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Subglacial Microbial Ecosystems and Biochemical Reservoirs: Insights into Metabolic Potential beneath the Glaciers and Ice Sheets

D19, D2.6 A catalogue of metabolites in subglacial habitats (M37, GFZ)

DC5- Anirban Majumder, GFZ

Subglacial environments

Subglacial environments are unique, dark, extreme habitats, located beneath glaciers and ice sheets. These environments are mostly isolated from the external inputs for long periods, and in the case of subglacial lakes, they can preserve biosignatures, including those representing paleoclimatic conditions (Livingstone *et al.* 2022; Davis *et al.* 2023). Subglacial streams, ice, and sediments also play crucial roles in regulating glacial-bed basal hydrology, ice melting and flow dynamics shown in **Figure 1** (Clarke 2005).In addition to their physical importance, subglacial systems are also the habitat of largely unexplored microbial communities that drive subglacial biogeochemical cycles, influencing subglacial fluxes (Tranter, Skidmore and Wadham 2005; Davis *et al.* 2023).

Subglacial systems beneath alpine glaciers and polar ice sheets consist of diverse aquatic habitats, including subglacial streams, water-saturated sediments, and subglacial lakes. These habitats have been identified across polar and glaciated regions worldwide primarily through remote sensing and ground-penetrating radar geophysical methods (Livingstone *et al.* 2022). These include Antarctica (Wadham *et al.* 2012; Christner *et al.* 2014; Achberger *et al.* 2016), Greenland (Palmer *et al.* 2013; Dieser *et al.* 2014), various mountain glaciers in Svalbard or the Canadian Arctic (Tranter *et al.* 1996; Boyd *et al.* 2010; Rutishauser *et al.* 2018) or under Iceland's ice caps (Marteinsson *et al.* 2013; Vannier *et al.* 2023).

At the surface of glaciers and ice sheets, microbial primary production is driven by photosynthesis, which is promoted by sunlight (Boetius *et al.* 2015; Kaczmarek *et al.* 2016; Lutz *et al.* 2016, 2017) and is dominated by mostly aerobic processes, with biomass in the melt season being controlled by snow and glacier algal processes (Anesio *et al.* 2017; Hoham and Remias 2020) or cyanobacteria in cryoconite holes (Segawa *et al.* 2017). In contrast, subglacial habitats are aphotic and mostly anoxic and fed by seasonally melting surface water delivered through englacial channels of melting at the bedrock–ice interface. Although we know relatively little about subglacial settings due to the difficulty of accessing these environments, from the few studies that exist, we know that subglacial environments are characterized by extremely low organic carbon contents [DOC ≈ 0.15 mg L^{-1}], resulting in an oligotrophic ecosystem for microbial life (Hood *et al.* 2015; Vick-Majors *et al.* 2016). This leads to chemolithoautotrophic and heterotrophic microbial communities being dominant in these low-carbon, dark habitats (Sattley and Madigan 2006; Boyd *et al.* 2014; Christner *et al.* 2014; Dieser *et al.* 2014; Achberger *et al.* 2016). Although organic matter, carbon or other nutrient inputs to fuel microbial life in these energy-limited environments are scarce, sometimes such inputs can also come from supraglacial meltwater or subglacial marine sediments, preserved beneath the ice surfaces (Wadham *et al.* 2012, 2016).

