The University of South Bohemia in České Budějovice
Faculty of Science

Microbial community composition of Arctic cryoconite holes

Bachelor thesis

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ANNOTATION

Cryoconite holes on glaciers are small holes filled with water, and microorganisms, and they are important habitats found on the surface of many glaciers worldwide. However, little research has been conducted on how cryoconite holes in close proximity and distributed over the same glacier differ in their microbial composition. This thesis focuses on the microbial composition of Arctic cryoconite holes from Svalbard glaciers situated in close proximity. A particular focus is placed on the comparison of their composition to already existing literature.

DECLARATION

I declare that I am the author of this qualification thesis and that in writing it, I have used the sources and literature displayed in the list of used sources only.

České Budějovice, 10th of August 2023.

……

Lara Marie Höscheler

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I am also grateful to my family, partner, and friends for all their emotional support and patience with me.

ABSTRACT

Cryoconite holes are small holes on glaciers filled with water and microorganisms. They are known for being habitats for diverse microbial communities on glacial surfaces. This study aimed to investigate the taxonomic composition and diversity of microbial communities within cryoconite holes across different glacial sites in Svalbard. In total, 43 samples from individual cryoconite holes from 10 different glacier sites were provided for this thesis. DNA isolation and polymerase chain reaction (PCR) targeting the 16S rRNA gene were performed, followed by the verification of gel electrophoresis. The DNA samples were sent for Illumina sequencing targeting the 16S rRNA gene. For the downstream analysis, data from 14 cryoconite samples were provided, and Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes were identified as the predominant phyla, collectively accounting for 86.7 % of the samples. This finding aligns with previous research, where these phyla were commonly reported. The remaining phyla, including Verrucomicrobia, Firmicutes, and Planctomycetes, comprised the remaining 13.3 %, with genera below 1 % abundance grouped as "Other." Moreover, an overall similarity in microbial compositions among samples, except for one site, was observed. This finding aligns with existing literature as the sample sites are in relatively close proximity, supporting the view of uniform communities in the Arctic due to open and connected cryoconite holes. Overall, this research provides insights into the taxonomic diversity and composition of microbial communities within cryoconite holes. Further studies should employ whole-genome sequencing and functional metagenomic analyses to gain a comprehensive understanding of microbial diversity, functional roles, and potential impacts on the glacial ecosystem.

Keywords: Arctic cryoconite holes, 16S rRNA gene, microbial composition, Svalbard, taxonomic composition

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1. INTRODUCTION

The cryosphere resulting from *cryo*, the Greek word for cold, describes an environment where water is frozen periodically or for an extended period (Laybourn-Parry et al., 2012). To this environment belong glaciers, ice caps and sheets, snow cover, sea ice, lake ice covers, frozen soil, and permafrost (Benn & Evans, 2010; Laybourn-Parry et al., 2012). Glaciers and ice sheets have been certified as biomes, entirely controlled by microbial processes (Anesio et al., 2017).

1.1. Glaciers

Glacial ice encompasses approximately 10 % of the Earth's surface (Anesio & Laybourn-Parry, 2012; Hornberger & Winter, 2009; Paterson, 1994). Almost 7 % of the ice is in the Antarctic and Greenland Ice Sheets (Benn & Evans, 2010). Ice caps, ice fields, and glaciers in alpine regions or high latitudes worldwide are responsible for the residual 3 %. Due to topography, temperature, and precipitation, they are more widespread in the northern hemisphere, especially in the Arctic Ocean basin, Norway, Alaska, the Alps, and the Himalayas. Andes, New Zealand, and sub-Antarctic islands are examples of the southern hemisphere. Glaciers become more extensive with proximity to the poles because solar irradiation becomes weaker to melt snow and ice with increasing latitude. Additionally, glaciers become more significant with increasing altitude due to colder temperatures with decreasing air density.

Fluctuations in temperature, precipitation, and other factors influence the growth and decrease of glaciers. Furthermore, glacial ice constantly exchanges mass and energy with other significant Earth systems, including the hydrosphere, atmosphere, biosphere, and lithosphere. Therefore, climate change plays an essential role in the existence of glacial ice and its influence on Earth's systems.

1.1.1. The formation of glaciers

Two processes lead to the formation of a glacier: the accumulation and the ablation, as illustrated in Fig. 1 (Laybourn-Parry et al., 2012; Paterson, 1994).

In the accumulation process, which occurs at or near the glacier's surface, glaciers acquire their mass primarily through snowfall and ice formed from refrozen snow melt, rain, and fog. Through firnification, snowflakes are progressively transformed into annual layers of glacier ice over several years.

Other factors that affect accumulation are the altitudinal gradients of the glacier and proximity to the oceans (Reijmer & van den Broeke, 2003). The rates of annual accumulation increase with increasing altitude and greater proximity to the sea. On the contrary, with reduced proximity to the coast, precipitation decreases on continental-scale ice masses, leading to a maximum accumulation zone at their margin or to no glacier formation (Benn & Evans, 2010).

Melting, sublimation, and iceberg production are the reasons for ice mass loss, called ablation, occurring at the glacier's surface or terminus (Paterson, 1994).
Ablation increases with decreasing altitude, causing a decrease in nutrients, debris, and the residence time of water (Laybourn-Parry et al., 2012). The ice surface of continental-scale ice sheets is influenced the least by air temperatures at the margin of the glacier, which has the highest ablation rate. Another critical factor for continental-scale ice sheets is the process of calving, referring to the breaking off of mass at the end of the ice flow as an iceberg into the ocean (Anesio & Laybourn-Parry, 2012).

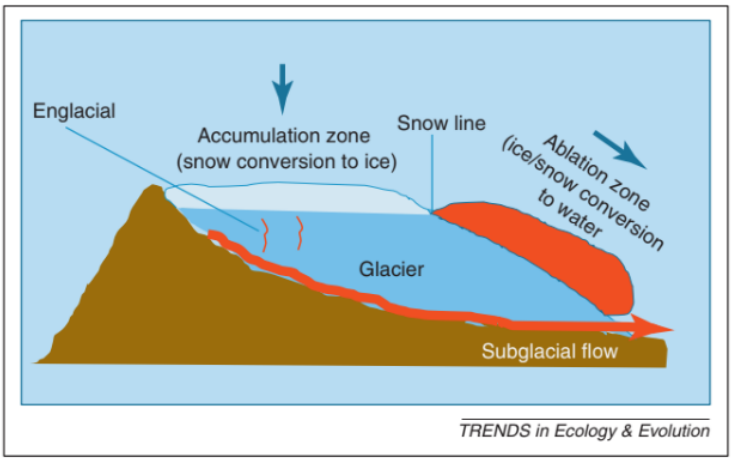


Fig. 1. Simplified picture of a glacier's accumulation and ablation zone divided by the equilibrium line (snow line) (Anesio & Laybourn-Parry, 2012).

The net mass balance of glacier ice depends on the difference in accumulation and ablation (Benn & Evans, 2010). When the accumulation exceeds the ablation, a glacier grows; when the gains are smaller than the losses, the glacier withdraws. Thus, glaciers consist of two zones divided by the equilibrium line: an accumulation zone where the input is higher than the output by ablation and an ablation zone, respectively, where the output is higher than the input. Depending on the local and regional climate and topography of the glacier, the altitude of the equilibrium line changes.

1.1.2. Classification of glaciers

Since the rapidity of snow transformation into ice depends on the environment's temperature, not all glaciers have a melting point at a rate of 0.07 °C per 100 m (Paterson, 1994). Thus, glaciers are subdivided into the following thermal regimes: cold ice with a temperature below the melting point, warm ice with a temperature around the pressure melting point, and polythermal, where the pressure melting increases with increasing depth.

Warm ice on the glacier bed causes glaciers to flow, which is termed crevassing (Laybourn-Parry et al., 2012). Furthermore, cold-based glaciers undertake less crevassing than warm-based valley glaciers due to higher ice deformation and basal sliding (Benn & Evans, 2010). In contrast, the crevassing of polythermal-based glaciers with their cold ice in their inner border lies in between the rate of cold-based and warm-based glaciers (Sugden & John, 1984). Crevassing is essential for the glacial biome because it improves the mobilization of resources, such as water and nutrients, and organisms between the surface and bottom of the glacier, thereby connecting the supraglacial and subglacial ecosystems (Hodson et al., 2008). However, this transport occurs only in the presence of meltwater because meltwater permeates the snow and, hence, transfers microbes and nutrients (Wadham et al., 2006).

1.1.3. Microbial life on glaciers

Due to the following abiotic factors, the biological activity of microorganisms in glacial environments is limited: the seasonal and daytime changes in climate (microbial activity stagnates at temperatures (Laybourn-Parry et al., 2012) below five degrees Celsius, and microbial activity starts during the summer season (Anesio & Laybourn-Parry, 2012)); the lack of light and oxygen for photosynthesis (Price, 2000); the thickness of glaciers and its resulting pressure; the acidic or highly concentrated environment due to interstitial freezing (Mader et al., 2006; Price, 2000); and the nearly impermeable ice surface for nutrients, microorganisms, and water (Lliboutry, 1996).

Water exists in the glacier biome as fine channels, water film, veins, and pockets (Paterson, 1994). Nutrients are obtained from gasses, precipitation, and debris (Hodson et al., 2008).

The glacial biome consists of supraglacial, englacial, and subglacial environments, as illustrated in Fig. 2 (Hodson et al., 2008). The supraglacial ecosystem revolves around the glacier surface, including snow, supraglacial streams, ponds, moraines, and cryoconite holes; the subglacial revolves around the ice-bed interface with its basal ice and subglacial lakes (Hodson et al., 2008).

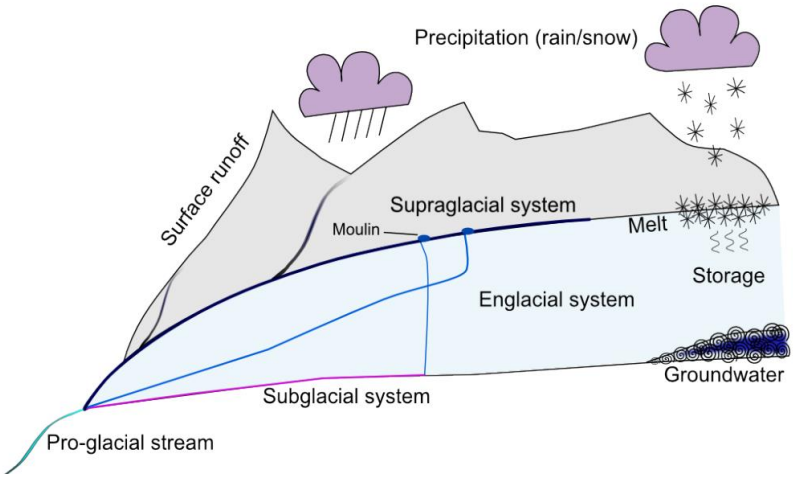


Fig. 2. Simplified picture of the three different glacial zones within the glacial biome: supraglacial, englacial, and subglacial (Ekblom Johansson, 2013).

Psychrotolerant or psychrotrophic as well as psychrophilic microorganisms inhabit the glacial biome and endure dehydration, extreme temperatures, and frequent lack of nutrients (Anesio & Laybourn-Parry, 2012). To prevent cell damage, they adapt by producing cold-active enzymes, anti-freezing proteins, and exopolymeric compounds (Gilbert et al., 2005, as cited in Anesio & Laybourn-Parry, 2012; Margesin & Miteva, 2011, as cited in Anesio & Laybourn-Parry, 2012; Siddiqui & Cavicchioli, 2006, as cited in Anesio & Laybourn-Parry, 2012).

