

## Chapter

# Microbial Community Structure and Metabolic Networks in Polar Glaciers

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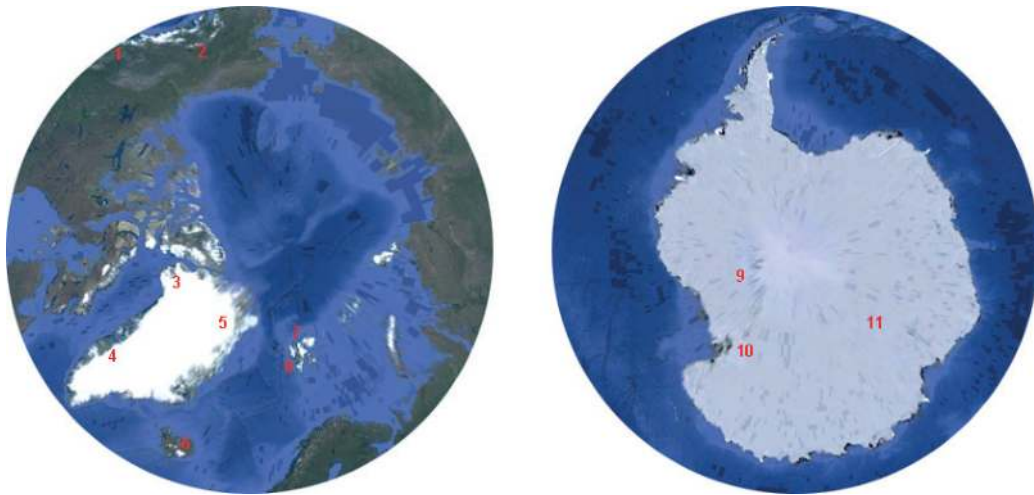
## Abstract

Polar glaciers are inhabited by numerous microorganisms including representatives of bacteria, archaea, microeukaryotes, and viruses. Low temperature is a main factor when considering polar glaciers as extreme environments. However, desiccation, low nutrients availability, ultraviolet irradiation, and photoreactive chemistry do also significantly influence their challenging life. Glaciers are highly selective and confined habitats, which make them favorable environments for adaptation and speciation. Depending on the glacier area studied, microorganisms establish a vertical food chain, from the surface photosynthesizers in upper illuminated layers to chemoautotrophs and heterotrophs confined to the inner part. These regions are rich not only in biodiversity but also in new mechanisms of adaptation to the environment, since selection acts with a particular intensity. Glaciers are retreating in many areas of the world due to global warming. When glaciers have ultimately withdrawn, microorganisms play a main role, carrying out key processes in the development of soil and facilitating plant colonization. These features make them unique and interesting for the study and protection of the biological heritage. Metagenomics have allowed a deeper understanding of microbial ecology and function of polar glacier microbial communities. In this review, we present a complete analysis of the microbial diversity in these ecosystems and include a thorough overview of the metabolic potentials and biogeochemical cycles in polar glacier habitats.

**Keywords:** polar glacier, metagenomics, diversity, community structure, metabolic networks

## 1. Introduction

Polar glaciers have aroused great interest over the last year, and their study has increased since they are sentinels of climate change. Although both poles are extreme environments (in terms of low temperatures, high UV radiation, lack of light in winter and permanent solar radiation in summer, scarce nutrients, etc.), Arctic and Antarctic glaciers are very different. The North Pole is an ocean surrounded by land, while the South Pole is a continent surrounded by water. This distinction confers them very unique geographical and environmental characteristics.



**Figure 1.** Polar maps and localization of the referenced glaciers. (1) Cascade Volcano Arc [4]; (2) Robertson Glacier, Alberta [5]; (3) Western margin of the Greenland ice sheet [6]; (4) southwest part of the Greenland ice sheet [7]; (5) North-Eastern Greenland [8]; (6) Icelandic glaciers [11, 12]; (7) Hamiltonbukta, Svalbard [9]; (8) Ny Ålesund, Svalbard [10]; (9) West Antarctic ice sheet [15]; (10) high Antarctic Plateau [13]; (11) Lake Vostok [14] (map source: Google Earth Pro).

