

A CRITICAL ASSESSMENT OF TECHNOLOGIES FOR THE STUDY OF ORGANIC
MATTER IN GLACIERS AND ICE SHEETS

by

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A professional paper submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Resources and Environmental Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2019

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DEDICATION

To
B.B.E.

ACKNOWLEDGEMENTS

Thank you to Dr. Christine Foreman for engaging conversation, honest opinions, and for reinvigorating my love for polar science. It was dearly missed.

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GLOSSARY and NOMENCLATURE

Ablation zone	Area of the glacier where there is net loss of ice
Labile	Highly bioreactive, can easily be consumed by heterotrophs within hours or days
Semi-labile	Bioreactive, can be consumed by heterotrophs within months
Recalcitrant	Can be consumed, but is resistant to biological degradation
Inner filter effect	The reabsorption of light that occurs at both excitation and emission. Must be removed from excitation emission matrices

ABSTRACT

Polar and temperate glaciers harbor active microbial communities and a substantial storage of organic carbon. These frozen ecosystems are especially sensitive to the effects of climate change and are expected to release roughly 15 teragrams of carbon by 2050. This creates a sense of urgency for further experimentation to increase our understanding of glacier ecosystem function and the impact glacier habitats have on local and global biogeochemical cycles. Due to the complex nature of organic matter, there is no single method which is suitable for every study. Technological advancements have improved methods for determining the quantity and quality of organic matter and emerging new technologies are providing faster and less-costly ways to overcome the challenges of working in these harsh environments. Consequently, a synthesis of peer-reviewed literature was conducted to summarize the current state of microbial ecology of glaciers and ice sheets, and to explore the techniques and new tools which are being developed to aid in the study of these rapidly disappearing ecosystems. The culmination of this work is an introduction and guide for analysts interested in examining the source, transformation history, and fate of organic matter in glacial systems. It was found that there is not one single technique superior to another, rather the appropriate technique is dependent on the questions being addressed and the resources available.

INTRODUCTION

The cryosphere covers 20% of the Earth's surface and approximately half of this frozen system is made up of glaciers and ice sheets (Fountain et al., 2012; Milner et al., 2017). Despite their expansiveness, glaciers and other icy environments have historically been excluded from studies of the biosphere. Initial studies of ice cores, such as the Camp Century core drilled from the Greenland ice sheet in 1963, focused primarily on stable isotope measurements and other inorganic compounds used for reconstruction of Earth's climate history. The first biological studies of ice cores were controversial (Abyzov, 1993), but they changed the scientific community's perception of glaciers and ice sheets and seeded an interest in observing the biological components of glacier systems (Fig. 1). Recent calculations estimate there may be on the order of 10^{29} cells in all of Earth's glaciers and ice sheets (Anesio et al. 2017) and roughly 6 petagrams of organic carbon (Hood et al., 2015).

Once established that biological materials, including prokaryotic cells, are present in natural ices, the prevailing view was that cells capable of living in these extreme conditions likely do so in a dormant state (Cowan & Tow, 2004; Castello & Rogers, 2005). However, over the last 15 years it has been demonstrated that many of these cells are not dormant. Instead they comprise diverse and distinct

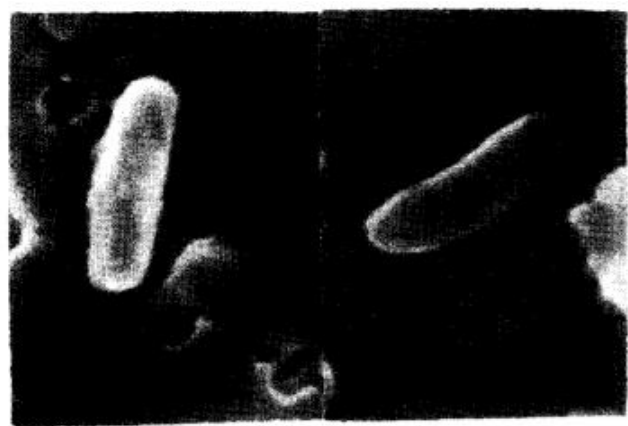


Figure 1. Scanning electron microscopy image (20k mag.) taken from Abyzov et al. (2001) and purportedly one of the earliest images of cells from a deep ice core. These cells were isolated from accretion ice above Lake Vostok, Antarctica.