Bacteria, algae, phytoflagellates, fungi, viruses, rotifers, tardigrades, and diatoms dominate the supraglacial ecosystem; aerobic and anaerobic bacteria, as well as viruses, dominate the subglacial ecosystem (Hodson et al., 2008).

The most biologically active environments are melting glaciers and snow surfaces due to presence of cryoconite and sunlight and glacier beds due to subglacial sediments and pressure melting (Laybourn-Parry et al., 2012). The englacial vein network creates an extreme environment through acidic pH, high pressure, and high ionic strength. Hodson et al. (2008) elucidate that microbial activity in the englacial ecosystem is insignificant at the glacier scale due to the aforementioned factors.

Subglacial habitats consist of low or no oxygen, no light, and high levels of carbon, and they are inhabited by psychrophilic bacteria, such as aerobic chemoheterotrophs, anaerobic nitrate reducers, sulphate reducers, methanogens (Skidmore et al., 2000), and fungi (Anesio & Laybourn-Parry, 2012). Subglacial lake systems exist primarily beneath the Antarctic ice sheet (Wright & Siegert, 2011). Until recently, limited research had been conducted on the microbial composition in these habitats due to the thick overlying ice layers. However, a recent research conducted on two subglacial lakes beneath the Vatnajökull ice cap in Iceland sheds light on the microbial composition of subglacial environments (see Vannier et al. (2023)).

1.2. Cryoconite holes

1.2.1. Development of cryoconite holes

Cryoconite originates from the combination of the Greek words *cryo*, cold, and *kónis*, dust (Rozwalak et al., 2022) and is the main component of debris in the middle section of glaciers (Hodson et al., 2008). It is developed in the process where small particles transported by wind are combined with microbial threads and their secretions (Hodson et al., 2008; Takeuchi et al., 2010) to oval structures called cryoconite granules (Rozwalak et al., 2022).

This process starts with debris and microorganisms, such as snow, ice algae, and Cyanobacteria, which are distributed on the surface of the snow and ice by wind (Laybourn-Parry et al., 2012), thereby darkening it (Anesio et al., 2017). It is followed by melting, causing a phase of water surrounding the particle (Bøggild et al., 2010), leading to cell-mineral and mineral-mineral interactions that induce a biofilm and extracellular polymeric substances (EPS) (Hodson et al., 2008; Langford et al., 2010), which consequently leads to the development of cryoconite granules (Laybourn-Parry et al., 2012).

Cryoconite holes differ in size from millimeters to tens of centimeters. They are cylindrical melt pools filled with water and sediment (Fountain et al., 2004), resulting from a process called biocryomorphology (Cook et al., 2015). Biocryomorphology occurs when cryoconite is melted into ice by solar heat because the particles and autochthonous as well as allochthonous organic matter are darker than their surroundings, reducing the albedo of cryoconite (Rozwalak et al., 2022), which is the absorption of solar radiation (Fountain et al., 2004).

Cryoconite holes develop on melting glaciers (Laybourn-Parry et al., 2012), in sea ice, lake ice (Fountain et al., 2004), and on ice sheets (Box & Ski, 2007). They cover approximately 4 to 6 % of the glacier (Fountain et al., 2004) and are mainly located in the weathering crust due to their permeability and sufficient cell-debris interaction during the summer season (Laybourn-Parry et al., 2012).

1.2.2. Different types of cryoconite holes

Three different types of cryoconite holes are distinguished: open holes, submerged holes, and closed holes, as illustrated in Fig. 3 (Hodson et al., 2008). They differ in shape concerning their location.

In the Antarctic, they are covered by ice, unable to interact with the atmosphere (Laybourn-Parry et al., 2012). However, during extremely warm seasons, their ice lid may melt (Foreman et al., 2007). Approximately 50 % of covered cryoconite holes are entirely isolated, the other half connected to water under the ice surface (Bagshaw et al., 2012; Fountain et al., 2004).

Whereas in the Arctic and alpine environments, cryoconite holes are open and in contact with the atmosphere (Laybourn-Parry et al., 2012), and they encounter frequent flushing by stream flow (Bagshaw et al., 2012). Compared to Antarctic cryoconite holes, cryoconite holes in the Arctic are closer to the state of balance regarding carbon dioxide (Hodson et al., 2008). Furthermore, they experience interference with meltwater due to the lack of an ice cap, which influences plankton and sediment surface communities (Laybourn-Parry et al., 2012).

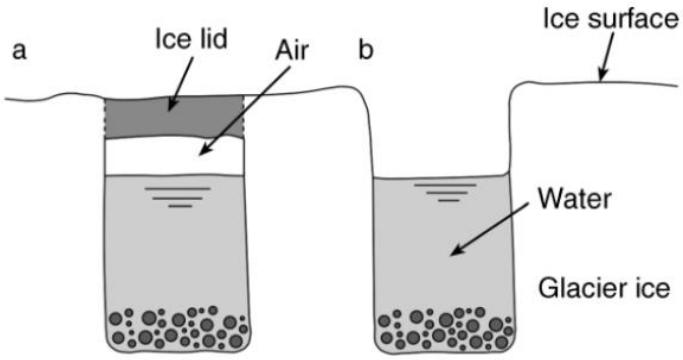


Fig. 3. Schematic picture of a cryoconite hole, illustrating (a) closed and (b) open one (Hodson et al., 2008).

1.2.3. Life in cryoconite holes

Cryoconite holes create a diverse environment compared to supraglacial habitats (Anesio et al., 2017) because microbial growth influences the size of cryoconite granules and, thus, the composition of the community (Uetake et al., 2019).

Another factor which plays a role in the community composition is the cryoconite which is immersed in water only above freezing temperature inside the cryoconite holes (Anesio et al., 2017). Various microorganisms, such as algae, prokaryotic photoautotrophs, heterotrophs, and viruses, inhabit cryoconite granules (Anesio et al., 2017). The community composition differs according to whether the cryoconite holes are located in the Arctic or Antarctica (Laybourn-Parry et al., 2012). In Antarctica, community compositions of different cryoconite holes are more individual and diverse due to their ice lid (Wharton et al., 1981). Whereas in the Arctic, more uniform communities are found because of the open and connected cryoconite holes (Hodson et al., 2008).

However, each cryoconite hole has a unique microbial composition (Cameron et al., 2012). The fauna of cryoconite holes is more similar to its surrounding aquatic ecosystem than to the soil's ecosystems (Porazinska et al., 2004). Furthermore, the microbial composition of cryoconite holes is different from ice-marginal ecosystems (Edwards et al., 2013). Most discovered species that inhabit cryoconite holes are cosmopolitan; few are endemic (Zawierucha et al., 2015). However, according to Zawierucha et al. (2015), this observation may be due to random and insufficient sampling of cryoconite holes, leading to an underestimation of true diversity.

Cyanobacteria (Anesio & Laybourn-Parry, 2012; Anesio et al., 2017), particularly the filamentous *Phormidesmis priestleyi*, are the leading primary producers in polar and alpine regions (Chrismas et al., 2015) and the key factor for the production of cryoconite granules (Uetake et al., 2016). They release EPS, which contain proteins, lipids, polysaccharides, and other secondary metabolites, responsible for the development of cryoconite and the stabilization of microbial films, creating a stabilized habitat (Anesio et al., 2017). EPS is also useful for others as a cryoprotectant protecting the organism from cell membrane disruption due to frigid conditions (Tamaru et al., 2005) and as a resistance to intense ultraviolet radiation (Pereira et al., 2009).

Gokul et al. (2016) state that although Cyanobacteria play a vital role in the supraglacial environment, they are scarcely present. Instead Proteobacteria are the most dominant microorganisms (Anesio et al., 2017).
According to Kaczmarek et al. (2015), 370 taxa without invertebrates occur worldwide in cryoconite holes, but most have not been identified into the species level. Approximately 38 % were identified: 39 species or subspecies of bacteria and Archaea, 11 fungi, 17 Cyanobacteria, 62 algae, and 13 Protista.

Cryoconite holes worldwide are inhabited by five phyla of invertebrates (Rotifera, Annelida, Tardigrada, Nematoda, and Arthropoda), with 25 taxa acting as higher-level consumers or predators in the glacial biome (Zawierucha et al., 2015).
To sustain the extreme conditions in the glacial biome, tardigrades and rotifers enter anabiosis. Anabiosis is a condition in which the organisms can either stop or drastically reduce their activity (Uzunova-Doneva & Donev, 2004-2005) or produce highly resistant forms, for instance, dormant eggs (Zawierucha et al., 2015). When more favorable conditions reoccur, their microbial activity returns to normal, and reproduction begins rapidly.
Rotifera and Tardigrada occur in both polar regions, Nematoda only in Antarctica (Mueller et al., 2001, as cited in Zawierucha et al., 2015).

1.3. Svalbard

The name Svalbard is derived from Old Norse and means ‘Cold Coast’ (The Editors of Encyclopaedia Britannica, 2020). It comprises an area of approximately 39 000 square km and several islands in the Arctic Ocean, northwest of the Barents Shelf (Fig. 4) (Harland, 1997). As part of Norway, the archipelago lies between 10 ° to 35 °E longitude and 74 ° to 81 °N latitude (The Editors of Encyclopaedia Britannica, 2020). Svalbard is comprised of nine main islands: the largest island Spitsbergen, Nordaustlandet, Barentsøya, Edgeøya, Prins Karls Forland, Storøya, Kong Karls Land, Hopen, and Bjørnøya (Harland, 1997). Two sources of ocean water are responsible for the climate in Svalbard: the warm West Spitsbergen Current, water flowing from the Gulf Stream north, and the cold East Spitsbergen Current, water flowing southwest around the archipelago.

Annual precipitation rates amount to 10 mm on the east coast and approximately 400 mm, the highest being 1 000 mm on the west coast, and consist mainly of snow or rain in the summer season.

Temperatures range from 4 °C to 5 °C in summer and about -12 °C in winter, to -20 °C in the west. Glacial ice dominates around 60 % of Svalbard; the most common are ice caps, ice fields, outlet glaciers, and ice streams (Hagen et al., 1993). Most of their ablation zones belong to cold-based glaciers, and their accumulation zones to warm-based glaciers.

Fig. 4. Location of Svalbard, marked in orange (The Editors of Encyclopaedia Britannica, 2020).

Säwström et al. (2002), as cited in Laybourn-Parry et al. (2012), were pioneers in conducting biological studies of cryoconite holes in Svalbard. To date, significant research has been conducted to explore the microbial diversity of cryoconite holes (see, e.g., Kaczmarek et al., 2015; Zawierucha et al., 2015). They have revealed a comprehensive list of invertebrates and microorganisms that inhabit cryoconite holes. However, it has not yet been established whether the microbial composition of cryoconite holes is influenced by their geographical location (such as margin, near the coast, or interior of the glacier) on the same glacier, particularly in Svalbard. Therefore, in this study, Svalbard was previously chosen from the research team due to the following reasons:

- Average yearly temperatures in the region are close to 0 °C.
- Winter precipitation leads to substantial snow accumulation.
- Temperatures in the remaining seasons are not high enough to completely melt the snow accumulated during the winter season.