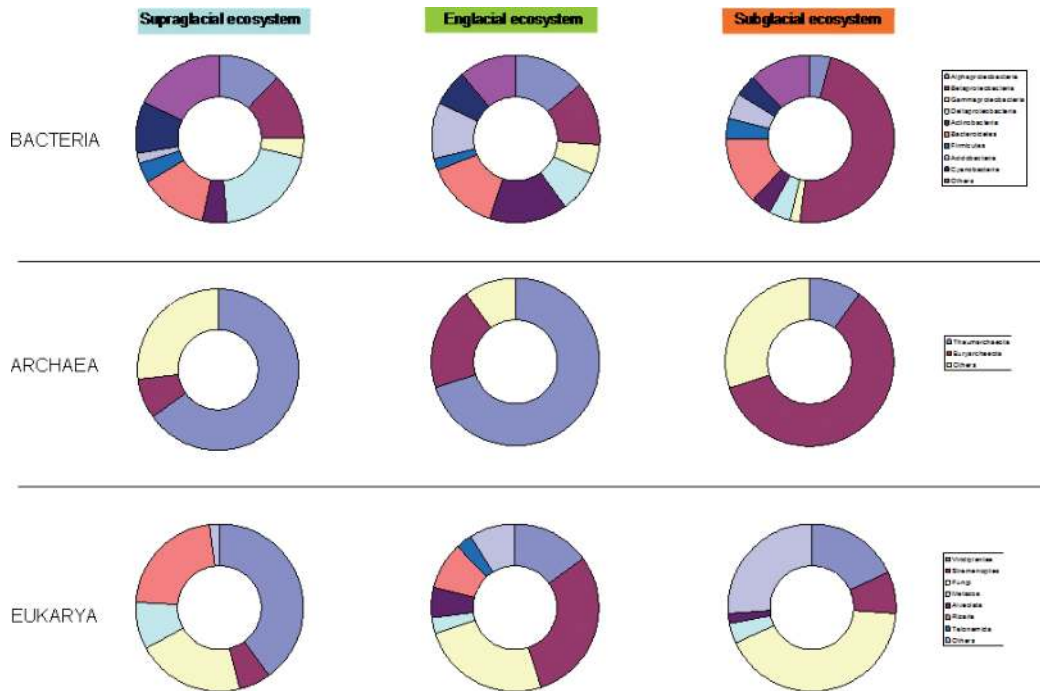
Metabolically active microbial communities have been identified in both the Arctic [1] and Antarctic glaciers (**Figure 1**) [2]. These microbial communities include bacteria, archaea, microeukaryotes, and viruses [3].

Arctic glaciers do not reach such distant latitudes or low temperatures as Antarctic glaciers do. These are some of the reasons why they are being extremely affected by global warming. There are glaciers around the entire Arctic Ocean, but polar glaciers in North America [4, 5], Greenland [6–8], Svalbard [9, 10], and Iceland [11, 12] (**Figure 1**) have been the most widely studied from a microbiological point of view. Antarctic glaciers present exceptional environmental conditions. Being in higher latitudes allows the existence of very low temperatures and high rates of solar radiation in summer. Some published reports on glacial and subglacial microbiology refer to very extreme latitudes that reach  $-75^{\circ}\text{S}$  in the high Antarctic Plateau [13],  $-77^{\circ}\text{S}$  in Lake Vostok [14], and  $-84^{\circ}\text{S}$  in the West Antarctic ice sheet [15] (**Figure 1**).

In the study of glacier microbiology, a variety of techniques have been traditionally used, such as microscopy techniques [16], cell cultures, and isolation of microorganisms [17]. However, the most significant advance has been achieved with the application of metagenomics. This discipline has allowed both the knowledge of the microbial communities' structure and the comprehension of their metabolic potential.

## 2. Microbial community structure through reconstruction of microbial genomes in polar glaciers

Glaciers have recently been considered authentic biomes [18]. It has been observed that microbial community composition depends on the area of the glacier studied [19]. In most of them, three well-defined and interconnected ecosystems can be defined: supraglacial, englacial, and subglacial ecosystems. These ecosystems are different in their solar radiation, water content, nutrient abundance, and redox potential [20]. These factors influence in the abundance and diversity of microbial populations inhabiting glaciers (**Figure 2**). They also affect the type of functionality and the biogeochemical cycles in these ecosystems.