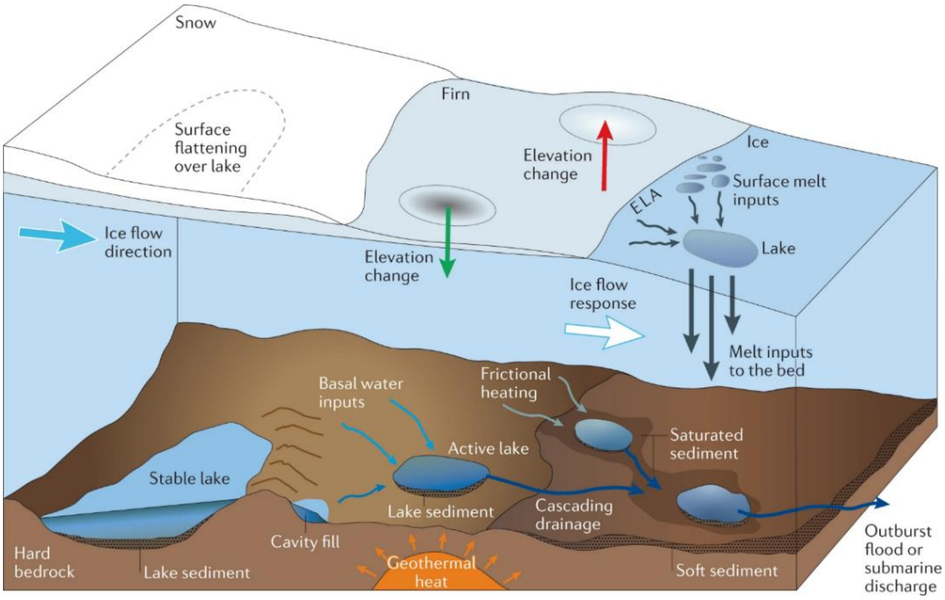


Figure 1- Schematic representation of the subglacial environment showing subglacial lakes, sediment and streams (from Livingstone *et al.* 2022).

Subglacial Microbial Ecosystems and Metabolic Potential

The subglacial environment is characterized by extreme conditions, including the absence of sunlight-derived energy, low temperatures, high pressure and limited nutrient availability, all of which pose significant challenges to microbial survival. These habitats were for a long time considered sterile and inhospitable to life; however, recent evidence revealed that metabolically active microbial communities can persist and adapt within these isolated ecosystems (Vick-Majors *et al.* 2016), and that both in subglacial water (Marteinsson *et al.* 2013; Christner *et al.* 2014) and sediments (Achberger *et al.* 2016; Davis *et al.* 2023), beneath glaciers and ice sheets, respectively, and these microbes contribute to regulating various subglacial biogeochemical cycles (Vannier *et al.* 2023).

Through many recent studies, the presence of microbial life in subglacial environments has now been relatively well documented, but the mechanisms by which these microorganisms generate energy to sustain life under extreme subglacial conditions remain poorly understood. In the dark and oligotrophic settings typical of such subglacial settings, microbial communities thrive by producing energy chemolithotrophically by 'mining' elements from subsurface minerals through various redox processes (Christner *et al.* 2006; Boyd *et al.* 2014; Gill-Olivas *et al.* 2021). For example, they can oxidize or reduce various forms of nitrogen (Boyd *et al.* 2011; Wadham *et al.* 2016), iron (Achberger *et al.* 2016), methane (Boyd *et al.* 2010; Wadham *et al.* 2012; Dieser *et al.* 2014), or sulfur (Purcell *et al.* 2014) to obtain the energy necessary for their survival (Vannier *et al.* 2023).

Although no metabolomics data sets related to subglacial microbial processes have been reported in the literature. Below are the major biogeochemical metabolisms that are likely to regulate microbial processes in the subglacial ecosystem. The data discussed below are derived from other omics data sets and not metabolomics data.