Moreover, to understand microbial diversity comprehensively, we employed high-throughput sequencing targeting the 16S rRNA gene, enabling us to uncover the unique microbial communities within the cryoconite holes. Additionally, we compared our results with previous investigations of the cryoconite communities (Anesio et al., 2017; Chrismas et al., 2015; Edwards et al., 2013; Gokul et al., 2016; Millar et al., 2021; Rozwalak et al., 2022; Uetake et al., 2016).

2. AIM OF THE WORK

1. To isolate DNA from selected glacial biotopes from different parts of Svalbard.
2. To use the data to determine the taxonomic composition of microbial communities.
3. To compare the obtained data with data from genetic databases and literature.
4. To characterize microbial communities based on their taxonomy and functional role in the glacier ecosystem.

3. MATERIALS AND METHODS

3.1. Sampling sites and sample collection

The sample collection was carried out by Mrs. Marie Šabacká and her team, and the following samples were provided for this bachelor's thesis:
A total of 43 individual cryoconite holes were sampled from 10 different sites (as shown in Table 1).

These samples were gathered during the regional summer melt season of the years 2017 and 2018, and the sample sites were selected based on their location on the glaciers, ranging from 2 km from the terminus to inland.

The samples were collected in triplicate in a sterile manner from the surface of the glaciers.

Subsequently, all the samples were immediately frozen after fieldwork (within a few hours) and transported while frozen to the home laboratory at the University of South Bohemia, where they were kept frozen at a temperature of -20 ℃ until further analysis.

Table 1: Summary of the samples: sample site, date and location of collection, and number of provided samples. The exact location of the sample sites were determined with the website TopoSvalbard by the Norwegian Polar Institute.

Sample site	Date	Location	No. of samples
Hansbreen	-	77.1 °N, 15.6 °E	1
Sefströmbreen	02/08/17	78.7 °N, 13.9 °E	1
Longyearbreen	26/10/17	78.2 °N, 15.5 °E	1
Nordbreen	27/04/18	79.6 °N, 16.0 °E	3
Franklinbreane	17/07/18	80.1 °N, 19.6 °E	9
Sørbreen	24/07/18	79.5 °N, 16.1 °E	16
Lingbreen	25/07/18	79.8 °N, 12.3 °E	9
Torellbreen	29/07/18	77.2 °N, 15.0 °E	1
Hørbyebreen	14/08/18	78.8 °N, 16.3 °E	1
Nordenskiöldbreen	20/08/18	78.7 °N, 17.2 °E	1

3.2. DNA isolation and measurement

DNA isolation is a crucial step in identifying the various microorganisms present in each cryoconite sample. The genomic DNA was extracted using the state-of-the-art Quick-DNA™ Fecal/Soil Miniprep Kit (Zymo Research, CatD6010).

Each sample underwent the following procedure:
Initially, the samples were thawed at 4 °C, followed by slow melting at room temperature.

About 200 mg of each soil sample was pipetted under sterile conditions into a ZR BashingBead™ Lysis tube of 0.1 and 0.5 mm, followed by the addition of 750 µL of BashingBead™ Buffer. The tube was placed in a bead beater with a 2 mL tube holder assembly (precellys 24 tissue homogenizer by Bertin) and processed for nine minutes at maximum speed.

The resulting mixture was centrifuged in the centrifuge miniSpin by Eppendorf at maximum speed for one minute, and 400 µL of the supernatant was transferred into a Zymo-Spin™ III Column in a Collection Tube and centrifuged at 6 000 x g for one minute.

Then, 1 200 µL of Genomic Lysis Buffer was added to the filtrate, and 800 µL of the resulting mixture was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at maximum speed for one minute.

The flow-through was discarded, and 800 µL of the mixture was transferred again to the Zymo-Spin™ IIC Column and centrifuged at maximum speed for one minute.

Afterwards, 200 µL of DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at maximum speed for one minute.

Subsequently, 500 µL of g-DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at maximum speed for one minute.

The Zymo-Spin™ IIC Column was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL DNA Elution Buffer was directly added to the column matrix and centrifuged at maximum speed for 30 seconds to elute the DNA.

The eluted DNA was filtered through a Zymo-Spin™ III-HRC Filter in a clean 1.5 mL microcentrifuge tube and centrifuged at maximum speed for 3 minutes.

Before filtering, the Zymo-Spin™ III-HRC Filter was prepared by adding 600 µL of Prep Solution to the filter in a clean Collection Tube and centrifuging it at 6 000 x g for three minutes.

Finally, the filtered DNA was frozen at -20 °C until further analysis.

In order to verify that enough DNA was isolated, Mrs. Marie Šabacká measured the quantity of DNA with the Qubit 3 fluorometer by Invitrogen™ Q33216 for all samples, except for the samples of Lingbreen.

3.3. Polymerase Chain Reaction (PCR)

3.3.1. PCR principle

Polymerase Chain Reaction (PCR) was first developed in the early 1980s by Saiki et al. (1985). However, since then, PCR has undergone various changes to adapt to various methods, including DNA sequencing (Green & Sambrook, 2019).

PCR involves the following three stages (Green & Sambrook, 2018):

1. Denaturation: In this step, the template DNA is denatured by heat at more than 90 °C.
2. Annealing: Prior to this step, two synthetic oligonucleotide primers were designed based on the known DNA sequence of the template.

They have to fulfil the following: they need to have a size between 20 and 25 nucleotides, and they need to be complementary on the opposite strands of the target DNA.
During annealing, the forward primer and backward primer attach themselves opposite to each other on the binding sites of the target DNA.

3. Extension: This step is initiated at between 55 °C and 70 °C by a DNA polymerase that is thermally stable.

The primers will be extended, which means that they will be synthesized at the 3’endso of the attached primers.

Those three stages are repeated in about 25 to 35 cycles using a programmable device called a thermal cycler.

The products of each cycle serve as a template for the next cycle, creating products with a length that matches the number of nucleotides between the primer binding sites.

3.3.2. PCR procedure

To ascertain the presence of eluted DNA, all samples, except the samples from Nordenskiöldbreen and Hansbreen, were prepared for PCR analysis. The prokaryotic 16S rRNA genes (V3 – V5 region) were amplified by PCR.

The following procedure was repeated twice:
The DNA samples were defrosted at room temperature.

Under UV exposure, the tubes were sterilized, 12.5 µL Plain PP Master Mix 2x conc. with Mg^{2+} (Top Bio, Cat.P201-203xl), 9.5 µL PCR Ultra H_2O (Top Bio), as well as 1 µL of the forward and backward primers were put into a PCR tube and vortexed.
The Plain PP Master mix contained 150 mMTris-HCl, pH 8.8 (at 25 °C), 40 mM $(NH_4)_2SO_4$, 0.02 % Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP, 100 U/mL Taq DNA polymerase, stabilizers, and additives. Additionally, the forward primer was specific for the V3 to V4 region, denoted by the sequence 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3', and the specific for region V3 to V5 reverse primer had the sequence 5'-GTCTCGTGGGCGGAGATGTGTATAAGAGACAG-3', and both of them contained overhand adapters.
After that, 1 µL of the corresponding DNA sample was added in a sterile manner, but not anymore under UV exposure.

The amplification of the 16S rRNA gene was accomplished using a thermocycler device, the BioRad T100 Thermal. The amplification process consisted of the following: an initial denaturation phase at 94 °C for 3 minutes, 30 cycles of denaturation at 94 °C for 45 seconds, annealing at 48 °C for 45 seconds, and elongation at 72 °C for 60 seconds. The denaturation to elongation steps were repeated 34 times.

The process finished with a final extension phase at 72 °C for a duration of 7 minutes. After that, the PCR products were placed on hold at 4 °C.

The PCR products were then frozen at -20 °C until further analysis.

3.4. Gel electrophoresis

3.4.1. Gel electrophoresis principle

Agarose gel electrophoresis is a common application in order to verify if the PCR reaction was carried out successfully (Haines et al., 2015).

When an electric current is applied, the nucleic acid fragments that are negatively charged will travel to the positive charge. While doing that, larger fragments travel less than smaller fragments. With the addition of a suitable dye like ethidium bromide, these fragments become visible under ultraviolet light since the dye binds to the DNA or RNA and emits green fluorescence at 268 nm and 294 nm.

3.4.2. Gel electrophoresis procedure

The PCR products were verified by gel electrophoresis. All the chemicals and their producers used for the gel electrophoresis are listed in Table 2.
This procedure was repeated three times, and the first two times, different loading dyes and nucleic acid stains were used. In each attempt, two gels were prepared and used.

Initially, a 1.5 % agarose gel was prepared by mixing 1.5 g of Agarose with 100 mL of 1x TAE, which was then heated slowly until boiling. Once the mixture cooled down to room temperature for the first time, 10 μL of 6x DNA Nucleic Acid Stain were added. For the second and third times, Gel Green Nucleic Acid Stain 10 000 x in DMSO was added.
The solution was stirred until a homogenous solution formed and then poured into gel formers to create wells, and it was let to solidify for 30 minutes.

The Bio-Rad of PowerPack Basic was filled with 1x TAE buffer, and the solidified gels were placed inside.

The first and last wells were filled with 3 μL GeneRuler 1 kb DNA ladder while every second well in between was loaded with 5 μL of the sample that had been previously loaded the first time with 6x DNA Loading Dye Buffer Double Blue, and the second and third time with 6x DNA Loading Dye. Only every second well was loaded.

Electrophoresis was performed for each gel using an electric current at a voltage of 100 V for 45 minutes.

After the electrophoresis was performed, the gel was put under UV light at 268 nm in the NuGenius machine by SYNGENE for 5 minutes, and pictures were taken. In total, three gel electrophoresis procedures were conducted with the use of two gels during each repetition.

Table 2: A detailed overview of used chemicals and their respective manufacturers for gel electrophoresis.

Substance	Manufacturer
GoodViewTM Nucleic Acid Stain	Beijing SBS Genetech Co. Ltd.
TAE BUFFER 50x SOLUTION	BIO BASIC CANADA INC.
Agarose SERVA for DNA electrophoresis	SERVA Electrophoresis GmbH
6x DNA Loading Dye Buffer Double Blue	SOLIS BIODYNE
GeneRuler 1 kb, DNA Ladder	Thermo Scientific
Gel Green Nucleic Acid Stain 10 000 x in DMSO	Biotium
6x DNA Loading Dye	Thermo Scientific

3.5. Illumina sequencing

All samples which showed enough DNA by the Qubit 3 fluorometer or were adequately verified by gel electrophoresis were sent to SEQme for DNA sequencing with Illumina.

Illumina sequencing technology is more and more often used in genomics, transcriptomics, and epigenomics due to its flexibility, rapidity, and accuracy for large-scale sequencing (Illumina Inc, 2010). Illumina sequencing is based on the principle of forming clonal arrays and using a unique process to label and read the DNA bases.

In Illumina sequencing, a "proprietary flow cell surface" plays a crucial role. This surface not only immobilizes the DNA samples and positions them in such a way that easy interaction with enzymes is possible but also stabilizes the DNA samples and prevents unwanted binding of fluorescently labeled nucleotides (Illumina Inc, 2010).

The sequencing procedure in Illumina involves fixing DNA samples onto the "proprietary flow cell surface," followed by the creation of individual high-density DNA clusters through solid-phase amplification resulting in 1 000 copies per cluster within an area of one micron or less in diameter. Subsequently, the technique called Sequencing by Synthesis (SBS) is applied, where four specially labeled fluorescent nucleotides simultaneously sequence the high-density DNA clusters. Each sequencing cycle initiates with the addition of a single labeled deoxynucleotide triphosphate (dNTP) to the DNA chain. This dNTP both terminates chain growth and allows for the identification of the incorporated base. After that, the fluorescent dye is imaged, and cleaved by enzymes so that the next building blocks can be incorporated.

