Stramenophiles. The samples collected in 2013, which lie on the coastal line of the western glacier to the southern glacier (except Vatnajokull, which is more to the east) were more dominant in Fungi (67-94.9%), with an abundant class of Microbotryomycetes (Basidiomycota). However, the samples collected in 2012 and 2014 (except Eyafjallajökull, ICE-14_1) had a higher abundance of Chloroplastida (35.4–60.6%) and Stramenophiles (28-53%; e.g., Chrysophyceae-golden algae; Volcanic samples of 2014) followed by lower count of Rhizaria and Alveolata.

To estimate the composition of the algal community, the OTUs of Chloroplastida were extracted from sequences. It revealed that the genera of *Chloromonas polyptera* (5-75.9%) and *Raphidonema sempervirens* (3.4-98.5%) were more abundant in all the samples followed by some uncultured *Chlamydomonadaceae* (2-43%; high similarity with *Chloromonas*). The abundance of *Chloromonas polyptera* (5-75.9%) and *Raphidonema sempervirens* (3.4-98.5%)

showed an antagonistic trend. Other sparse algal genera belonged to *Chloromonas* (up to 16.7%), *Chlamydomonas* (<0.1%), *Ancylonema* (<0.1%), and *Mesotaenium* (<0.1%).

The Shannon indices in **Table. 1** showed no significant difference in diversity (H'=3.88-4.51) among the 2012 and 2013 samples. The volcanic samples of 2014 had a very low Shannon index of H'=1.07-1.81 which is indicative of low algal diversity. This sample was only dominated by *Raphidonema sempervirens* (90.9-98.5%) followed by a low count of *Chloromonas polyptera* (0.9-6.5%). Hence, one dominant species does not increase the diversity.

Chloromonas polyptera were highly abundant in all the samples and is also known for astaxanthin production (Remias et al., 2013). The corresponding pigment analyses also revealed that the samples were rich in chlorophylls (chl a and chl b; 31-100%) and secondary carotenoids (up to 69%) such as astaxanthin which attributed to the red color of the snow. The samples (2013 and 2014) were collected in the late melt season and stress-induced secondary metabolism leads to secondary carotenoid production.

Table. 1 The Shannon diversity indices of the samples collected from Iceland. Adapted from (Lutz et al., 2015)

Location **Arctic**	**Sample type**	**Shannon**			**Reference**
		Eukarya/Fungi (OTUs)	**Algae (OTUs)**	**Bacteria (OTUs)**	
Drangajökull-12_2/3	Red Snow	5.55	4.5 (714)	5.13 (513)	(Lutz et al., 2015)
Laugafell-12_4	Red Snow	6.02	4.47 (89)	5.27 (120)	
Hofsjökull-12_6/7	Red Snow	5.61	3.88 (792)	5.38 (339)	
Vatnajökull-13_13/14/15	Red Snow	5.02	4.51 (66)	5.06 (14)	
Langjökull-13_16/18	Red Snow	5.65	4.26 (340)	5.29 (111)	
Langjökull-13_19	Red Snow	4.99	4.26 (38)	5.14 (444)	
Snaefellsjökull-13_21/23/24	Red Snow	5.51	4.04 (41)	3.97 (486)	
Eyafjallajökull-13_4/5/6	Red Snow	5.14	4.21 (37)	5.27 (55)	
Mýrdalsjökull-13_8/9	Red Snow	5.14	0 (8)	0 (2)	
Eyafjallajökull-14_1	Red Snow	5.11	1.74 (3357)	4.53 (938)	
Eyafjallajökull-14_2	Red Snow	3.75	1.07 (3307)	4.64 (1158)	
Eyafjallajökull-14_3	Red Snow	4.96	1.81 (4460)	4.59 (3390)	

Bacterial diversity

The sequencing of 16S amplicons yielded 24,221 sequences clustered at 97% sequencing identity with QIIME. The OTUs aligned against the Greengenes database revealed the most abundant phyla of Proteobacteria, Bacteroidetes, and Cyanobacteria. In Proteobacteria, the

Betaproteobacteria was prevalent in the samples of Snaefellsjökull (95.1%; 2012), Langjökull (80.3 and 71.9%; 2013) and Eyafjallajökull (28.7–65.4%; 2014) and the Alphaproteobacteria was dominant in the samples of Vatnajökull (49.5%; 2013) and Eyafjallajökull (42.6%; 2013), and Drangajökull (42.0 %; 2012).

The Bacteroidetes phyla was dominated by the *Sphingobacteria* in the samples of Hofsjökull (32.2%) and Drangajökull (18.8%), whereas *Saprospirae* dominated Hofsjökull (38.4%), Eyafjallajökull (28.0–45.5%; 2014), Laugafell (28.5%; 2012) and Drangajökull 24.1%; 2012). A few classes of Cytophagia were also found in almost all of the samples but in low abundance (0.1-5.4%). Similarly, Actinobacteria were also present in almost all of the samples (0.3-15.9%).

The phyla of Cyanobacteria were represented by the Nostocophycideae and Oscillatoriophycideae families in the samples of Eyafjallajökull (13.7% and 4.3%; 2013) and, Vatnajökull (30.8% and 9%; 2013).