Methane metabolism

Under anoxic conditions, methane plays an essential role in microbial carbon cycling, serving as an energy source for methanotrophs and as an end product for methanogens, as shown in **Figure 2** (Niu, et al. 2018). For example, in subglacial lakes and sediments beneath the Greenland Ice Sheet, methanogenic and methanotrophic communities have been identified, such as the archaeal members *Methanosarcinales and Methanomicrobiales,* and the bacterial taxa *Methylococcales* (Dieser *et al.* 2014). The detection of functional genes such as methane monooxygenase (*pmoA*) in methanotrophic bacteria further supports the presence of active methane cycling within these subglacial ecosystems (Dieser *et al.* 2014). Similarly, in the subglacial sediment of the West Antarctica ice sheet, the bacteria *Methylobacter* – a facultative methylotroph - has been described (Christner *et al.* 2014; Achberger *et al.* 2016; Michaud *et al.* 2017). Subglacial methane reservoirs in Antarctica also show insights into the presence of methane-metabolizing microorganisms (Wadham *et al.* 2012). In such subglacial environments, metabolically active microbial communities perform methane oxidation, chemoautotrophic carbon fixation and methanogenesis, all of which represent widespread and significant metabolic processes in these ecosystems (Boyd *et al.* 2010; Wadham *et al.* 2012).

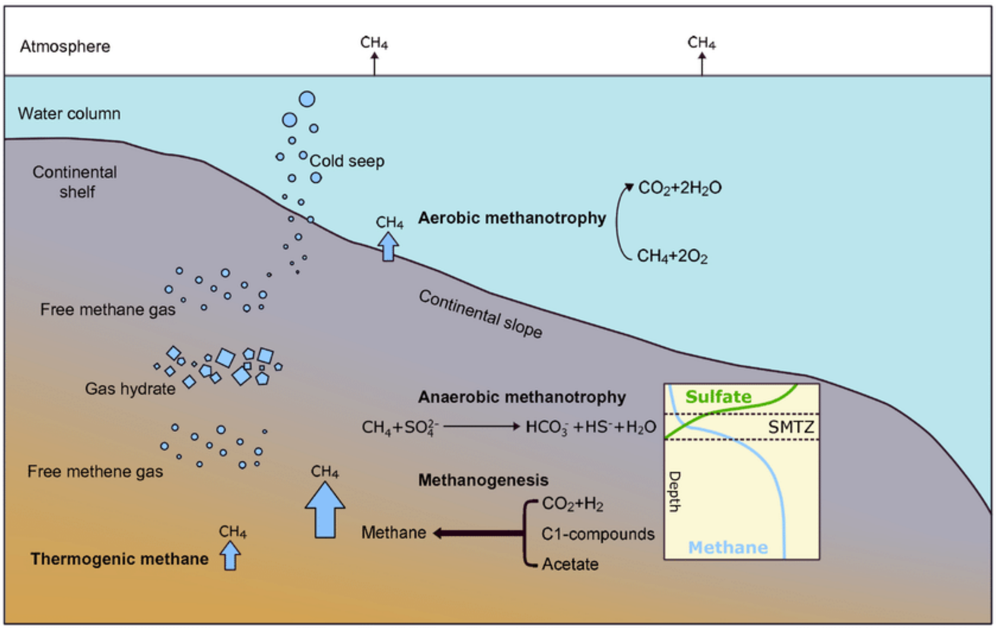


Figure 2- Predicted methane metabolism in subglacial habitat mimicking from marine subsurface (from Niu et al. 2018).

Sulfur metabolism

Another abundant element that microbes can utilize as electron donors or acceptors, and that is often present in minerals underlying glaciers and ice sheets, is sulfur (Gill-Olivas *et al.* 2021). Sulfur is present in sulfate and sulfide minerals (i.e., $CaSO_4$ *2 H_2O, or FeS_2), typical of sedimentary, volcanic or metamorphic rock formations. For example, sulfides in the form of the mineral pyrite (FeS_2) can be used by sulfide- and iron-oxidising bacteria that are often present as key organisms of subglacial microbial communities. From amplicon sequencing data sets, we know that, for example, in Antarctic subglacial sediments, microorganisms including *Thiobacillus, Sulfurifustis, Sideroxydans,* and *Gallionella* are present (Purcell *et al.* 2014). These bacteria play crucial roles in sulfur (**Figures 3 and 4**) and iron biogeochemical cycling by oxidising these compounds and producing energy in these oligotrophic environments (Achberger *et al.* 2016; Davis *et al.* 2023).