Due to the presence of individual molecules representing all four types of dNTPs (A, C, T, G), a natural competition occurs during incorporation, leading to a reduction in biases.

3.6. Data analysis

3.6.1. Provided data and data editing

The data from the following 14 samples from seven glaciers from central Svalbard (Billefjorden, Petuniabukta) were provided for this bachelor's thesis, as illustrated in Table 3.

Table 3: Summary (sample site, location and number of samples) of the provided data. The exact location of the sample sites were determined with the website TopoSvalbard by the Norwegian Polar Institute.

Sample site	Location	No. of samples
Bertilbreen	78.69 °N, 16.26 °E	3
Ferdinandbreen	78.71 °N, 16.32 °E	2
Hørbyebreen	78.76 °N, 16.28 °E	1
Jotunfonna	78.61 °N, 16.10 °E	2
Nordenskiöldbreen	78.69 °N, 17.16 °E	2
Ragnarbreen	78.75 °N, 16.70 °E	2
Svenbreen	78.73 °N, 16.30 °E	2

The sample preparation for sequencing the Illumina MiSeq V3 – V4 regions was carried out analogously to the procedure in 3.1. to 3.5., with the only difference being the timing of when the samples were sent for sequencing.

The data was processed by Mrs. Marie Šabacká using QIIME 2 (Bolyen et al., 2019), a software platform, and for taxonomic classification, the SILVA database was employed. Taxa were assigned to the sequences based on their similarity to known taxonomic information within the database.

The data was received as an Operational Taxonomic Units (OTUs) table in the BIOM format (McDonald et al., 2012). This table was subsequently employed for additional taxonomical analysis using METAGENassist (Arndt et al., 2012) and EzMAP (Shanmugam et al., 2021), as further described in 3.6.2.

3.6.2. Downstream processing

The from 3.6.1. provided data was used for downstream processing with the tool METAGENassist (Arndt et al., 2012), whereby the taxonomic analysis was carried out by constructing pie charts, a Principal Component Analysis (PCA), and a dendrogram. For the different genera assigned by METAGENassist, the phyla were identified with NCBI (Schoch et al., 2020). Alpha Diversity results were provided by Mrs. Marie Šabacká and they were calculated with the program EzMAP (Shanmugam et al., 2021).

The following steps were chosen for the analysis of the data in METAGENassist (Arndt et al., 2012):

First, OTU ID labels with the same taxonomic assignment were stripped and combined. This approach was chosen to avoid the removal of OTUs by data filtering in the subsequent steps. Then, data filtering was performed to identify and remove variables that were not likely useful for data modeling. It removed two types of variables that are non-informative: variables with extremely small values and variables with constant values (detected by robust estimate interquantile range (IQR)).

The following rules were applied during data filtering based on the number of variables present, as illustrated in Table 4.

Table 4: Rules for data filtering based on the number of variables present.

Number of variables	Variables filtered
< 15	0 %
15 – 250	5 %
250 – 500	10 %
500 – 1 000	25 %
> 1 000	40 %

If more than 5 000 variables remained after filtering, only the top 5 000 variables were used for the analysis. Furthermore, data tables that were derived from specific taxonomic ranks and mapped phenotypes underwent filtering, except when a low number of variables (less than 15) was present.

Additionally, it was chosen to exclude unassigned and unmapped reads, which could impact downstream analysis, and to remove variables with over 50 % zeros.
As the filtering process did not consider metadata information, the filtered results were able to be used in downstream analysis.
In the data filtering results, the main taxonomy table initially contained 406 variables, where no variables with unassigned reads were found. A total of 257 variables with over 50 % zeros were removed, and 7 variables (5 %) were filtered out based on IQR. After filtering, 142 variables remained. The derived data tables underwent a reduction in the number of variables, ranging from the genus, family, class, order, phylum, and super kingdom to specific phenotypic categories, such as energy source, metabolism, diseases, and habitat.

For data normalization, row-wise normalization (sample against sample) by sum was chosen in order to ensure that values within a sample are proportionally distributed based on their relative contribution to the total sum in order to be able to compare each sample with different sizes to each other.

4. RESULTS AND EVALUATION

4.1. Qubit 3 fluorometer

The DNA concentration in all samples, except those from Lingbreen, was measured using the high-sensitivity kit, which detected DNA in the range from 0.005 to 120 ng/μL. The results from the fluorometer are presented in Table 5. Samples displaying a DNA concentration above 10 ng per μL were sent to SEQme for DNA sequencing with Illumina.

Table 5: Measuring results from Qubit 3 fluorometer.

Sample site	ng of DNA per μL
Hansbreen	12.70
Sefströmbreen	9.74
Longyearbreen	27.80
Nordbreen	
Nordbreen_2	2.72
Nordbreen_3	> 120.00
Nordbreen_6	> 120.00
Franklinbreane	
NF_1	> 120.00
NF_2	> 120.00
NF_3	> 120.00
NF_4	> 120.00
NF_5	> 120.00
NF_6	> 120.00
NF_7	> 120.00
NF_8	> 120.00
NF_9	> 120.00
Sørbreen	
SOR_1	14.60
SOR_2a	52.00
SOR_2b	6.84
SOR_3a	10.00
SOR_3b	> 120.00
SOR_4	2.46

Sample site	ng of DNA per μL
SOR_5a	2.33
SOR_5b	> 120.00
SOR_6a	2.36
SOR_6b	> 120.00
SOR_7a	5.96
SOR_7b	> 120.00
SOR_8	1.34
SOR_9a	> 120.00
SOR_9b	1.63
SOR_10	8.88
Lingbreen	all 9 samples were not measured
Torellbreen	27.80
Hørbyebreen	12.10
Nordenskiöldbreen	23.20

All samples, except eight from Sørbreen (SOR_2b, SOR_4, SOR_5a, SOR_6a, SOR_7a, SOR_8, SOR_9b, SOR_10), one from Nordbreen (Nordbreen_2), and the only sample from Sefströmbreen, exhibited sufficient ng of DNA per μL. Samples from Lingbreen were not tested; however, the abundance of DNA in these samples was confirmed through PCR and gel electrophoresis (as further described in 4.2.). Consequently, all samples meeting the DNA threshold of 10 ng of DNA per μL, along with those from Lingbreen, were sent to SEQme for DNA sequencing with Illumina.

4.2. Gel electrophoresis

Fig. 5 and Fig. 6 display the results obtained from the third attempt at gel electrophoresis. As mentioned in section 3.4.2., it is important to note that only every second well was loaded with samples. The first and last wells were loaded with GeneRule 1 kb DNA Ladder.

During the first try, no bands were visible on both gels, indicating an issue during gel preparation; thus, the chemicals were swapped. In the second attempt, only two samples and the ladder on one gel were visible, suggesting a problem with PCR preparation. On the other gel, no visible bands were observed due to overrunning the electrophoresis, causing the samples to wash out.

Subsequently, PCR preparation was redone, and on the third try, the first gel (Fig. 5) ran for too little, which can be seen in the DNA Ladder, which is not entirely separated. The second gel (Fig. 6) worked well for most samples, except for the eighth and last samples, which were from Torellbreen and one sample from Sørbreen (SOR_9b), respectively, which showed low and insufficient DNA and aligns with the results from the Qubit 3 fluorometer measurements (as illustrated in 4.1.).

All other samples demonstrated enough DNA at approximately 300 bp during gel electrophoresis.
The following PCR products were verified: 5 samples from Lingbreen (LING_2, LING_3, LING_4, LING_9, LING_10), 1 sample from Sørbreen (SOR_6b), 2 samples from Franklinbreane (NF_3, NF_4), and the sample from Longyearbreen.

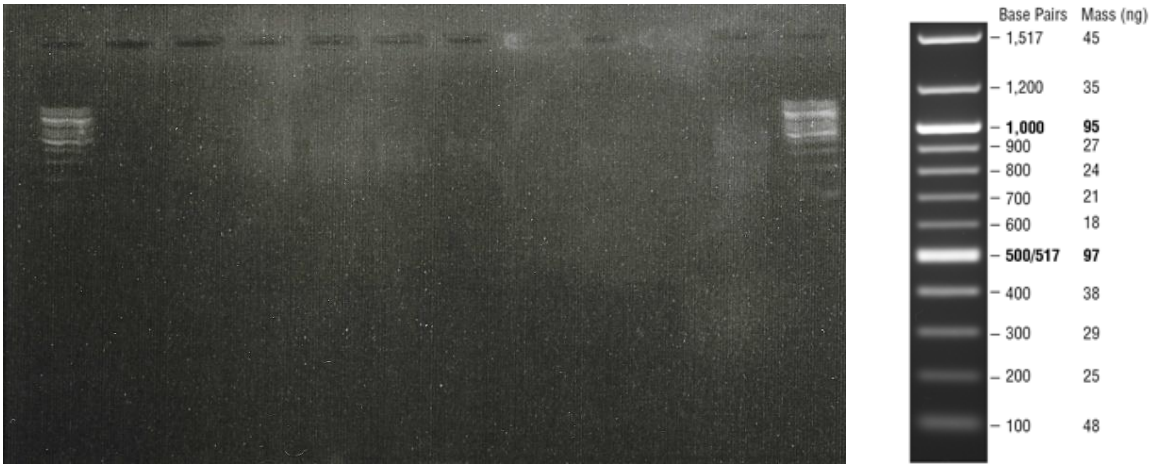


Fig. 5. The first 1.5 % TAE agarose gel of the third attempt. Only every second well was filled with samples. On the right is the 100 bp DNA Ladder, observed through ethidium bromide staining on a 1.3 % TAE agarose gel, with mass values assigned to each lane (0.5 μg per lane). The last and first wells were filled with GeneRule 1 kb DNA Ladder by Thermo Scientific. The gel was subjected to UV light at 268 nm for 5 minutes. The base pairs were visualized by 6x DNA Nucleic Acid Stain in 10 000 x in DMSO. As can be seen, by the ladders, the gel electrophoresis was terminated too early before the samples could separate enough.