**Figure 2.** Bacterial community structure in polar glacier ecosystems based on 16S and 18S rRNA gene sequences. Pie charts represent relative abundances of bacteria, archaea, and eukarya for three glacier ecosystems: supraglacial, englacial, and subglacial. The data are from [20, 11, 10, 4, 13, 8] for bacteria, from [11, 15, 5, 6, 14] for archaea, and from [19, 7, 12, 14] for eukarya.

## 2.1 The supraglacial ecosystem

The supraglacial ecosystem is the one which has best been studied. It has been reported that the main habitats in the supraglacial ecosystem are the snowpack, cryoconite holes (vertical cylindrical melt holes in a glacier surface), supraglacial streams, and moraines [20].

### 2.1.1 The glacial snow

The sunlit and oxygenated supraglacial surface is populated by autotrophic microorganisms such as cyanobacteria, microalgae, and diatoms [21], by chemolithotrophic bacteria, which feed on inorganic sand particles, and by heterotrophic bacteria and microeukaryotes [22] (**Figure 2**). The main bacterial classes that have been described in this ecosystem are *Betaproteobacteria*, *Actinobacteria*, and *Bacteroidetes* [11, 23]. The genus *Polaromonas* is one of the most abundant in the supraglacial area of Arctic [9] and Antarctic glaciers [24].

Among microeukaryotes, snow is mainly populated by pigmented algae, which have been observed in Arctic and Antarctic glaciers [25]. They belong to several taxa, mainly *Chlamydomonas*, *Chloromonas*, *Raphidonema*, and *Chrysothrix* [11].

Fungi, especially basidiomycetous yeasts and *Chytridiomycota*, have been reported in glacial snow and ice [26]. It is believed that they act as saprophytes and parasites, yet their diversity and function in this ecosystem are poorly known [25].

Archaea have also been identified in glacial snow and ice, although they have not been found in all the studies that have been carried out. They belong to Nitrososphaerales, which are known as important ammonia oxidizers [11].

### 2.1.2 Ice surfaces

Under the recently fallen snow, there is a layer of hard ice. This layer arises to the surface in the episodes of melting that occur during the polar summer. The ice surface constitutes a distinct type of supraglacial microhabitat that is different from cryoconite holes. It is mainly populated by microalgae (Zygnematophyceae) and by cyanobacteria [17].

### 2.1.3 Cryoconite holes

Cryoconite holes are predominantly inhabited by cyanobacteria [27]. Filamentous cyanobacteria such as *Phormidesmis*, *Oscillatoria*, *Leptolyngbya*, *Phormidium*, and *Nostoc* play an important role in cryoconites [28]. They produce organic material and extracellular polymeric substances (EPS), which act as cryo- and osmo-protectants [27]. Additionally, bacteria of the class *Actinobacteria* (*Microbacteriaceae* and *Intrasporangiaceae*) are also important members of cryoconite holes, followed by *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*. Archaea and eukarya are the least abundant and the least representative members of this environment [10].

## 2.2 The englacial ecosystem

In englacial ecosystems, live motile bacteria can reach more than 3000 m of depth. These bacteria reside in clay particles and ice channels. According to their metabolism, they can be both chemoautotrophs (i.e., *Streptomyces*, *Nocardia*, *Bacillus*) and heterotrophs (i.e., *Proteobacteria*, *Actinobacteria*) (Figure 2). The later bacteria feed on solubilized organic products from pollen grains and from other dead microorganisms. At great depth, anaerobic respiration takes place [29] and methanogens (for instance, *Firmicutes* and *Euryarchaeota*) are also active [20].

## 2.3 The subglacial ecosystem

The subglacial ecosystem is dominated by aerobic and anaerobic bacteria in basal bedrock and subglacial lakes. It does also contain diverse and metabolically active archaeal, bacterial, and fungal species [25] (Figure 2).

Among bacteria, species with chemolithotrophic activity have been identified; an example is *Sideroxydans lithotrophicus*, which is an iron sulfide oxidizer. Other bacterial taxa found in this ecosystem are *Thiobacillus* and *Thiomicrospira*, both associated with the sulfur and iron cycles [15].

Archaea in these anoxic environments are mainly represented by methanogenic and methanotrophic species [25]. Methanogenesis, the production of methane in an anaerobic process mediated exclusively by methanogenic archaea, is a very plausible process in the subglacial ecosystem. In glacier samples from this environment, methanogenic archaea of the euryarchaeal orders Methanosarcinales [5] and Methanomicrobiales have been detected [6].