The Shannon indices (H'= 5.13-5.38.) showed no significant difference in bacterial diversity among several samples (**Table. 1**). They all shared similar microbial structures. The sample of volcanic snow from Eyafjallajökull in 2014 had an index of H'=4.52–4.64 which was only dominated by Bacteroidetes and Proteobacteria and similarly, the sample of Snaefellsjökull was solely dominated by Proteobacteria (97.8%) which gave a lower index of H'= 3.97.

Microbial diversity of the alpine samples

For the alpine habitat, the study conducted by (Hotaling et al., 2022) illustrates the microbial composition of the snow collected from the paradise glacier of Mount Rainier in Washington, USA. The samples were collected from the same spot every month for May, June, July, August, September Pre-snow and September Post-snow 2019 (**Fig. 4**).

The samples were processed for amplicon sequencing. For bacteria and archaebacteria, the 515F and 806R primers were used to amplify the V4 hypervariable region of the 16S rRNA gene. For the eukaryotes, E572F and E1009R primers were used to amplify the V9 hypervariable region of the 18S rRNA genes. For the fungal community, ITS1F and ITS2R primers were used to amplify the ITS1 region of the eukaryotic rRNA cistron.

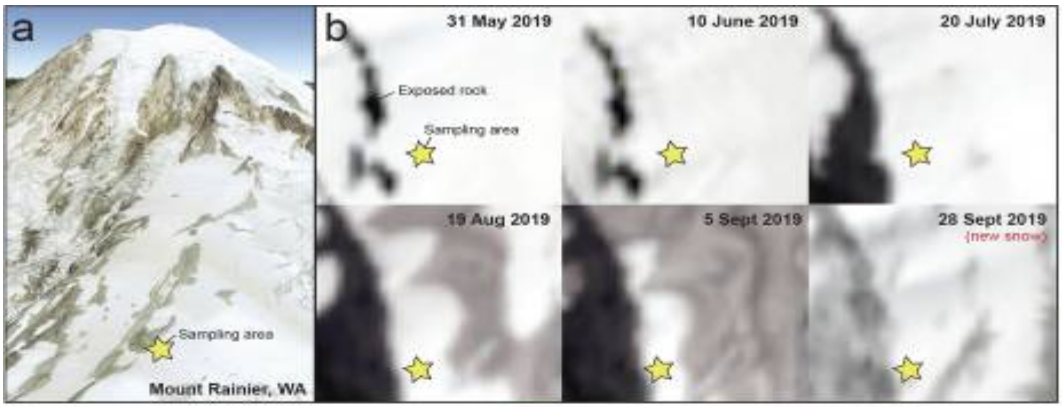

Fig. 4 Map of Mount Rainer indicating the sampling site (as golden star) and temporal changes in snow cover. Adapted from (Hotaling et al., 2022)

Algal and Fungal diversity

The sequencing of 18S rRNA and ITS amplicons yielded 1246 and 3007 eukaryotic OTUs respectively, including all the time points. The 18S amplicons were aligned against the PR2 database (v.4.12.0) and were clustered at 99% sequence similarity with the OptiClust algorithm in Mothur. The ITS amplicons were aligned against the UNITE database and were clustered at 97% sequence similarity with the agc algorithm on Mothur.

The taxonomic assignment of 18S OTUs revealed Chlorophyta (up to 90%) as the most dominant phyla in all the samples but was least abundant in June. Within Chlorophyta, the *Chlainomonas* group dropped in abundance from May (20%) to June (2-5%) and then showed a gradual increase with a peak in August (30-40%) which indicates the feature of *Chlainomonas* growth in high water content. Another variant of *Chlainomonas* was only abundant in May (38%; buried from snowfall) and late September (2-10%). The Shannon

indices (**Table. 2**) indicate a fall in diversity in June (H'=1.5) as compared to May (H'=2.8). The diversity indices from July onwards (H'=2.4-2.9) show no significant changes except for September pre-snow with an index of H'=3.4. The months of August and September showed more dynamic changes in terms of microbial dominance and proportions.

The taxonomy of ITS revealed that Basidiomycota dominated the fungal community. Within the Basidiomycota clade, the *Microbotryomycetes* group dominated the first half of the melting season such as in May (25%), June (65%), peaking in July (75%) and gradually decreased to 5% in late September. Another group of *Cystobasidiomycetes* was also found dominant only during the June month (50%). The fresh snow of late September was more dominant in *Ascomycota* (50%). The Shannon indices (**Table. 2**) indicate a fall in diversity in June (H'=1.8) and July (H'=1.5) as compared to May (H'=3). It could be due to changes in even distribution and dominance of a single species. The Shannon index shows an increase in diversity from H'=1.5 in July to a peak of H'=3.4 in September pre-snow which also indicates the reduction in the dominance of *Microbotryomycetes* and an increase in richness of other species.

Table. 2 The Shannon diversity indices of the samples collected from Mount Rainer. Adapted from (Hotaling et al., 2022)

Location Alpine	**Sample type**	**Shannon**			**Reference**
		Fungi	**Eukarya/Algae**	**Bacteria**	
Mount Rainier-USA	Snow May	3	2.8	2.9	(Hotaling et al., 2022)
Mount Rainier-USA	Snow June	1.8	1.5	2.2	
Mount Rainier-USA	Snow July	1.5	2.4	2.6	
Mount Rainier-USA	Snow August	2.5	2.5	3.6	
Mount Rainier-USA	Pre-snow September	3.4	3.4	4.6	
Mount Rainier-USA	Post-snow September	2.4	2.9	2.5	

Bacterial Diversity

The sequencing of the 16S rRNA gene yielded 4724 bacterial OTUs including all the time points i.e., May to September. The amplicons were aligned against the Silva (v.138) database and clustered at 97% sequence similarity by using the OptiClust algorithm in Mothur.