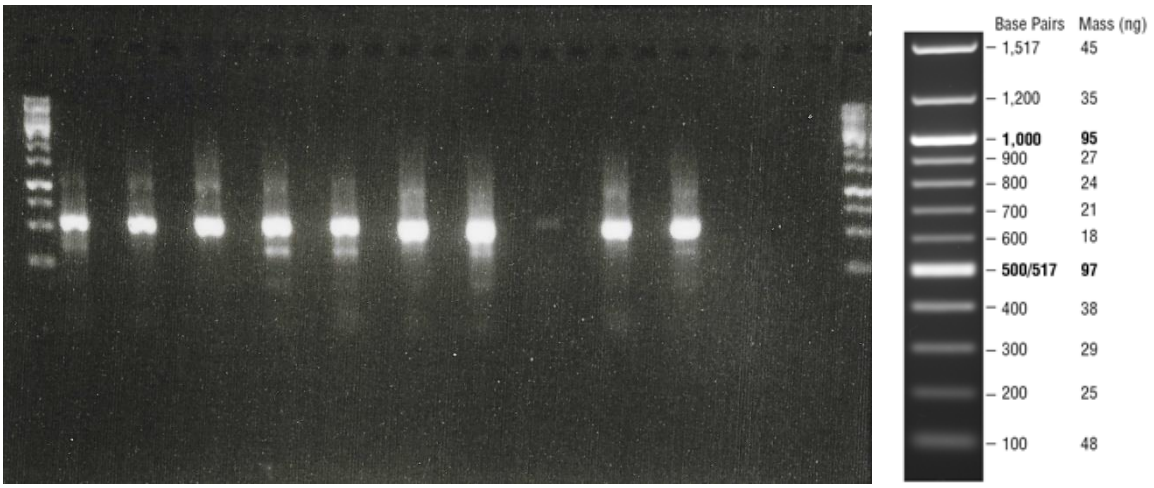


Fig. 6. Second 1.5 % TAE agarose gel of the third attempt. Only every second well was filled with samples. The last and first wells were filled with GeneRule 1 kb DNA Ladder by Thermo Scientific. The gel was subjected to UV light at 268 nm for 5 minutes. On the right is the 100 bp DNA Ladder, observed through ethidium bromide staining on a 1.3 % TAE agarose gel, with mass values assigned to each lane (0.5 μg per lane). The base pairs were visualized by 6x DNA Nucleic Acid Stain in 10 000 x in DMSO. Every sample, except the eighth and last sample, showed sufficient DNA at around 300 bp. Loaded samples from left to right were LING_9, SOR_6b, NF_4, LING_4, LING_3, NF_3, LING_2, Torellbreen, Longyearbreen, LING_10, SOR_9b.

4.3. Data analysis

With the program METAGENassist (Arndt et al., 2012), as described in 3.6.2., the data of the different glaciers, as illustrated in Table 3, were analyzed. The relative abundance of bacteria in the cryoconites, averaged by glacier, was investigated. First, each glacier's relative abundance of the phyla and genera is described individually (4.3.1. to 4.3.7.). Then the phyla are compared as a PCA (4.3.8.), and dendrogram (4.3.8.). and lastly, the alpha diversity was calculated (4.3.9.).

4.3.1. Bertilbreen

Fig. 7 and Fig. 8 show the relative abundance results of the phyla and genera for the glacier Bertilbreen (Bertil).

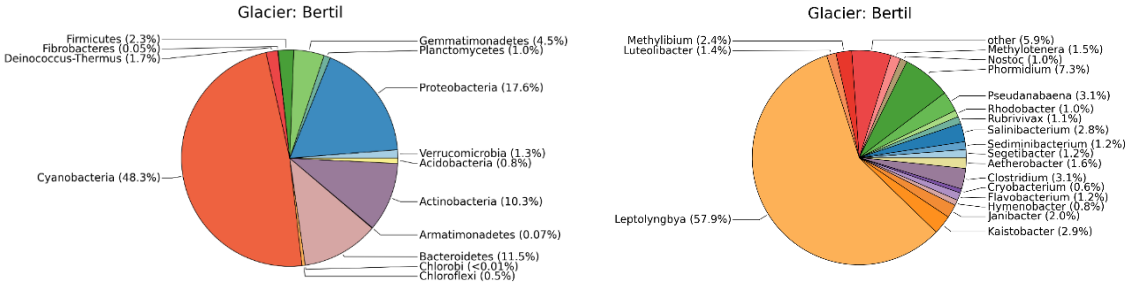


Fig. 7. and **Fig. 8.** Relative abundance of the phyla (left) and genera (right) in Bertil.

The four most abundant bacterial phyla in the cryoconite sites of Bertil are Cyanobacteria (48.3 %), Proteobacteria (17.6 %), Actinobacteria (10.3 %), and Bacteroidetes (11.5 %).

The phylum Proteobacteria (17.6 %) is composed of 13.9 % *Alphaproteobacteria*, 19.9 % *Betaproteobacteria*, 1.2 % *Gammaproteobacteria*, and 5.2 % *Deltaproteobacteria*. 57.9 % *Leptolyngbya*, 7.3 % *Phormidium*, 3.1 % *Pseudanabaena*, and 1.0 % *Nostoc* belong to the phylum Cyanobacteria.

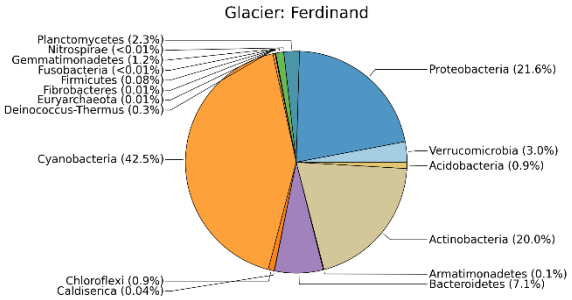
Among *Alphaproteobacteria* are 2.9 % *Kaistobacter*, and 1.0 % *Rhodobacter*; among *Betaproteobacteria* are 2.4 % *Methylibium*, 1.1 % *Rubrivivax*, and 1.5 % *Methylotenera*; and among *Deltaproteobacteria* are 1.6 % *Aetherobacter*.

1.2 % *Sediminibacterium*, 1.2 % *Segetibacter*, 1.2 % *Flavobacterium*, and 0.8 % *Hymenobacter* belong to the phylum Bacteroidetes.

Actinobacteria are comprised of 2.8 % *Salinibacterium*, 2.0 % *Janibacter*, and 0.6 % *Cryobacterium*. 1.4 % *Luteolibacter* belong to the phylum Verrucomicrobia (1.3 %), and the 3.1 % *Clostridium* belong to the phylum Firmicutes (2.3 %). The 5.9 % labeled as "Other" comprises genera collectively contributing to less than 1 %.

4.3.2. Ferdinandbreen

The abundance results in percentages from the glacier Ferdinandbreen (Ferdinand) are illustrated in Fig. 9 and Fig. 10.



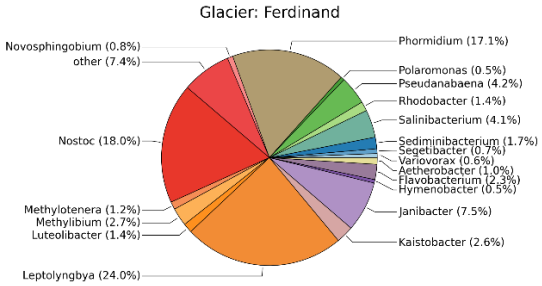


Fig. 9. and **Fig. 10.** Relative abundance of the phyla (left) and genera (right) in Ferdinand.

Cyanobacteria with 42.5 %, Proteobacteria with 21.6 % which is composed of 13.6 % *Alphaproteobacteria*, 20.9 % *Betaproteobacteria*, 4.4 % *Deltaproteobacteria*, and 3.7 % *Gammaproteobacteria*, Actinobacteria with 20.0 %, and Bacteroidetes with 7.1 % are the four most abundant bacterial phyla in Ferdinand.

To the phylum Cyanobacteria belong 24.0 % *Leptolyngbya*, 18.0 % *Nostoc*, 17.1 % *Phormidium*, and 4.2 % *Pseudanabaena*.

Alphaproteobacteria are comprised of 2.6 % *Kaistobacter*, 1.4 % *Rhodobacter*, and 0.8 % *Novosphingobium*; *Betaproteobacteria* are comprised of 2.7 % *Methylibium*, 1.2 % *Methylotenera*, 0.6 % *Variovorax*, and 0.5 % *Polaromonas*; and *Deltaproteobacteria* are comprised of 1.0 % *Aetherobacter*.

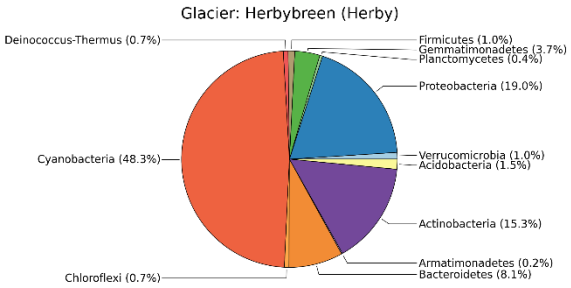
2.3 % *Flavobacterium*, 1.7 % *Sediminibacterium*, 0.7 % *Segetibacter*, and 0.5 % *Hymenobacter* belong to the phylum Bacteroidetes.

The phylum Actinobacteria includes 7.5 % *Janibacter* and 4.1 % *Salinibacterium*.

Among Verrucomicrobia (3.0 %) are 1.4 % *Luteolibacter*. 7.4 % are recognized as “Other.”

4.3.3. Hørbyebreen

Fig. 11 and Fig. 12 display the relative abundance of bacteria on the glacier Hørbyebreen (Herbybreen (Herby)).



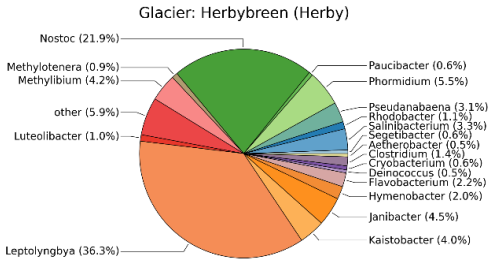


Fig. 11. and **Fig. 12.** Relative abundance of the phyla (left) and genera (right) in Herby.

The four most abundant phyla in Herby are Cyanobacteria (48.3 %), Proteobacteria (19.0 %), Actinobacteria (15.3 %) and Bacteroidetes (8.1 %).

Proteobacteria are comprised of 15.6 % *Alphaproteobacteria*, 22.0 % *Betaproteobacteria*, 2.5 % *Deltaproteobacteria*, and 1.3 % *Gammaproteobacteria*.

Among Cyanobacteria are 36.3 % *Leptolyngbya*, 21.9 % *Nostoc*, 5.5 % *Phormidium*, and 3.1 % *Pseudanabaena*.

To the class *Alphaproteobacteria* belong 4.0 % *Kaistobacter* and 1.1 % *Rhodobacter*; to the class, *Betaproteobacteria* belong 4.2 % *Methylibium*, 0.9 % *Methylotenera*, and 0.6 % *Paucibacter*; and to the class *Deltaproteobacteria* belong 0.5 % *Aetherobacter*.

The phylum Bacteroidetes include 2.2 % *Flavobacterium*, 2.0 % *Hymenobacter*, and 0.6 % *Segetibacter*.

4.5 % *Janibacter*, 3.3 % *Salinibacterium*, and 0.6 % *Cryobacterium* are assigned to the phylum Actinobacteria.

Verrucomicrobia (1.0 %) include 1.0 % *Luteolibacter*, Firmicutes (1.0 %) include 1.4 % *Clostridium*, and Deinococcus-Thermus (0.7 %) include 0.5 % *Deinococcus*. 5.9 % are labeled as “Other.”

4.3.4. Jotunfonna

The abundance of bacteria phyla and genera in percentages of the cryoconite sites on the glacier Jotunfonna (Jote) are illustrated as a pie chart in Fig. 13 and Fig. 14.