In subglacial lakes of Iceland, analysis of 16S rRNA genes, other bacterial genera, such as *Sulfuricurvum* and *Sulfurospirillum*, have also been detected. Genes involved in sulfate and sulfite reduction, for example, were identified in *Desulfosporosinus*, whereas genes responsible for sulfate oxidation were detected in *Sulfuricurvum* (Marteinsson *et al.* 2013; Vannier *et al.* 2023). The presence of these microorganisms and their functional genes provides valuable insights into sulfur cycling (**Figures 3 and 4**), showing both oxidation and reduction processes and highlighting the complex chemolithotrophic metabolic networks that sustain microbial life within subglacial environments (Boyd *et al.* 2014).

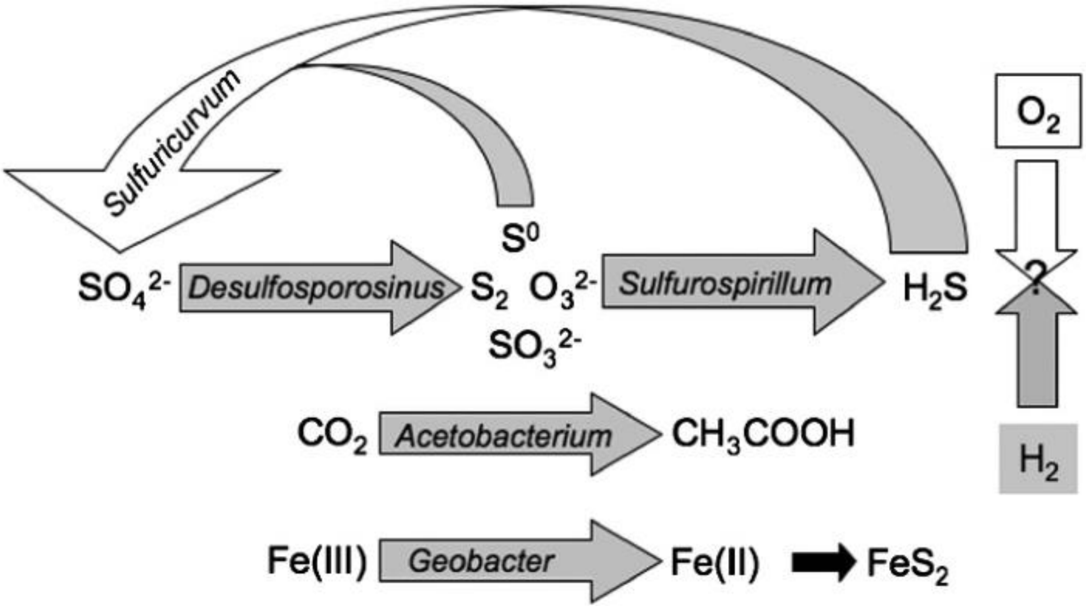
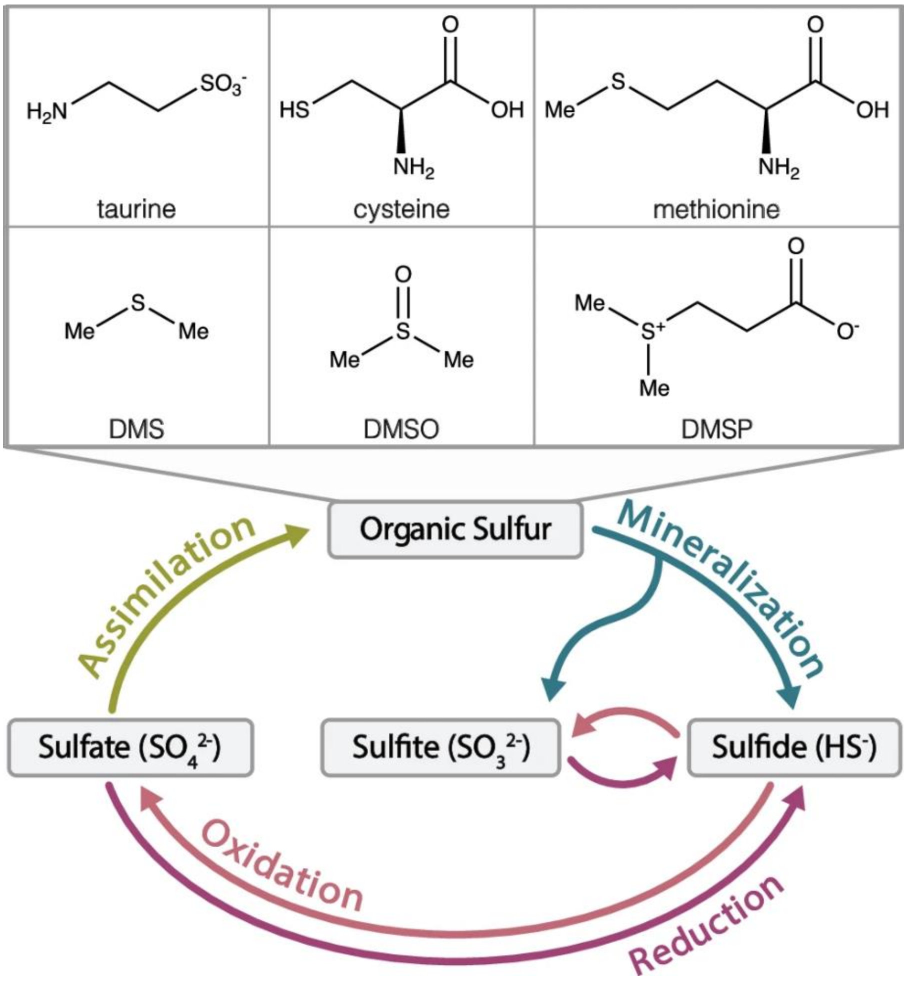