The taxonomic assignment of OTUs showed that the phyla of Bacteroidetes represented 10-45% (May), 15% (June), peaked at 5-75% (July), and then reduced to 45% (August), 20-25% (September-Pre and post-snow). Whereas Proteobacteria maintained an overall dominance of

20-60% in all the melting duration (May and August) and were most dominant in September pre and post-now conditions (50-55%). Actinobacteria represented up to 20% (May), 10% (June), a slight peak of 10-30% (July), followed by a drop to 10-15% (August) and, 20-25% (Pre-snow and Post-snow in September). July month experienced hikes in abundance values. Among Bacteroidetes, the classes of Ferruginibacter and Solitalea were most abundant and Pseudomonas (Gammaproteobacteria) were abundant in the Proteobacteria phyla.

As per the Shannon indices (**Table. 2**), the first 3 months had no significant differences in bacterial diversity (H'=2.2-2.9), and the months of August and September Pre-Snow had an index of H'=3.6 and H'=4.6 respectively. It indicates a significant change in diversity and it could be a result of snow melting and constant summer temperature which lead to increased microbial activity. The September-post-snow index dropped to H'=2.5, where fresh snowfall and temperature drops reduced or replaced the previous microbial community.

Microbial diversity of the Antarctic samples

For the Antarctic habitat, the study conducted by (Luo et al., 2020) explains the microbial community of snow samples (red and green snow) collected near the freshwater lake Changhu (RS3, RS4, GS5) and a lake beside the Teniente R. Marsh airport (RS1, RS2). The samples were collected at the end of January.

The samples were processed for amplicon sequencing. For eukaryotes, the V4 hypervariable region of the 18S rRNA gene was amplified with the forward 3NDf (5′-GGCAAGTCTGGTGCCAG-3′) and reverse V4_euk_R2 (5′-ACGGTATCTRATCRTCTTCG-3′) primers. For prokaryotes, the V4-V5 hypervariable region of the 16S rRNA gene was amplified with 515F-Y (5′-GTG YCA GCMGCC GCGGTAA-3′) and 926R (5′-CCG YCA ATTYMTTT RAGTTT-3′).

Eukaryotic diversity

The sequencing of the 18S rRNA gene yielded 30,264 reads for each sample. It was aligned against the SILVA database and clustered at 98% sequence similarity. A total of 379 OTUs were generated from the 18S amplicons.

The taxonomic assignment of the OTUs revealed that 29 OTUs were shared among all the samples. The OTUs were designated with 5 phyla i.e., Chlorophyta, Ochrophyta, Fungi, Cercozoa, and others as unclassified. The two most dominant phyla in red snow samples were Chlorophyta (RS2-53%; RS4-88.9%) and Ochrophyta (RS1-30.91%; RS3-12%). The green snow (GS5) was prevalent in Chlorophyta (>78%), Ochrophyta (20.6%), Fungi (0.83%), and a few Cercozoa (0.22%).

At the genera level, RS1, RS2, and RS3 were dominant in *Psuedochlorella*, *Sanguina,* and *Ochromonas* whereas GS5 was mostly dominated by Ulothrix followed by some less abundant *Spumella*, *Chloromonas*, *Pseudochlorella,* and *Chlainomonas*. In the RS4 sample, *Chlainomonas* and *Pseudochlorella* represented 84% and 4%, respectively. The presence of Chlainonomas is representative of high water activity in samples from slush and wet snow (Procházková et al., 2018). *Sanguina*, *Chloromonas,* and *Chlainonomas* are also well known for secondary carotenoid production under stress which is a characteristic of red snow samples (Procházková et al., 2018, 2019; Remias et al., 2013). The dominant phyla of Fungi

were represented by Chytridiomycota (GS5-0.7%; RS3-12.6%), and Basidiomycota (GS5-0.17; RS2-5.9%).

As per the diversity indices **(Table. 3)**, all the samples were low in eukaryotic diversity as observed by the Shannon indices (H'=1.89-2.76) and the sample RS4 was extremely low H'=0.8. As per the Simpson index, all (D=0.11-0.25) but RS4 (D=0.71) had an even distribution of species. As RS4 was highly dominated by the Chlorophyceae family (especially 84% of *Chlainomonas*), it reflected poor diversity values of the Shannon and Simpson indexes. Similarly, the Chao index reflects high proportions of eukaryotes in RS3 (S=101.3) and lowest in RS1 (S=53.42) and RS4 (76). Since the number of OTUs (S_{obs}) in RS1 is 52 and RS4 is 74, it may give lower estimates as per the formula. ($S = S_{obs} + \frac{f_1^2}{2f_2}$)

Table. 3 The Shannon, chao and Simpson diversity indices of the sample collected from Fildes peninsula. Adapted from(Luo et al., 2020)