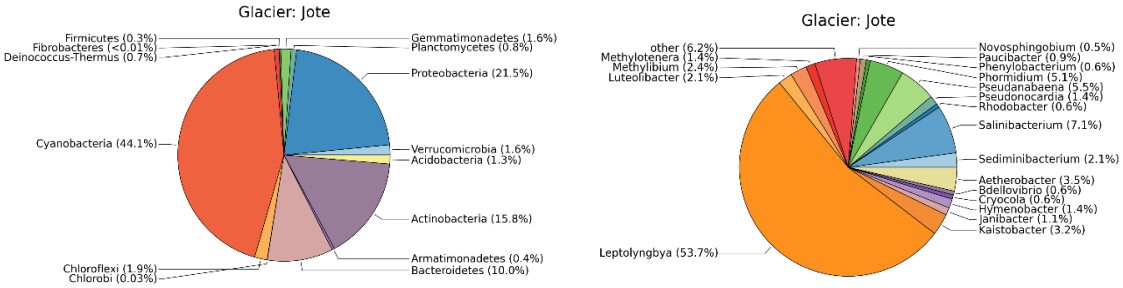


Fig. 13. and **Fig. 14.** Relative abundance of the phyla (left) and genera (right) in Jote.

The four most abundant phyla are Cyanobacteria with 44.1 %, Proteobacteria with 21.5 %, Actinobacteria with 15.8 %, and Bacteroidetes with 10.0 %.

Proteobacteria are classified into 18.9 % *Alphaproteobacteria*, 14.8 % *Betaproteobacteria*, 8.4 % *Deltaproteobacteria*, and 0.2 % *Gammaproteobacteria*.

53.7 % of *Leptolyngbya*, 5.5 % of *Pseudanabaena*, and 5.1 % of *Phormidium* are assigned to the phylum Cyanobacteria.

Among *Alphaproteobacteria* are 3.2 % *Kaistobacter*, 0.6 % *Rhodobacter*, 0.6 % *Phenylobacterium*, and 0.5 % *Novosphingobium*; among *Betaproteobacteria* are 2.4 % *Methylibium*, 1.4 % *Methylotenera*, and 0.9 % *Paucibacter*; and among *Deltaproteobacteria* are 3.5 % *Aetherobacter*, and 0.6 % *Bdellovibrio*.

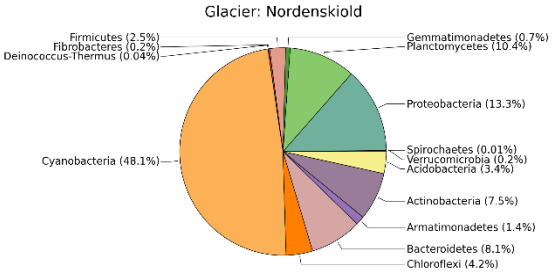
To the phylum, Bacteroidetes belong 2.1 % *Sediminibacterium* and 1.4 % *Hymenobacter*.

The phylum Actinobacteria includes 7.1 % *Salinibacterium*, 1.4 % *Pseudonocardia*, 1.1 % *Janibacter* and 0.6 % *Cryocola*.

2.1 % of *Luteolibacter* are assigned to Verrucomicrobia (1.6 %), and 6.2 % are identified as “Other.”

4.3.5. Nordenskiöldbreen

Fig. 15 and Fig. 16 show the relative abundance results of the phyla and genera for the glacier Nordenskiöldbreen (Nordenskiold).



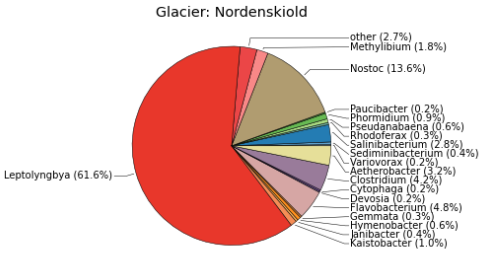


Fig. 15. and **Fig. 16.** Relative abundance of the phyla (left) and genera (right) in Nordenskiold.

The four most abundant phyla of Nordenskiold are Cyanobacteria (48.1 %), Proteobacteria (13.3 %), classified in 11.1 % *Alphaproteobacteria*, 11.7 % *Betaproteobacteria*, 8.7 % *Deltaproteobacteria*, and 1.1 % *Gammaproteobacteria*, 7.5 % Actinobacteria, and 8.1 % Bacteroidetes.

The phylum Cyanobacteria includes 61.6 % *Leptolyngbya*, 13.6 % *Nostoc*, 0.9 % *Phormidium*, and 0.6 % *Pseudanabaena*.

Among *Alphaproteobacteria* are 1.0 % *Kaistobacter*, and 0.2 % *Devosia*; among *Betaproteobacteria* are 1.8 % *Methylibium*, 0.3 % *Rhodoferax*, 0.2 % *Paucibacter*, and 0.2 % *Variovorax*; among *Deltaproteobacteria* are 3.2 % *Aetherobacter*.

The phylum Bacteroidetes compromise 4.8 % *Flavobacterium*, 0.6 % *Hymenobacter*, 0.4 % *Sediminibacterium*, and 0.2 % *Cytophaga*.

2.8 % *Salinibacterium* and 0.4 % *Janibacter* belong to the phylum Actinobacteria.

4.2 % *Clostridium* is assigned to Firmicutes (2.5 %), 0.3 % *Gemmata* to Planctomycetes (10.4 %), and 2.7 % to “Other.”

4.3.6. Ragnarbreen

Fig. 17 and Fig. 18 display the relative abundance of bacteria on the glacier Ragnarbreen (Ragnar).

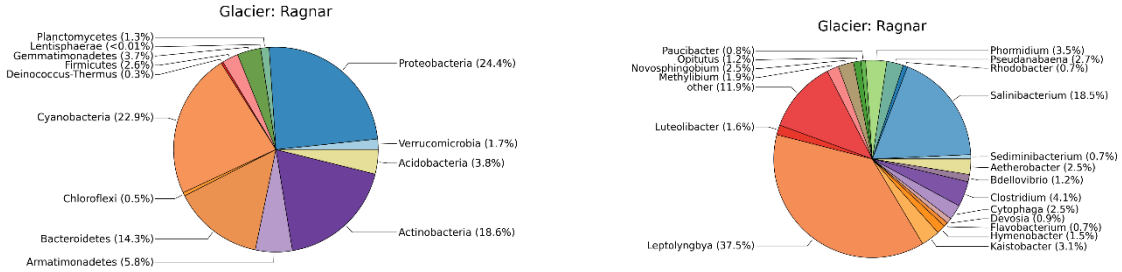


Fig. 17. and **Fig. 18.** Relative abundance of the phyla (left) and genera (right) in Ragnar.

Cyanobacteria, with 22.9 %; Proteobacteria, with 24.4 %; Actinobacteria, with 18.6 %; and Bacteroidetes, with 14.3 %, are the four most abundant phyla in Ragnar.

The phylum Proteobacteria is classified into 18.0 % *Alphaproteobacteria*, 10.5 % *Betaproteobacteria*, 4.1 % *Deltaproteobacteria*, and 3.2 % *Gammaproteobacteria*.

To the phylum Cyanobacteria belong 37.5 % *Leptolyngbya*, 3.5 % *Phormidium*, and 2.7 % *Pseudanabaena*.

Alphaproteobacteria comprise 3.1 % *Kaistobacter*, 2.5 % *Novosphingobium*, 0.9 % *Devosia*, and 0.7 % *Rhodobacter*; *Betaproteobacteria* comprise 1.9 % *Methylibium*, and 0.8 % *Paucibacter*; and *Deltaproteobacteria* comprise 2.5 % *Aetherobacter*, and 1.2 % *Bdellovibrio*.

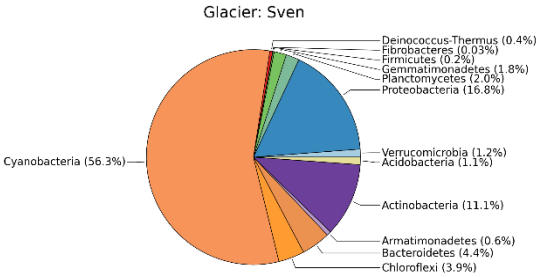
Among Bacteroidetes are 2.5 % *Cytophaga*, 1.5 % *Hymenobacter*, 0.7 % *Sediminibacterium*, and 0.7 % *Flavobacterium*.

18.5 % of *Salinibacterium* belong to the phylum Actinobacteria.

Verrucomicrobia (1.7 %) include 1.6 % *Luteolibacter*, and 1.2 % *Opitutus*, Firmicutes (2.6 %) include 4.1 % *Clostridium*, and 11.9 % are identified as “Other.”

4.3.7. Svenbreen

The abundance results in percentages from the glacier Svenbreen (Sven) are illustrated in Fig. 19 and Fig. 20.



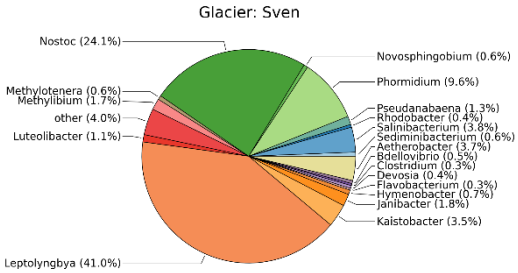


Fig. 19. and **Fig. 20.** Relative abundance of the phyla (left) and genera (right) in Sven.

Among the four most abundant phyla on the glacier Sven are Cyanobacteria (56.3 %), Proteobacteria (16.8 %), Actinobacteria (11.1 %), and Bacteroidetes (4.4 %).

Proteobacteria are classified into 20.2 % *Alphaproteobacteria*, 14.0 % *Betaproteobacteria*, 12.3 % *Deltaproteobacteria*, and 0.9 % *Gammaproteobacteria*.

To the phylum Cyanobacteria belong 41.0 % *Leptolyngbya*, 24.1 % *Nostoc*, 9.6 % *Phormidium*, and 1.3 % *Pseudanabaena*.

Alphaproteobacteria include 3.5 % *Kaistobacter*, 0.6 % *Novosphingobium*, 0.4 % *Devosia*, and 0.4 % *Rhodobacter*; *Betaproteobacteria* include 1.7 % *Methylibium* and 0.6 % *Methylotenera*; and *Deltaproteobacteria* include 3.7 % *Aetherobacter* and 0.5 % *Bdellovibrio*.

0.7 % *Hymenobacter*, 0.6 % *Sediminibacterium*, and 0.3 % *Flavobacterium* are assigned to Bacteroidetes.

3.8 % *Salinibacterium* and 1.8 % *Janibacter* are Actinobacteria.

Among Verrucomicrobia (1.2 %) are 1.1 % *Luteolibacter*, among Firmicutes (0.2 %) are 0.3 % *Clostridium* and 4.0 % are labeled as “Other.”

4.3.8. Data evaluation

Table 6 summarizes the findings of the four most abundant phyla in all glaciers, and Table 7 summarizes the relative results of the genera in each glacier.

Table 6: Summary of the four most abundant phyla in their relative amount (%) among all glaciers.

	Bertil	Ferdindand	Herby	Jote	Nordenskiold	Ragnar	Sven
Cyanobacteria	48.3	42.5	48.3	44.1	48.1	22.9	56.3
Proteobacteria	17.6	21.6	19.0	21.5	13.3	24.4	16.8
Actinobacteria	10.3	20.0	15.3	15.8	7.5	18.6	11.1
Bacteroidetes	11.5	7.1	8.1	10.0	8.1	14.3	4.4

Table 7: Summary of the relative abundance of the present genera grouped by their phylum or class in all glaciers. If the genus is not present, it is abbreviated as NA. "Other" comprises genera that collectively contribute to less than 1 % of the overall abundance.