Figure 3- Schematic of possible chemolithoautotrophic sulfur metabolic pathway in a subglacial lake in Iceland (from Marteinsson *et al.* 2013).

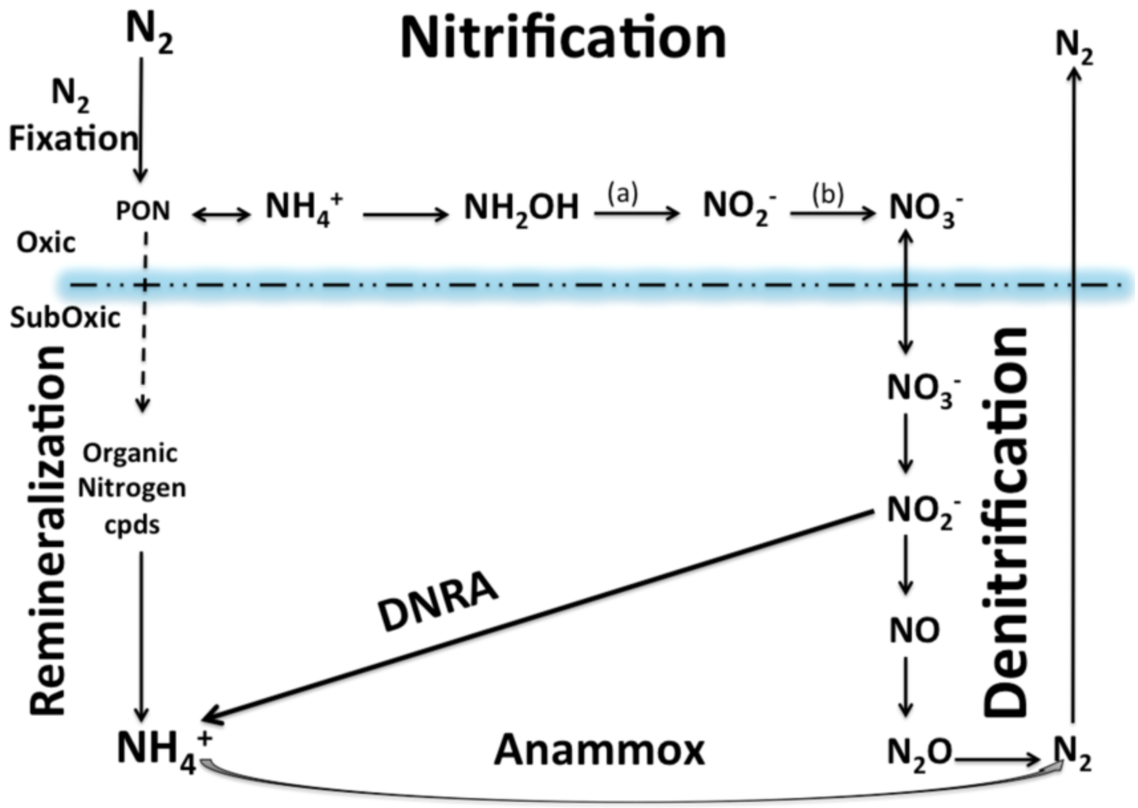


<u>Figure 4</u>- Proposed model of recycling of organic and inorganic sulfur compounds and their interaction/remobilization in the biogeochemical sulfur metabolism in subglacial ecosystems (from Patsis *et al.* 2025).

Nitrogen metabolism

In these oligotrophic environments, besides methane and sulfur cycling, other chemolithoautotrophic bacterial and archaeal communities that are involved in nitrogen cycling have also been characterized in Antarctic subglacial lake sediments (Boyd *et al.* 2011). Among bacterial genera, *Candidatus Nitrotoga* and *Nitrospira* were identified as the dominant nitrite-oxidizers, playing a key role in the conversion of nitrite to nitrate. Among archaea, *Candidatus Nitrosopumilus* and *Candidatus Nitrosoarchaeum* were reported to perform the first step of nitrification, oxidizing ammonia to nitrite. Similarly, ammonia oxidation to nitrite and nitrate was also found to be performed by bacterial members such as *Nitrosomonas* and *Nitrosospira* (Christner *et al.* 2014; Davis *et al.* 2023). These studies all show that subglacial microbial communities can mediate processes such as nitrification, denitrification, ammonification and nitrogen fixation, thus highlighting the importance of nitrogen metabolism in sustaining life beneath the ice (Boyd *et al.* 2011).

Furthermore, metagenomic analyses also revealed the presence of genes associated with diverse nitrogen metabolic pathways. For example, in Icelandic subglacial lakes, genes involved in nitrate and nitrite reduction were linked in bacterial genera such as *Geobacter* and *Pedobacter*, suggesting active nitrogen cycling in these environments (Vannier *et al.* 2023). This same study also identified nitrogen fixation genes that convert nitrogen to ammonia linked to bacterial genera, including *Acetobacterium* (Vannier *et al.* 2023). Together, these microbial taxa illustrate the functional diversity and metabolic adaptability of subglacial ecosystems in maintaining active nitrogen turnover under cold, low-nutrient, and oxygen-limited conditions.



<u>Figure 5</u>- Theoretical pathways showing nitrogen species exchange between oxic supraglacial and anoxic subglacial systems. This might be possible, driven by meltwater transport and microbial transformations (taken from Farouk *et al.* 2012, a study about marine processes, but the similar principles apply).