Location Antarctic	Sample type	Shannon		Simpson		Chao		Reference
		Eukarya (OTUs)	Bacteria (OTUs)	Eukarya	Bacteria	Eukarya	Bacteria	
Fildes Peninsula-Antarctica	Red Snow 1	1.89 (52)	2.47 (95)	0.25	0.11	53.42	107.21	(Luo et al., 2020)
Fildes Peninsula-Antarctica	Red Snow 2	2.39 (82)	2.6 (111)	0.16	0.13	89.5	128.76	
Fildes Peninsula-Antarctica	Red Snow 3	2.76 (101)	2.69 (113)	0.11	0.109	101.3	123.46	
Fildes Peninsula-Antarctica	Red Snow 4	0.8 (74)	3.05 (124)	0.71	0.08	76	130.6	
Fildes Peninsula-Antarctica	Green Snow 5	2.03 (70)	2.21 (84)	0.19	0.21	72.62	92.66	

Bacterial Diversity

The sequencing of the 16S rRNA gene yielded 27,880 sequences for each sample. It was aligned against the SILVA database and clustered at 98% sequence similarity. A total of 527 OTUs were obtained from all the samples.

A total of 33 OTUs were shared among all the 5 samples. The taxonomic assignment identified the most dominant bacterial phyla of Proteobacteria (RS2-36.9%; GS5-71.9%), Bacteroidetes (GS5-25.3%; RS2-53.86%), and Cyanobacteria (GS5-2.61; RS1-15.5%).

Actinobacteria were present in very low abundance (RS2- 0.17%; RS3-0.19%; RS4-1.14% and <10 reads in RS1 and GS5).

The most abundant phyla of Proteobacteria were majorly represented by Betaproteobacteria (RS3-23.98%; GS5-71.2%), Alphaproteobacteria (GS5-0.38%; RS3-20.8%), Gammaproteobacteria (GS5-0.09%; RS3-1.93%) and some rare Deltaproteobacteria (<10 reads). The top 10 abundant OTUs from Betaproteobacteria were classified under the order Burkholderiales where most of these OTUs were classified under the genera *Polaromonas* (72.03% of Betaproteobacteria) followed by *Rhizobacter*, *Actimicrobium*, *Rhodoferax*, *Variovorax*, *Simplicispira*, *Piscinibacter*, *Hydrogenophaga* and some rare genera of *Roseateles*, *Janthinobacterium* and *Achromobacter*.

The second most abundant phyla of Bacteroidetes were very diverse and the majority of it was categorized under the family of Sphingobacteriia (71% of Bacteriodetes), Cytophagia (18.68% of Bacteroidetes) and Flavobacteria (10.31% of Bacteroidetes). Around 67.8% of reads from the Sphingobacteria family were assigned to the genera of *Ferruginibacter* and 93.7% of reads of the Cytophagia family were assigned to the *Flectobacillus* genera. Some genera of *Flavobacterium* and *Chryseobacterium* were found in the family of Flavobacteria. Although the majority of the reads from Cyanobacterial phyla were categorized under the chloroplast category few reads were assigned to the species of uncultured *Cyanobacterium* clone BMPI-24.

As per the Shannon index, most of the samples indicate low bacterial diversity (H'=2.21-2.69) except RS4 (H'=3.05). Similarly, the Simpson index confirms the highest diversity (or more even distribution) in RS4 (D=0.08) sample whereas others exhibited similar even distribution (D=0.109-0.21). Even the Chao index supports the same conclusion that RS4 (S=130.6) has the highest bacterial diversity among all the samples i.e., RS2 (S=128.76), RS3 (S=123.46), RS1 (S=107.21) and GS5 (S=92.66). The percent abundance of taxa in the RS4 sample is not properly indicated by the author but the heatmap plot reflects the values of the diversity indices (Luo et al., 2020).

4. Conclusion

The objective of this deliverable is to provide a general idea about the diversity of the microbial community of snow over the glacier surfaces. By microbial we mean the algal, fungal, and bacterial communities. This report discusses the metrics and methods used for the diversity analyses. The information provided in this study will allow the user to quantify the microbial community and address the relevance and dissimilarities with a statistical approach. It also utilizes an example to describe microbial species or classes found in the snow samples of Arctic, Alpine, and Antarctic habitats. Based on the literature, the phyla of Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria were commonly found in all the snow samples but in varying richness and abundance. Especially the orders associated with the recycling of algal and fungal biomass (Flavobacteriales, Burkholderiales, Cytophagales). In eukaryotes, the genera of *Chloromonas*, and *Chlainomonas* were commonly found under the phyla of Chlorophyta, and some fungal communities of Basidiomycota and Ascomycota were predominantly found in all the snow samples. The abundance of the algal community was restricted to the late melting season when the temperature was stable and the fungal community showed a constant dominance, especially before algal bloom between late winter and early spring.

The diversity estimates are not universal and could vary based on several factors. The Shannon, Simpson, Chao, and Gini indices are frequently used in studies. Utilizing these multiple tests strengthens the credibility of our data. It is recommended to address both the eukaryotic and prokaryotic faction of the microbial community and provide a sufficient statistical comparison with additional information such as the number of reads, OTUs, percent abundance, and at least the dominant genera or family-level taxonomy.