	Bertil	Ferdinand	Herby	Jote	Nordenskiold	Ragnar	Sven
Cyanobacteria (phylum)							
Leptolyngbya	57.9	24.0	36.3	53.7	61.6	37.5	41.0
Nostoc	1.0	18.0	21.9	NA	13.6	NA	24.1
Phormidium	7.3	17.1	5.5	5.1	0.9	3.5	9.6
Pseudanabaena	3.1	4.2	3.1	5.5	0.6	2.7	1.3
α-proteobacteria (class)							
Devosia	NA	NA	NA	NA	0.2	0.9	0.4
Kaistobacter	2.9	2.6	4.0	3.2	1.0	3.1	3.5
Novosphingobium	NA	0.8	NA	0.5	NA	2.5	0.6
Phenylobacterium	NA	NA	NA	0.6	NA	NA	NA
Rhodobacter	1.0	1.4	1.1	0.6	NA	0.7	0.4
β-proteobacteria (class)							
Methylibium	2.4	2.7	4.2	2.4	1.8	1.9	1.7
Methylotenera	1.5	1.2	0.9	1.4	NA	NA	0.6
Paucibacter	NA	NA	0.6	0.9	0.2	0.8	NA
Polaromonas	NA	0.5	NA	NA	NA	NA	NA
Rhodoferax	NA	NA	NA	0.1	0.3	NA	NA
Rubrivivax	1.1	NA	NA	NA	NA	NA	NA
Variovorax	NA	0.6	NA	NA	0.2	NA	NA
δ-proteobacteria (class)							
Aetherobacter	1.6	1.0	0.5	3.5	3.2	2.5	3.7
Bdellovibrio	NA	NA	NA	0.6	NA	1.2	0.5

	Bertil	Ferdinand	Herby	Jote	Nordenskiold	Ragnar	Sven
Actinobacteria (phylum)							
Cryobacterium	0.6	NA	0.6	NA	NA	NA	NA
Cryocola	NA	NA	NA	0.6	NA	NA	NA
Janibacter	2.0	7.5	4.5	1.1	0.4	NA	1.8
Pseudonocardia	NA	NA	NA	1.4	NA	NA	NA
Salinibacterium	2.8	4.1	3.3	7.1	2.8	18.5	3.8
Bacteroidetes (phylum)							
Cytophaga	NA	NA	NA	NA	0.2	2.5	NA
Flavobacterium	1.2	2.3	2.2	NA	4.8	0.7	0.3
Hymenobacter	0.8	0.5	2.0	1.4	0.6	1.5	0.7
Sediminibacterium	1.2	1.7	NA	2.1	0.4	0.7	0.6
Segetibacter	1.2	0.7	0.6	NA	NA	NA	NA
Firmicutes (phylum)							
Clostridium	3.1	NA	1.4	NA	4.2	4.1	0.3
Deinococcus-Thermus (phylum)							
Deinococcus	NA	NA	0.5	NA	NA	NA	NA
Planctomycetes (phylum)							
Gemmata	NA	NA	NA	NA	0.3	NA	NA
Verrucomicrobia (phylum)							
Luteolibacter	1.4	1.4	1.0	2.1	NA	1.6	1.1
Opitutus	NA	NA	NA	NA	NA	1.2	NA
“Other”	5.9	7.4	5.9	6.2	2.7	11.9	4.0

Furthermore, the phyla for each sample are depicted in a PCA, as illustrated in Fig. 21, and their similarity in a dendrogram (Fig. 22).

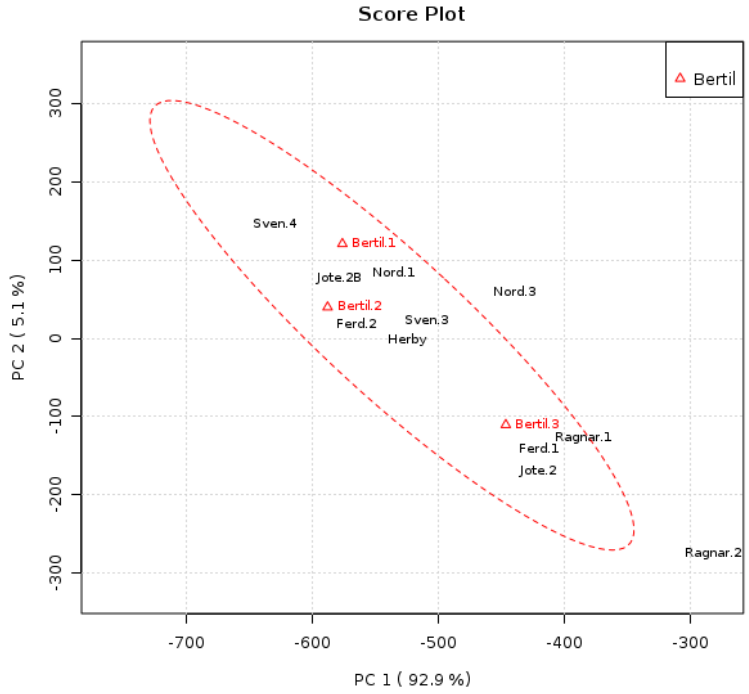


Fig. 21. PCA: 2 D score plot. Ellipsis represents a confidence region of 95.0 %.

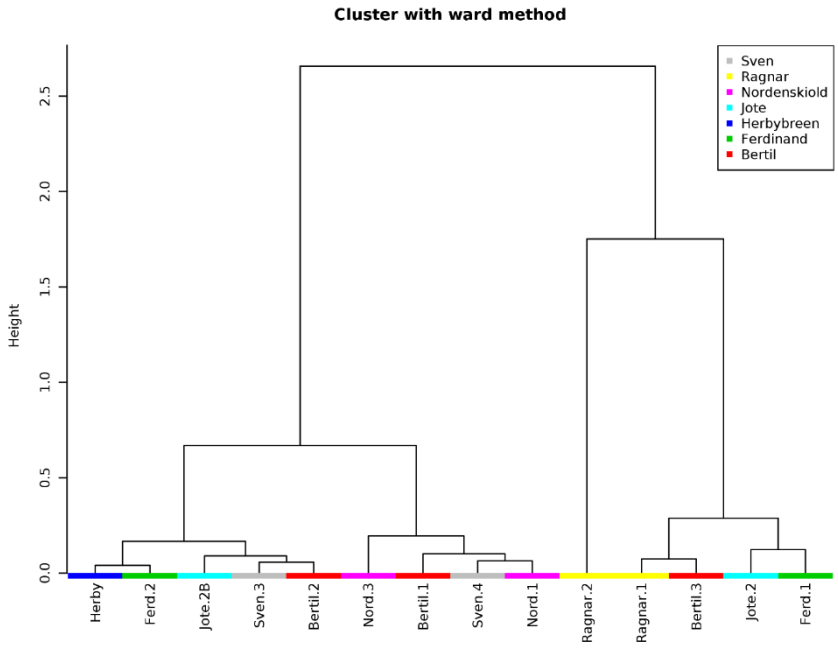


Fig. 22. Dendrogram. Constructed with Pearson's method and the clustering algorithm Ward.

4.3.9. Alpha diversity

Fig. 23 illustrates the Alpha Diversity which was calculated with the program EzMAP (Shanmugam et al., 2021) by Mrs. Marie Šabacká.

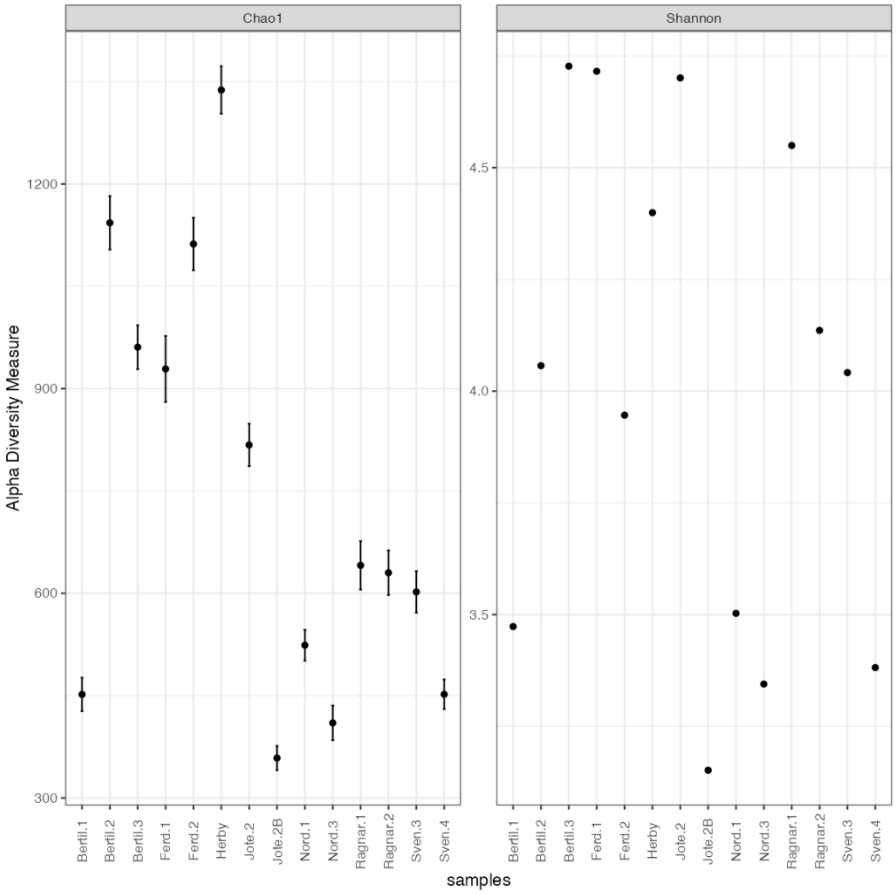


Fig. 23. Alpha Diversity: Chao1 estimates the total richness of each sample on the left and the Shannon Index, which provides information about richness and evenness, on the right.

5. DISCUSSION

When comparing all glacial sites with each other, the predominant phyla are Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes, collectively accounting for 86.7 %. This finding aligns with previous research, which also reported Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes as the four most abundant phyla (Anesio et al., 2017; Edwards et al., 2013; Gokul et al., 2016; Millar et al., 2021).

The remaining 13.3 % includes other phyla, such as Verrucomicrobia, Firmicutes, and Planctomycetes.

Not all genera could be assigned to a taxon. Genera with < 1 % of the total abundance were grouped together and labeled as "Other." We would have to sequence additional genes or the entire genome instead of only the 16S rRNA gene to improve the taxonomic resolution. However, the 16S rRNA gene was chosen in this study because it is a crucial element of cell function and a highly conserved gene, making it more tolerant to mutations (Clarridge, 2004). Only a few genes exhibit such high conservation as the 16S rRNA gene. Even though the precise rate of change in the sequence of the 16S rRNA gene remains unknown, it serves as a marker for assessing evolutionary distance and the relationships between organisms (Kimura, 1980).

As seen in the PCA and verified by the dendrogram, two clusters arose.

Within the first cluster, two distinct clades emerged. One clade consists of the samples Herby, Ferd.2, Jote.2B, Sven.3, and Bertil.2, with Herby and Ferd.2 exhibiting the highest similarity, followed by Sven.3, Bertil.2, and Jote.2B. The second clade within the first cluster comprises Nord.3, Bertil.1, Sven.4, and Nord.1, with Sven.4 and Nord.1 demonstrating the most significant similarity.

The second cluster is divided into two clades: one consisting solely of the sample Ragnar.2, and the other including Ragnar.2, Bertil.3, Jote.2 and Ferd.1, where Ragnar.1 and Bertil.3, as well as Jote.2 and Ferd.1 show the most similarity.