Eukaryotes have only been found in some of the studied subglacial environments [19]. Among them, mainly fungi have been described [26]. *Basidiomycetes* predominate, among which *Cryptococcus* and *Rhodotorula* are the dominant genera.

## 3. Metabolic potentials and biogeochemical cycles in polar glaciers through reconstruction of microbial metagenomes

Living in such extreme environments implies coping with low temperatures, desiccation, low nutrients availability, and ultraviolet irradiation [30]. Over the last

years, metagenomics have allowed a great understanding of metabolic potentials and biogeochemical cycles in polar glaciers through reconstruction of microbial genomes (Figure 3).

Regarding the supraglacial ecosystem, metagenomic studies have demonstrated the wide diversity of functions in cryoconite holes, with a range of metabolic pathways which depend on their competence to acquire and degrade available nutrients [10]. Functional analyses highlighted the importance of stress responses and efficient carbon and nutrient recycling.

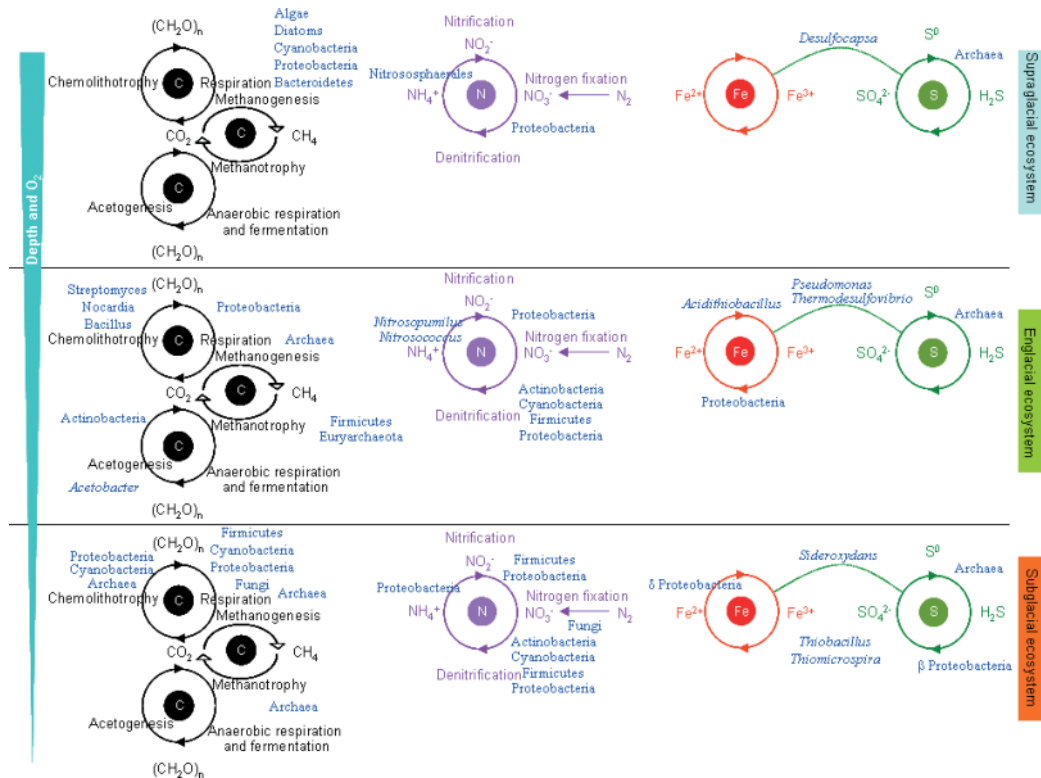
Metagenomic techniques have also been used to identify algal communities in the supraglacial ecosystem and their relationship with geochemical factors [12].

The potential of archaea as important ammonia oxidizers has been another finding achieved by metagenomics [11].

Little is known about the metabolic potential and the biogeochemical cycles of microbial communities inhabiting the englacial ecosystem. It has been reported that microorganisms enclosed in the englacial ice present very low metabolic rates, using energy only to repair damaged biomolecules and not to grow and reproduce [31].

In the subglacial ecosystem, some metagenomics data implied that the most abundant and active component were bacteria within the order *Methylococcales* [6]. Transcripts of the particulate methane monoxygenase from these taxa were detected, demonstrating that methanotrophic bacteria were functional members of this subglacial ecosystem.