5. References

Aalismail, N. A., Ngugi, D. K., Díaz-Rúa, R., Alam, I., Cusack, M., & Duarte, C. M. (2019). Functional metagenomic analysis of dust-associated microbiomes above the Red Sea. *Scientific Reports*, *9*(1). https://doi.org/10.1038/S41598-019-50194-0

Alsante, A. N., Thornton, D. C. O., & Brooks, S. D. (2021). Ocean Aerobiology. *Frontiers in Microbiology*, *12*, 764178. https://doi.org/10.3389/FMICB.2021.764178/BIBTEX

Amato, P., Hennebelle, R., Magand, O., Sancelme, M., Delort, A. M., Barbante, C., Boutron, C., & Ferrari, C. (2007). Bacterial characterization of the snow cover at Spitzberg, Svalbard. *FEMS Microbiology Ecology*, *59*(2), 255–264. https://doi.org/10.1111/J.1574-6941.2006.00198.X

Amato, P., Joly, M., Besaury, L., Oudart, A., Taib, N., Moné, A. I., Deguillaume, L., Delort, A. M., & Debroas, D. (2017). Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS ONE*, *12*(8). https://doi.org/10.1371/JOURNAL.PONE.0182869

Amato, P., Parazols, M., Sancelme, M., Mailhot, G., Laj, P., & Delort, A. M. (2007). An important oceanic source of micro-organisms for cloud water at the Puy de Dôme (France). *Atmospheric Environment*, *41*(37), 8253–8263. https://doi.org/10.1016/J.ATMOSENV.2007.06.022

Anesio, A. M., & Laybourn-Parry, J. (2012). Glaciers and ice sheets as a biome. *Trends in Ecology and Evolution*, *27*(4), 219–225. https://doi.org/10.1016/j.tree.2011.09.012

Anne Chao. (1984). Nonparametric Estimation of the Number of Classes in a Population. *Scandinavian Journal of Statistics*, *11*(4), 265–270.

Azzoni, R. S., Tagliaferri, I., Franzetti, A., Mayer, C., Lambrecht, A., Compostella, C., Caccianiga, M., Minora, U. F., Garzonio, C. A., Meraldi, E., Smiraglia, C., Diolaiuti, G. A., & Ambrosini, R. (2018). Bacterial diversity in snow from mid-latitude mountain areas: Alps, Eastern Anatolia, Karakoram and Himalaya. *Annals of Glaciology*, *59*(77), 10–20. https://doi.org/10.1017/AOG.2018.18

B Murray, D. O. J. A. M. W. (2012). Ice nucleation by particles immersed in supercooled cloud droplets. *Chem. Soc. Rev.*, *41*, 6519–6554. https://doi.org/10.1039/c2cs35200a

Bastida, F., Eldridge, D. J., García, C., Kenny Png, G., Bardgett, R. D., & Delgado-Baquerizo, M. (2021). Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *The ISME Journal 2021 15:7*, *15*(7), 2081–2091. https://doi.org/10.1038/s41396-021-00906-0

Bradley, J. A., Trivedi, C. B., Winkel, M., Mourot, R., Lutz, S., Larose, C., Keuschnig, C., Doting, E., Halbach, L., Zervas, A., Anesio, A. M., & Benning, L. G. (2023). Active and dormant microorganisms on glacier surfaces. *Geobiology*, *21*(2), 244–261. https://doi.org/10.1111/GBI.12535

Cameron, K. A., Stibal, M., Hawkings, J. R., Mikkelsen, A. B., Telling, J., Kohler, T. J., Gözdereliler, E., Zarsky, J. D., Wadham, J. L., & Jacobsen, C. S. (2017). Meltwater export of prokaryotic cells from the Greenland ice sheet. *Environmental Microbiology*, *19*(2), 524–534. https://doi.org/10.1111/1462-2920.13483

Centurion, V. B., Lacerda-Júnior, G. V., Duarte, A. W. F., Silva, T. R., Silva, L. J., Rosa, L. H., & Oliveira, V. M. (2021). Dynamics of microbial stress responses driven by abiotic changes along a temporal gradient in Deception Island, Maritime Antarctica. *Science of The Total Environment*, *758*, 143671. https://doi.org/10.1016/J.SCITOTENV.2020.143671

Cooper, T. F., Rozen, D. E., & Lenski, R. E. (2003). Parallel changes in gene expression after 20,000 generations of evolution in Escherichiacoli. *Proceedings of the National Academy of Sciences of*

the United States of America, *100*(3), 1072–1077. https://doi.org/10.1073/PNAS.0334340100

Després, V. R., Alex Huffman, J., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M. O., Pöschl, U., & Jaenicke, R. (2012). Primary biological aerosol particles in the atmosphere: a review. *Tellus B: Chemical and Physical Meteorology*, *64*(1). https://doi.org/10.3402/TELLUSB.V64I0.15598

Duan, P., Hu, W., Wu, Z., Bi, K., Zhu, J., & Fu, P. (2023). Ice nucleation activity of airborne pollen: A short review of results from laboratory experiments. *Atmospheric Research*, *285*, 106659. https://doi.org/10.1016/J.ATMOSRES.2023.106659

Eiler, A., Langenheder, S., Bertilsson, S., & Tranvik, L. J. (2003). Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology*, *69*(7), 3701–3709. https://doi.org/10.1128/AEM.69.7.3701-3709.2003