This clustering pattern aligns with the earlier data analysis, where the glaciers Bertil, Ferdinand, Herby, Jote, Nordenskiold, and Sven displayed similar abundances in their four most prominent phyla (Cyanobacteria: 42.5 – 56.3 %; Proteobacteria: 16.8 – 21.6 %; Actinobacteria: 7.5 – 20.0 %; Bacteroidetes: 4.4 – 11.5 %). In contrast, Ragnar exhibited differences in the relative abundance of these phyla (Cyanobacteria: 22.9 %, Proteobacteria: 24.4 %, Actinobacteria: 18.6 %, and Bacteroidetes: 14.6 %).

The observed similarities among most samples, except for Ragnar, can be attributed to the proximity of glaciers Bertil, Ferdinand, and Sven to each other. While Jote, Herby, and Nordenskiold are relatively farther away, they still remain in close proximity and inland. These findings also align with previous research, which states that more uniform communities are present in the Arctic because the cryoconite holes are open and connected (Hodson et al., 2008). One key difference in Ragnar is the presence of a lake between the sea and the glacier, which is not the case in the other glacier sites.

The impact of abiotic factors on microbial community composition has been well-documented in numerous studies. Surface hydrology, distance from the ice margin, melting season duration, geometry, and lithology have all been shown to influence microbial communities (Anesio & Laybourn-Parry, 2012; Edwards et al., 2013; Gokul et al., 2016). These factors likely play a role in shaping the distinct microbial profiles observed, especially with regard to the Ragnar sample compared to the others.

When examining the alpha diversity, Chao1 estimates the total richness in each sample, i.e., the total number of OTUs. The results indicate significant variation among the samples. For instance, some samples, like Ferdinand and Herby, display higher OTU counts, while others, such as Ragnar and Sven, show lower OTU numbers. This variation might be attributed to the use of only 16S rRNA sequencing, as previously discussed. However, the Shannon Index reveals that the spread of abundance between the samples differs significantly from one another.

We also wanted to compare our data to those from the literature. In all glacier samples, except for those from Ragnar, the most abundant phylum is Cyanobacteria, with two prominent families, Pseuanabaenaceae (*Leptolyngbya*, *Pseudanabaena*) and Phormidicaceae (*Phormidium*). Another family of Cyanobacteria, Nostocaceae (*Nostoc*), is present in a relative abundance ranging from 14 % to 24 % across all sample sites, except for Bertilbreen (1 %), and Ragnarbreen and Jotundfonna, where none were found. These findings coincide with existing literature, which also identifies Cyanobacteria as the most common taxa with *Leptolyngbya* and *Pseudanabaena* as the most frequently occurring genera in cryoconite samples (Gokul et al., 2016; Rozwalak et al., 2022; Uetake et al., 2016).

The occurrence of Cyanobacteria in cryoconite holes is hardly surprising, given their characteristics as aerobic photoautotrophs, relying on carbon dioxide, inorganic substances, and light to produce energy by oxygenic photosynthesis (Garcia-Pichel, 2009). As highlighted by Laybourn-Parry et al. (2012), the presence of Cyanobacteria is extremely common, as they possess the capacity to inhabit extreme environments and are widely distributed in ice and snow. According to Chrismas et al. (2015), specific genera of Cyanobacteria show strong cold tolerance traits. *Pseudanabaena* is frequently found in cold environments; *Leptolyngbya* displays a strong indication of cold tolerance; *Phormidium* has been discovered in Antarctic, Arctic, and Alpine regions, indicating a high likelihood of withstanding cold conditions; and *Nostoc* shows a very high probability of having ancestors with cold tolerance. This suggests that most Cyanobacteria found in glaciers (cold environments) were identified as psychrotolerant rather than psychrophilic (Anesio et al., 2017).

Cyanobacteria undergo acclimation processes in response to reduced temperature or changes in light and temperature conditions, which is the case in glacial environments (Huner et al., 1998; Miśkiewicz et al., 2000). These acclimation processes involve synthesizing carotenoids and making alterations to their photosynthetic apparatus.

Furthermore, Cyanobacteria play a significant role in the food web, as their photosynthesis produces 75 % and 95 % of available carbon (Stibal & Tranter, 2007). Especially, filamentous Cyanobacteria, such as *Nostoc* and *Phormidium*, possess the ability to produce EPS (as described in 1.2.3) (Anesio et al., 2017), which is a nutritional source for heterotrophs and can be re-absorbed by Cyanobacteria (Stuart et al., 2016). Apart from that, Cyanobacteria are significant in the formation of cryoconite holes (as described in 1.2.1.).

In the sample sites of Ragnar, Proteobacteria, not Cyanobacteria, are the most common phylum. This finding aligns with diversity studies that have also identified Proteobacteria as the most abundant phylum; however, in those studies, the phylum Cyanobacteria only contributes to a small degree to the overall microbial composition, which in Ragnar is not the case (Edwards et al., 2013; Millar et al., 2021). When looking at those studies, according to Gokul et al. (2016), the lower abundance of Cyanobacteria in comparison to their role in those environments marks them as “ecosystem engineers” and “keystone species.”

In the other sample sites, Proteobacteria are the second most abundant phylum.
In Jotunfonna, Nordenskiold, Ragnar, and Sven, *Alphaproteobacteria* dominate with 11 % to 20 % over other classes, followed by *Betaproteobacteria* at 11 % to 15 %. Contrary to Bertil, Ferdinand, and Herby, *Betaproteobacteria*, with 20 % to 22 % abundance is the most abundant class of Proteobacteria, followed by *Alphaproteobacteria* at 14 % to 16 %. The latter finding coincides with existing literature, which also states that *Betaproteobacteria* are the predominant class on glaciers (Edwards et al., 2013; Gokul et al., 2016).
Proteobacteria are Gram-negative and are classified as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, and *Zetaproteobacteria* (Boundless, n.d.-a). Al*phaproteobacteria* include the majority of phototrophic genera (Boundless, n.d.-b). Among *Betaproteobacteria* are aerobic or facultative bacteria. Especially *Methylotenera* belongs to the order *Nitrosomanadles*, chemoautotrophic bacteria oxidizing ammonia into nitrite, and the genus *Rubrivivax* are phototrophs (Boundless, n.d.-c). The genus *Mehtylotenera* is present in an abundance of 0.6 % to 1.5 % in all sample sites except for Nordenskiold and Ragnar. *Rubrivivax* is only present Bertil. The prevalence of *Betaproteobacteria* over *Alphaproteobacteria* in Ferdinand and Bertil can be attributed to their proximity in snow, slush, and ice (Hell et al., 2013). Furthermore, Gokul et al. (2016) suggest that Proteobacteria are responsible for heterotrophic processes.

Except for Bertil and Nordenskiold, Actinobacteria represent the third largest phylum across all glacier sites, comprising approximately 11 % to 20 % of the microbial community. Gokul et al. (2016) label Actinobacteria as “keystone taxa” due to their significant influence on the microbial community composition by facilitating “key processes or biotic interactions.” In addition, this study reveals that Actinobacteria have a previously unknown function in shaping the composition of cryoconite bacterial communities. When comparing the genera of all sample sites, it is noticeable that only the genus *Salinibacterium* can be found in all sites and, with 18.5 %, is most abundant on Ragnar.

In Bertil and Nordenskiold, the third largest phylum is Bacteroidetes, which in all the other glacier samples ranks as the fourth most abundant. According to Edwards et al. (2013), this phylum is found in a variety of environments, ranging from the gastrointestinal tract to the cryosphere. The abundance of Bacteroidetes in cryoconite sites is likely associated with their potential role in breaking down organic material derived from plants. This aligns with the prevalence of external matter, as noted by Stibal et al. (2008).

As mentioned by Edwards et al. (2013), the presence of the bacteria discussed above can be attributed to the necessity for efficient recycling of external carbon and nutrients in glacial environments. This requirement arises due to the demanding physical, chemical, and nutritional conditions prevailing in such habitats, as described in section 1.1.3.

Additionally, it is important to acknowledge that Mrs. Marie Šabacká provided the data used for this, as time constraints prevented the original data for this thesis from being adequately sequenced by SEQme.
Nevertheless, the data closely resembled the process of the original data, with the only difference being the location of the samples and the timing of their submission to SEQme for sequencing.

6. CONCLUSION

In this study, I investigated the taxonomic composition and diversity of microbial communities within cryoconite holes across different glacial sites in Svalbard.

One of this thesis' primary aims was to isolate DNA from selected glacial biotopes and use the data to determine the taxonomic composition of microbial communities. As seen in the gel electrophoresis and fluorometer results, this was achieved after several attempts. However, it must be noted that working with cryoconite samples proved to be particularly challenging. The DNA in such environments is prone to rapid degradation and thus requires careful protocols and adjustments throughout the experimental process.

By using sequencing, which targets the 16S rRNA gene, I uncovered the microbial communities present within cryoconite holes at various glacier sites.

My findings support previous research, highlighting the significance of Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes as the predominant phyla in glacial ecosystems. Additionally, the clustering patterns observed among most samples, except for Ragnar, highlight the influence of abiotic factors, such as surface hydrology and distance from the ice margin, on microbial community composition. As the samples were relatively close in distance to each other, it was not a surprise that their microbial community composition was closely similar. Further research is needed to determine the exact factors that contribute to the composition of the microbial community.

Another key aim of this work was to compare my results with previous investigations of cryoconite communities. My findings align with existing literature, which also identifies Cyanobacteria, especially the genera *Leptolyngbya* and *Pseudanabaena*, as the most common taxa in cryoconite samples. Moreover, the prevalence of specific genera, such as *Pseudanabaena*, *Leptolyngbya*, *Phormidium*, and *Nostoc*, in cryoconite holes aligns with their known cold tolerance traits, indicating their psychrotolerance in glacial environments. Additionally, I found that Proteobacteria, particularly *Betaproteobacteria* and *Alphaproteobacteria*, were the second most abundant phylum in most samples, playing significant roles in heterotrophic processes. Furthermore, Actinobacteria, labeled as "keystone taxa" were the third most abundant phylum across all glacier sites, indicating their importance in shaping the microbial community composition.

Another important objective of this study was to characterize microbial communities based on their taxonomy and functional role in glacier ecosystems. While 16S rRNA gene sequencing provided insights into microbial diversity, it must be said that this approach shows limitations in understanding the exact function of the bacteria. Further studies employing whole-genome sequencing and functional metagenomic analyses should be conducted to determine the specific metabolic pathways and functional roles of the microbial communities.

Considering the physical, chemical, and nutritional conditions dominating glacial environments, efficient recycling of external carbon and nutrients is crucial. Microbial communities, particularly Cyanobacteria, play a significant role in the food web, with their photosynthesis producing 75 % to 95 % of available carbon.
Additionally, filamentous Cyanobacteria, such as *Nostoc* and *Phormidium*, produce EPS, a nutritional source for heterotrophs, contributing to the complex interactions within the ecosystem. Moreover, Cyanobacteria are involved in forming cryoconite holes, highlighting their importance in shaping glacial ecosystems. However, determining the precise functional role of the predominating phyla in these extreme environments is challenging. Thus, further studies should focus on the investigation of the functional roles of microbial communities to comprehend their ecological role and potential impacts on global biogeochemical cycles.

To conclude, it can be said that this thesis provides valuable insights into the taxonomic composition and diversity of microbial communities within cryoconite holes across different glacial sites in Svalbard.

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