Subglacial Metabolomics Challenges and Future Perspective

Importance of studying subglacial metabolomics

Metabolomics serves as a powerful approach for elucidating the real-time biochemical activity of microbial life. Although so far not applied to the study of subglacial processes directly, metabolomics approaches can enable the identification of active metabolic pathways and provide insights into the physiological adaptations and survival strategies employed by microorganisms in these extreme environments. Through analyses of the metabolomes, specific metabolites can be detected and their importance, for example, in identifying stress response compounds (Atasoy *et al.* 2024; Doting *et al.* 2024), cryoprotectants (Cleland *et al.* 2004), or antioxidants (Carballo-Cárdenas *et al.* 2003; Doting *et al.* 2024; Arslan *et al.* 2025), can be elucidated. From the studies mentioned above, which are either laboratory stress induced in cultures (Atasoy *et al.* 2024) or light-induced stress in supraglacial habitat (Doting *et al.* 2024). We know that these metabolites play crucial roles in protecting cellular structures, and these may also facilitate chemolithoautotrophic processes under freezing subglacial conditions. Although not yet available, it is well known that metabolomics provides a critical link between the other available omics datasets. For example, while genomics, transcriptomics and proteomics can provide insights into the metabolic and functional potential of subglacial microbial communities, metabolomics bridges microbial gene expression with actual metabolic fluxes and end products from microbial physiological responses to their environment. This approach can reveal temporal and spatial dynamics of microbial activity, capturing changes driven by seasonal variations, environmental chemistry, or mineral composition within subglacial ecosystems. Thus, metabolomics provides a unique window into subglacial microbial biogeochemical cycling and energy flow, processes that cannot be fully predicted by other omics techniques alone. However, as already mentioned as yet there are no metabolomics data sets reported in the literature about microbial processes in subglacial environments. Below are outlined some challenges that have hindered subglacial research work so far in sample collection and processing.

Challenges in sampling for subglacial metabolomics

The primary challenge in studying subglacial metabolomics is access to samples from subglacial environments that are collected with the specific aim of studying metabolomic processes. This is linked most often to the extremely low microbial biomass, which increases the risk of contamination during sampling and often leads to samples being preferentially used for DNA sequencing. Although not targeting metabolite analyses, to minimize the risk of contamination, previous research on subglacial ice cores, for example, reported the use of chemically and microbiologically clean drilling methods with filtration, UV-treated and pasteurized hot water (Priscu *et al.* 2013; Achberger *et al.* 2016; Michaud *et al.* 2020; Makinson *et al.* 2021). Another significant difficulty lies in sample handling and sample processing. In a recent supraglacial study, it was documented that the conditions under which, for example, ice samples are thawed will compromise metabolite stability and alter the native biochemical profiles of the microorganisms (Peter *et al.* 2024). If subglacial samples were to be obtained, another challenge that has to be overcome is the preservation of the samples. For such extreme settings and low biomass, there are currently no standardized protocols to process such unique subglacial samples. Finally, the absence of bioinformatics tools and specialized databases to detect low-abundance metabolites and unknown compounds hinders the comprehensive characterization and understanding of the complex metabolite profiles and compositions in the subglacial samples. These challenges make the study of subglacial metabolomes difficult, but with the development of new approaches in the future, this will also be possible.

Conclusion and Outlook

Metabolomic processes are so far unexplored in subglacial research. They represent a major frontier for understanding microbial activity and biochemical processes beneath the glaciers and ice sheets. Ideally, in a future world, we will be able to integrate metabolomic data with other omics datasets, metabolic flux measurements, and isotope labelling experiments. Such a multi-tiered approach would provide deeper insights into subglacial microbial and biogeochemical processes and enable more accurate predictions of active metabolic pathways in these unique environments. The lack of data opens diverse research opportunities, not only for characterizing novel metabolites and their ecological roles but also for improving our understanding of subglacial ecosystem function, biogeochemical cycling and energy flow. Future research should prioritize method development, stringent contamination control, and comparative metabolomics across cryospheric systems, including the establishment of a dedicated cryosphere-specific metabolite database to promote future investigations.

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Declaration

The author has used ChatGPT (OpenAI) and Grammarly to assist with a review structure, language editing, grammar improvement, synonyms and making the sentences concise and scientific. All the content in this article was reviewed by the author with no plagiarism.