Els, N., Greilinger, M., Reisecker, M., Tignat-Perrier, R., Baumann-Stanzer, K., Kasper-Giebl, A., Sattler, B., & Larose, C. (2020). Comparison of Bacterial and Fungal Composition and Their Chemical Interaction in Free Tropospheric Air and Snow Over an Entire Winter Season at Mount Sonnblick, Austria. *Frontiers in Microbiology*, *11*, 529778. https://doi.org/10.3389/FMICB.2020.00980/BIBTEX

Fay, J. C., & Wittkopp, P. J. (2007). Evaluating the role of natural selection in the evolution of gene regulation. *Heredity 2008 100:2*, *100*(2), 191–199. https://doi.org/10.1038/sj.hdy.6801000

Ferea, T. L., Botstein, D., Brown, P. O., & Rosenzweig, R. F. (1999). Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(17), 9721–9726. https://doi.org/10.1073/PNAS.96.17.9721

Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R., & Raff, R. A. (1988). Molecular phylogeny of the animal kingdom. *Science (New York, N.Y.)*, *239*(4841 Pt 1), 748–753. https://doi.org/10.1126/SCIENCE.3277277

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(3), 626–631. https://doi.org/10.1073/PNAS.0507535103/SUPPL_FILE/07535FIG4.PDF

Gini, C. (1912). *Variabilità e mutabilità: contributo allo studio delle distribuzioni e delle relazioni statistiche. [Fasc. I.]*. Tipogr. di P. Cuppini. https://books.google.fr/books?id=fqjaBPMxB9kC

Harding, T., Jungblut, A. D., Lovejoy, C., & Vincent, W. F. (2011). Microbes in High Arctic Snow and Implications for the Cold Biosphere. *Applied and Environmental Microbiology*, *77*(10), 3234. https://doi.org/10.1128/AEM.02611-10

Hell, K., Edwards, A., Zarsky, J., Podmirseg, S. M., Girdwood, S., Pachebat, J. A., Insam, H., & Sattler, B. (2013). The dynamic bacterial communities of a melting High Arctic glacier snowpack. *The ISME Journal 2013 7:9*, *7*(9), 1814–1826. https://doi.org/10.1038/ismej.2013.51

Hotaling, S., Price, T. L., & Hamilton, T. L. (2022). Summer Dynamics of Microbial Diversity on a Mountain Glacier. *MSphere*, *7*(6). https://doi.org/10.1128/MSPHERE.00503-22/ASSET/C9E73BA1-1F0A-49BD-AFF5-91DC630FCE73/ASSETS/IMAGES/MEDIUM/MSPHERE.00503-22-F002.GIF

Hugerth, L. W., Muller, E. E. L., Hu, Y. O. O., Lebrun, L. A. M., Roume, H., Lundin, D., Wilmes, P., & Andersson, A. F. (2014). Systematic Design of 18S rRNA Gene Primers for Determining Eukaryotic Diversity in Microbial Consortia. *PLoS ONE*, *9*(4), 95567. https://doi.org/10.1371/JOURNAL.PONE.0095567

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S.

R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications 2019 10:1*, *10*(1), 1–11. https://doi.org/10.1038/s41467-019-13036-1

Lappan, R., Thakar, J., Molares Moncayo, L., Besser, A., Bradley, J. A., Goordial, J., Trembath-Reichert, E., & Greening, C. (2024). The atmosphere: a transport medium or an active microbial ecosystem? *The ISME Journal*, *18*(1), 92. https://doi.org/10.1093/ISMEJO/WRAE092

Larose, C., Dommergue, A., & Vogel, T. M. (2013a). The Dynamic Arctic Snow Pack: An Unexplored Environment for Microbial Diversity and Activity. *Biology 2013, Vol. 2, Pages 317-330*, *2*(1), 317–330. https://doi.org/10.3390/BIOLOGY2010317

Larose, C., Dommergue, A., & Vogel, T. M. (2013b). The Dynamic Arctic Snow Pack: An Unexplored Environment for Microbial Diversity and Activity. *Biology 2013, Vol. 2, Pages 317-330*, *2*(1), 317–330. https://doi.org/10.3390/BIOLOGY2010317

Le Gall, T., Darlu, P., Escobar-Páramo, P., Picard, B., & Denamur, E. (2005). Selection-driven transcriptome polymorphism in Escherichia coli/Shigella species. *Genome Research*, *15*(2), 260–268. https://doi.org/10.1101/gr.2405905

Lee, J., Kim, H. S., Jo, H. Y., & Kwon, M. J. (2021). Revisiting soil bacterial counting methods: Optimal soil storage and pretreatment methods and comparison of culture-dependent and -independent methods. *PLoS ONE*, *16*(2). https://doi.org/10.1371/JOURNAL.PONE.0246142

Locey, K. J., & Lennon, J. T. (2016). Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(21), 5970–5975. https://doi.org/10.1073/PNAS.1521291113/SUPPL_FILE/PNAS.1521291113.SAPP.PDF

Lopatina, A., Krylenkov, V., & Severinov, K. (2013). Activity and bacterial diversity of snow around Russian Antarctic stations. *Research in Microbiology*, *164*(9), 949–958. https://doi.org/10.1016/J.RESMIC.2013.08.005

Ludwig, J. A., & Reynolds, J. F. (1988). *Statistical Ecology: A Primer in Methods and Computing - John A. Ludwig, James F. Reynolds* (pp. 107–202).