At least three modes of carbon fixation were inferred [14]. The most common mode of carbon fixation was the reductive pentose phosphate cycle. The second in frequency was the reductive tricarboxylic acid pathway. This cycle also produces



**Figure 3.** Overview of the metabolic potentials between dominant microorganisms in the three polar glacial ecosystems. The data are from [20, 11, 10, 4, 13, 8] for bacteria, from [11, 15, 5, 6, 14] for archaea, and from [19, 7, 12, 14] for eukarya.

precursors for nucleic acid and aromatic amino acid syntheses. The third type of carbon fixation, the reductive acetyl-CoA pathway, is the one used by archaea [14].

These investigations did also identify genes that carry out various parts of the nitrogen cycle, including nitrogen fixation (*Actinobacteria*, *Cyanobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*), nitrification (*Alphaproteobacteria* and *Betaproteobacteria*), denitrification (*Gammaproteobacteria*), nitrate reduction (*Betaproteobacteria* and *Gammaproteobacteria*), anammox (*Planctomycetes*), assimilation (most microorganisms from these investigations), and decomposition (fungi and other heterotrophs) [14].

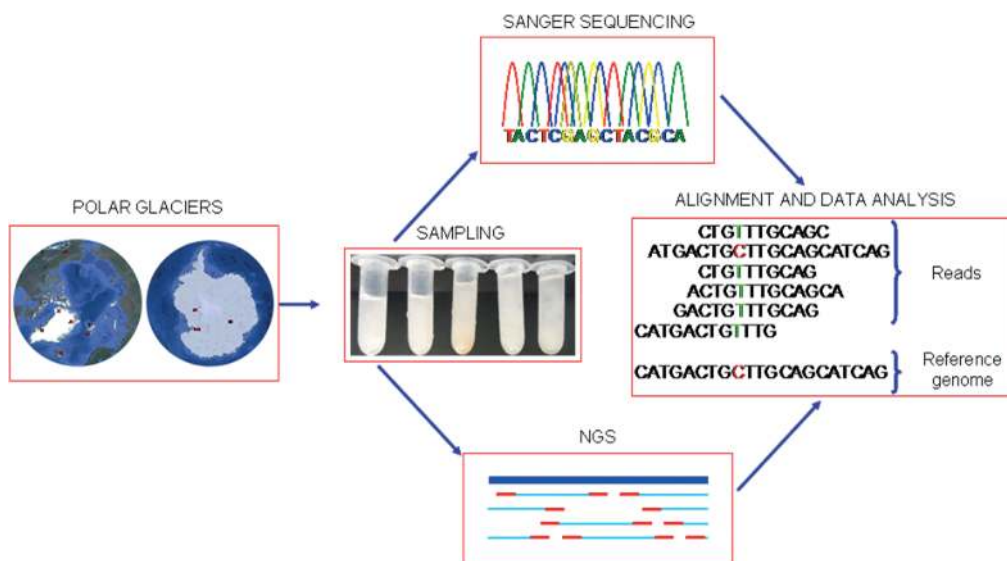
Characterization of the Antarctic Blood Falls microbial assemblage revealed taxa that could participate in active sulfur cycling, including autotrophs and heterotrophs such as *Desulfocapsa*, *Geopsychrobacter*, *Thiomicrospira*, and *Thermacetogenium* [32]. Although these microorganisms usually inhabit the subglacial ecosystem, in Blood Falls, they have been identified in brines collected from outflowing fluids (**Figure 3**).

#### 4. Comparison of metagenome analysis techniques

The metagenome of polar microorganisms has been widely studied in recent years. Their results can provide a great amount of information about the biodiversity, survival capacity, and functioning of microbial communities in these extreme environments. In addition, information about ancient communities preserved within glacial ice through time can be obtained [33].