Luo, W., Ding, H., Li, H., Ji, Z., Huang, K., Zhao, W., Yu, Y., & Zeng, Y. (2020). Molecular diversity of the microbial community in coloured snow from the Fildes Peninsula (King George Island, Maritime Antarctica). *Polar Biology*, *43*(9), 1391–1405. https://doi.org/10.1007/S00300-020-02716-0/METRICS

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2015). Microbial diversity on icelandic glaciers and ice caps. *Frontiers in Microbiology*, *6*(MAR), 136619. https://doi.org/10.3389/FMICB.2015.00307/ABSTRACT

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2017a). Linking microbial diversity and functionality of arctic glacial surface habitats. *Environmental Microbiology*, *19*(2), 551–565. https://doi.org/10.1111/1462-2920.13494

Lutz, S., Anesio, A. M., Edwards, A., & Benning, L. G. (2017b). Linking microbial diversity and functionality of arctic glacial surface habitats. *Environmental Microbiology*, *19*(2), 551–565. https://doi.org/10.1111/1462-2920.13494

Maccario, L., Carpenter, S. D., Deming, J. W., Vogel, T. M., & Larose, C. (2019). Sources and selection of snow-specific microbial communities in a Greenlandic sea ice snow cover. *Scientific Reports 2019 9:1*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-38744-y

Malard, L. A., Šabacká, M., Magiopoulos, I., Mowlem, M., Hodson, A., Tranter, M., Siegert, M. J., & Pearce, D. A. (2019a). Spatial variability of Antarctic surface snow bacterial communities. *Frontiers in Microbiology*, *10*(MAR), 431749. https://doi.org/10.3389/FMICB.2019.00461/BIBTEX

Malard, L. A., Šabacká, M., Magiopoulos, I., Mowlem, M., Hodson, A., Tranter, M., Siegert, M. J., & Pearce, D. A. (2019b). Spatial variability of Antarctic surface snow bacterial communities. *Frontiers in Microbiology*, *10*(MAR). https://doi.org/10.3389/FMICB.2019.00461/PDF

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S., Øvreås, L., Reysenbach, A. L., Smith, V. H., & Staley, J. T. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology 2006 4:2*, *4*(2), 102–112. https://doi.org/10.1038/NRMICRO1341

Mayol, E., Arrieta, J. M., Jiménez, M. A., Martínez-Asensio, A., Garcias-Bonet, N., Dachs, J., González-Gaya, B., Royer, S. J., Benítez-Barrios, V. M., Fraile-Nuez, E., & Duarte, C. M. (2017a). Long-range transport of airborne microbes over the global tropical and subtropical ocean. *Nature Communications*, *8*(1). https://doi.org/10.1038/S41467-017-00110-9

Mayol, E., Arrieta, J. M., Jiménez, M. A., Martínez-Asensio, A., Garcias-Bonet, N., Dachs, J., González-Gaya, B., Royer, S. J., Benítez-Barrios, V. M., Fraile-Nuez, E., & Duarte, C. M. (2017b). Long-range transport of airborne microbes over the global tropical and subtropical ocean. *Nature Communications*, *8*(1). https://doi.org/10.1038/S41467-017-00110-9

Mayol, E., Jiménez, M. A., Herndl, G. J., Duarte, C. M., & Arrieta, J. M. (2014). Resolving the abundance and air-sea fluxes of airborne microorganisms in the North Atlantic Ocean. *Frontiers in Microbiology*, *5*(OCT). https://doi.org/10.3389/FMICB.2014.00557

Oró, J., Miller, S. L., Lazcano, A., Oró, J., Miller, S. L., & Lazcano, A. (1990). The Origin and Early Evolution of Life on Earth. *AREPS*, *18*(1), 317. https://doi.org/10.1146/ANNUREV.EA.18.050190.001533

Procházková, L., Procházkov, P., Procházková, P., Leya, T., Kří, H., Křížkov, K., Křížková, K., Nedbalová, L., & Nedbalová, N. (2019). Sanguina nivaloides and Sanguina aurantia gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognised algae causing red and orange snow. *FEMS Microbiology Ecology*, *95*(6). https://doi.org/10.1093/FEMSEC/FIZ064

Procházková, L., Remias, D., Holzinger, A., Řezanka, T., & Nedbalová, L. (2018). Ecophysiological and morphological comparison of two populations of Chlainomonas sp. (Chlorophyta) causing red snow on ice-covered lakes in the High Tatras and Austrian Alps. *European Journal of Phycology*, *53*(2), 230–243. https://doi.org/10.1080/09670262.2018.1426789

Remias, D., Wastian, H., Lütz, C., & Leya, T. (2013). Insights into the biology and phylogeny of Chloromonas polyptera (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarctic Science*, *25*(5), 648–656. https://doi.org/10.1017/S0954102013000060

Rifkin, S. A., Houle, D., Kim, J., & White, K. P. (2005). A mutation accumulation assay reveals a broad capacity for rapid evolution of gene expression. *Nature*, *438*(7065), 220–223. https://doi.org/10.1038/NATURE04114

Roeters, S. J., Golbek, T. W., Bregnhøj, M., Drace, T., Alamdari, S., Roseboom, W., Kramer, G., Šantl-Temkiv, T., Finster, K., Pfaendtner, J., Woutersen, S., Boesen, T., & Weidner, T. (2021). Ice-nucleating proteins are activated by low temperatures to control the structure of interfacial water. *Nature Communications 2021 12:1*, *12*(1), 1–9. https://doi.org/10.1038/s41467-021-21349-3

Sánchez-Parra, B., Núñez, A., García, A. M., Campoy, P., & Moreno, D. A. (2021). Distribution of airborne pollen, fungi and bacteria at four altitudes using high-throughput DNA sequencing. *Atmospheric Research*, *249*, 105306. https://doi.org/10.1016/J.ATMOSRES.2020.105306

Šantl-Temkiv, T., Gosewinkel, U., Starnawski, P., Lever, M., & Finster, K. (2018). Aeolian dispersal of bacteria in southwest Greenland: their sources, abundance, diversity and physiological states.