##### 4.1 Sanger

Between 1975 and 2005, most of the DNA sequences were obtained through the application of the Sanger techniques [34], which led to the first generation of automated DNA sequencers [35] (**Figure 4**). For 16S or 18S rRNA sequencing, PCR amplification was carried out with specific primers (**Table 1**) and sequencing instruments based on capillary electrophoresis. Nowadays, Sanger sequencing achieves high read lengths of up to 1000 bp and per base and accuracies of 99.999%



**Figure 4.** Typical workflow in polar glacier metagenomics researches.

	Specificity	Primers	Sequence (5' to 3')	Product length (bp)	Authors	Reference
Sanger	Bacteria	16S-F	AGAGTTTGATCCTGGCTCAG	1000	Lane, 1991	[45]
	Bacteria	16S-R	CACGAGCTGACGACAGCC	1000	Lane, 1991	[45]
	Archaea	20F	TTCCGGTTGATCCYGCCRG	1372	Massana et al., 1997	[46]
	Archaea	U1392R	ACGGGGGGTGTGTRC	1372	Massana et al., 1997	[46]
	Eukarya	EukalF	CTGGTTGATCCTGCCAG	500	Lefranc et al., 2005	[47]
	Eukarya	Euk502R	TGATCCTTCTGCAGGTTACCTAC	500	Amann et al., 1990	[48]
	Bacteria	(V3-V4) 341F	ACACTGACGACATGGTTCTACACCTACGGGGGCGWGCAG	100	Herlemann et al., 2011	[49]
	Bacteria	(V3-V4) 805R	TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC	100	Herlemann et al., 2011	[49]
	Eukarya	(V9) 1380F	GCCTCCCTCGCGCCCATCAGXXXXCCCTGCCCHTTTGTACACAC	43	Amaral-Zettler et al., 2009	[50]
	Eukarya	(V9) 1510R	GCCTTGCCAGCCCCGCTCAGCCTTCYGCAGGTTACCTAC	39	Amaral-Zettler et al., 2009	[50]

**Table 1.**  
 Primer sequences for 16S or 18S rRNA sequencing.

[36]. In the de novo metagenomics, randomly fragmented DNA was cloned into a high-copy-number plasmid and then transformed in *Escherichia coli*. However, whole-genome sequencing by this technology was extremely expensive and time consuming.

Some examples of microorganisms from polar glaciers analyzed with these technologies were Antarctic bacteria from the Dry Valleys [37] and the Arctic ice pack [38]. In general, the number of sequences identified by this technique was scarce. However, this method has the advantage of generating long reference sequences, which are very useful for studies of taxonomy and biodiversity.

## 4.2 NGS

Next-generation sequencing (NGS) technology is similar to capillary sequencing (**Figure 4**). The main difference is that, instead of sequencing a single DNA fragment, NGS develops this process with millions of DNA fragments.

The introduction of pyrosequencing technology by 454 life sciences in 2005 began the NGS innovation. This allowed the identification of thousands of short-sequencing reads without the need for cloning. This technique was used to research the microbial life in the Dry Valleys, Antarctica [39], and Ace Lake, Antarctica [40], and in Arctic glaciers from Svalbard [10].

Since then, many other NGS technologies have been developed. The Illumina platform (MiniSeq, MiSeq, NextSeq, HiSeq, and NovaSeq instruments) is based on sequencing by synthesis of the complementary strand and fluorescence-based detection of reversibly blocked terminator nucleotides. The platform includes multiple instruments with varying read length. For example, Illumina sequencing has been employed in a metagenomic research into diazotrophic communities across Arctic glacier forefields [41] and in the metagenomic analysis of basal ice from an Alaskan glacier [42]. Sequencing of 16S and 18S rDNA PCR amplicons is the most common approach to investigating environmental prokaryotic diversity, despite the known biases introduced during PCR. Recently this method has been improved with the use of 16S rDNA fragments derived from Illumina-sequenced environmental metagenomes [43]. Furthermore, newer Illumina sequencers produce longer reads (e.g., the HiSeq2500 and MiSeq produce  $2 \times 150\text{bp}$  and  $2 \times 250\text{bp}$  reads, respectively, which after merging can generate reads up to, e.g., 290 and 490 bp).

Other metagenomic studies based on the Ion Torrent platform were also based on sequencing by synthesis, but the detection was performed using semiconductor technology. Ion Torrent technology was applied to analyze red snow microbiomes and their role in melting Arctic glaciers [12].

The main drawback of the aforementioned second-generation sequencing platforms is that they generate relatively fragmented genome assemblies. In order to produce closed reference genomes, longer reads are required [36]. To meet this demand, third-generation sequencing platforms have been developed. These technologies directly target single DNA molecules without the need for PCR amplification. The PacBio RSII platform uses single-molecule real-time (SMRT) sequencing technology which allows to obtain extremely long DNA fragments of 20 kb and even longer [43].

## 4.3 Genome analysis tools

Environmental microbiome sequencing analysis consists of binning sequencing reads into taxonomic units to compare the microbial composition of samples. This information will allow the knowledge of the microbial population taxonomy, diversity, and functioning. When these data are correlated to certain environmental parameters,



Category	Tool <sup>1</sup>	Taxonomy <sup>2</sup>	Function	URL
Use all available sequences	MEGAN/MEGAN4	B	+	<a href="https://ab.inf.uni-tuebingen.de/software/megan4">https://ab.inf.uni-tuebingen.de/software/megan4</a>
	MG-RAST	B/E	+	<a href="https://www.mg-rast.org/">https://www.mg-rast.org/</a>
	Genometa	B	–	<a href="http://genomics1.mh-hannover.de/genometa/">http://genomics1.mh-hannover.de/genometa/</a>
	Kraken	B	–	<a href="https://ccb.jhu.edu/software/kraken/">https://ccb.jhu.edu/software/kraken/</a>
	LMAT	B	+	<a href="https://computation.llnl.gov/projects/livermore-metagenomics-analysis-toolkit">https://computation.llnl.gov/projects/livermore-metagenomics-analysis-toolkit</a>
	Taxator-tk	B	–	<a href="https://github.com/fungs/taxator-tk">https://github.com/fungs/taxator-tk</a>
	CLARK	B	–	<a href="http://clark.cs.ucr.edu/">http://clark.cs.ucr.edu/</a>
	GOTTCHA	B/E	–	<a href="http://lanl-bioinformatics.github.io/GOTTCHA/">http://lanl-bioinformatics.github.io/GOTTCHA/</a>
	EBI	B	+	<a href="https://www.ebi.ac.uk/metagenomics/">https://www.ebi.ac.uk/metagenomics/</a>
Use a set of genes	MetaPhyler	B	–	<a href="http://metaphyler.cbcb.umd.edu/">http://metaphyler.cbcb.umd.edu/</a>
	QIIME6	B	–	<a href="http://qiime.org/">http://qiime.org/</a>
	mOTU	B	–	<a href="https://omictools.com/motu-tool">https://omictools.com/motu-tool</a>
	MetaPhlAn	B/E	–	<a href="http://huttenhower.sph.harvard.edu/metaphlan2">http://huttenhower.sph.harvard.edu/metaphlan2</a>
	One Codex	B/E	–	<a href="https://onecodex.com/">https://onecodex.com/</a>

<sup>1</sup>Incomplete list compiled from sources.

<sup>2</sup>B, bacterial taxa; E, eukaryotic taxa.

**Table 2.**  
*Metagenome analysis tools.*

both ecological and biogeochemical analysis can be performed. Taxonomic binning of 16S and 18S rRNA reads is usually based on one of these four databases: SILVA, Ribosomal Database Project, Greengenes, and NCBI [44]. For instance, the Ribosomal Database Project was used to perform a metagenomic analysis of Illumina sequences to identify bacterial communities in Antarctic surface snow [45].

Several tools have been developed to investigate the taxonomic composition of metagenomes and, in some cases, the functional composition of the community. These tools can be classified into two groups: those that use all the available sequences (MEGAN/MEGAN4, MG-RAST, Genometa, Kraken, LMAT, Taxator-tk, CLARK, GOTTCHA, EBI) and those that use a set of genes (MetaPhyler, QIIME6, mOTU, MetaPhlAn, One Codex) [33]. These genome analysis tools are summarized in **Table 2**.

An example of the use of these tools is the metagenomics analysis with MG-RAST performed to study Arctic microbial communities [41]. Sequence analysis with QIIME was performed with cryoconite samples from Arctic glaciers [10] and with permafrost samples from the Antarctic Dry Valleys [39].

Although metagenomics is changing rapidly, still new improvements in the development of analytical tools and databases are required to answer important questions in polar glacier microbiology.

## 5. Conclusions

- Extraordinary advances in metagenomics have allowed a great understanding of microbial ecology and function of polar glacier microbial communities.

- Important novel tools to study environmental microbiology based on metagenomics are being developed.
- Third-generation technologies may further revolutionize metagenomic research.
- The application of new technologies to metagenomic studies of polar glaciers will enable to link the diversity and functionality of these habitats.
- Significant challenges for metagenomics remain, especially in data processing and genome analysis.

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## **Conflict of interest**


The authors declare that none of us has any competing commercial interests in relation to the submitted work.

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