FEMS Microbiology Ecology, *94*(4). https://doi.org/10.1093/FEMSEC/FIY031

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K. D., Bai, F. Y., Barreto, R. W., Begerow, D., Bergeron, M. J., Blackwell, M., … Schindel, D. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(16), 6241–6246. https://doi.org/10.1073/PNAS.1117018109/-/DCSUPPLEMENTAL

Schuler, C. G., & Mikucki, J. A. (2023). Microbial ecology and activity of snow algae within a Pacific Northwest snowpack. *Arctic, Antarctic, and Alpine Research*, *55*(1). https://doi.org/10.1080/15230430.2023.2233785

Simpson, E. H. (1949). Measurement of Diversity. *Nature 1949 163:4148*, *163*(4148), 688–688. https://doi.org/10.1038/163688a0

Smith, D. J., Timonen, H. J., Jaffe, D. A., Griffin, D. W., Birmele, M. N., Perry, K. D., Ward, P. D., & Roberts, M. S. (2013). Intercontinental dispersal of bacteria and archaea by transpacific winds. *Applied and Environmental Microbiology*, *79*(4), 1134–1139. https://doi.org/10.1128/AEM.03029-12/SUPPL_FILE/ZAM999104100SO1.PDF

Song, S., & Wood, T. K. (2021). "Viable but non-culturable cells" are dead. *Environmental Microbiology*, *23*(5), 2335–2338. https://doi.org/10.1111/1462-2920.15463

Stibal, M., Šabacká, M., & Kaštovská, K. (2006). Microbial communities on glacier surfaces in Svalbard: Impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microbial Ecology*, *52*(4), 644–654. https://doi.org/10.1007/S00248-006-9083-3/METRICS

Stoppiello, G. A., Coleine, C., Moeller, R., Ripa, C., Billi, D., & Selbmann, L. (2023). Seasonality Is the Main Determinant of Microbial Diversity Associated to Snow/Ice around Concordia Station on the Antarctic Polar Plateau. *Biology*, *12*(9), 1193. https://doi.org/10.3390/BIOLOGY12091193/S1

Tignat-Perrier, R., Thollot, A., Magand, O., M. Vogel, T., & Larose, C. (2020). Microbial functional signature in the atmospheric boundary layer. *Biogeosciences*, *17*(23), 6081–6095. https://doi.org/10.5194/BG-17-6081-2020

Uetake, J., Kohshima, S., Nakazawa, F., Suzuki, K., Kohno, M., Kameda, T., Arkhipov, S., & Fujii, Y. (2006). Biological ice-core analysis of Sofiyskiy glacier in the Russian Altai. *Annals of Glaciology*, *43*, 70–78. https://doi.org/10.3189/172756406781811925

Usyk, M., Zolnik, C. P., Patel, H., Levi, M. H., & Burk, R. D. (2017). Novel ITS1 Fungal Primers for Characterization of the Mycobiome. *MSphere*, *2*(6). https://doi.org/10.1128/MSPHERE.00488-17

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(12), 6578–6583. https://doi.org/10.1073/PNAS.95.12.6578

Whittaker, R. H. (1972). EVOLUTION AND MEASUREMENT OF SPECIES DIVERSITY. *TAXON*, *21*(2–3), 213–251. https://doi.org/10.2307/1218190

Winkel, M., Trivedi, C. B., Mourot, R., Bradley, J. A., Vieth-Hillebrand, A., & Benning, L. G. (2022). Seasonality of Glacial Snow and Ice Microbial Communities. *Frontiers in Microbiology*, *13*, 876848. https://doi.org/10.3389/FMICB.2022.876848/BIBTEX

Woese, C. R., & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*,

74(11), 5088. https://doi.org/10.1073/PNAS.74.11.5088

Wolber, P. K. (1993). Bacterial ice nucleation. *Advances in Microbial Physiology*, *34*, 203–237. https://doi.org/10.1016/S0065-2911(08)60030-2

Yang, G. L., Hou, S. G., Le Baoge, R., Li, Z. G., Xu, H., Liu, Y. P., Du, W. T., & Liu, Y. Q. (2016). Differences in Bacterial Diversity and Communities Between Glacial Snow and Glacial Soil on the Chongce Ice Cap, West Kunlun Mountains. *Scientific Reports 2016 6:1*, *6*(1), 1–8. https://doi.org/10.1038/srep36548

Zhang, Y., & Steiner, A. L. (2022). Projected climate-driven changes in pollen emission season length and magnitude over the continental United States. *Nature Communications 2022 13:1*, *13*(1), 1–10. https://doi.org/10.1038/s41467-022-28764-0