microbial communities which are active in the liquid water regions of the ice (Campen et al., 2003; Singer et al., 2012; Musilova et al., 2017; Smith et al., 2016, 2018). Metabolically active microbial communities have been reported across every region of glaciers and ice sheets, from seasonally dynamic ablation zones, down to subglacial lakes and streams where the ice meets bedrock. Due to progressive research in this area of study, the cryosphere has been recognized as a microbially dominated biome and glaciers are considered integral parts of biogeochemical cycles (Anesio & Laybourn-Parry, 2012; Foreman et al., 2007).

Although advancements have been made, the extent to which these microbial communities influence nutrient cycling on regional and global scales is not well-defined. One of the most critical gaps in knowledge is the limited understanding of how organic matter (OM) interacts with and influences concomitant microbial communities within glacier systems. OM is a heterogeneous mixture of carbon compounds of varying molecular weight and structure. OM in glacier systems is ecologically significant for proglacial marine and freshwater systems. The quantity and composition of OM exported in glacial runoff during summer seasons can affect microbial respiration and downstream aquatic food webs (Fellman et al., 2015). Additionally, the presence of microbial communities and other organic materials may decrease the surface albedo of glaciers, quickening their retreat (Williamson et al., 2019; Cook et al., 2015).

Beyond their role in Earth systems, icy ecosystems such as glaciers and sea ice are receiving increased attention as proxies for potential extraterrestrial habitats (Garcia-Lopez & Cid, 2018). Jupiter's smallest Galilean moon, Europa, and Saturn's moon, Titan, have both been identified by NASA as targets in the search for life on ocean worlds in the outer solar system (Hendrix et al., 2019). Spectral data from the Cassini spacecraft revealed the presence of water

ice on the surface of Titan (Griffith et al., 2019) and there is evidence that Europa is covered in a water ice shell <25-km thick (Hand et al., 2017). The Europa Clipper, a multiple flyby mission to Europa, is scheduled to launch in the 2020s and will seek more information about the chemical makeup of the moon's icy crust. This mission and the potential for a future lander mission have fueled interest in studying organics in ices here on Earth. Understanding how microorganisms adapt and alter the composition of OM in cold, high-radiation environments such as the Earth's polar ice sheets may inform the search for biosignatures and potential life in extraterrestrial water ice.

Increased interest in glacial OM composition across many scientific disciplines, as well as its inherent molecular complexity, has led to varied approaches to quantification and characterization of OM in ice. Additionally, there are many reviews in the literature on soil and aquatic OM and reviews of microbial activity in glaciers, but very little has focused on the character of OM in frozen systems and associated microbial activity. Therefore, to address this gap in the literature, this review will summarize the current knowledge of OM in glaciers and ice sheets, and the role of active microbial communities in its production and transformation. I then discuss the gold standards and the emerging new technologies for OM characterization and microbial detection and their potential application in future studies of organic material in glaciers and ice sheets.

METHODS

All literature cited in this synthesis was peer-reviewed and published within accredited journals and institutions. Most of the articles were found through literature searches conducted on the Montana State University Library System, and the full list of source material can be found in the 'References Cited' section starting on page 28. The references were selected based on relevance to the subject matter, the scientific methods used by the researchers, and the publication date. Preference was generally given to more contemporary research, but foundational papers were also sought out for a thorough examination of each topic and analytical method discussed within this review.

My experience conducting field work in polar regions and working with ice samples was taken into consideration when writing this review. I have worked with sea ice as part of the Long-Term Ecological Research (LTER) project at Palmer Station, Antarctica. Separately, I worked on the LTER in the McMurdo Dry Valleys, as well as the NASA-funded PSTAR project, WATSON, in southwestern Greenland. These experiences undoubtedly shaped my perspective of working with ice samples, polar field science, and the analytical methods mentioned in this synthesis.

At the time of this writing many discrepancies remain as to the general composition, source, and age of glacial organic matter. The full significance and role of microbial metabolism in these systems is also subject to debate. This state of the field of study reinforces the need for a thorough investigation of current literature, however, it was not be possible to cover every article written on this broad topic. This synthesis was conducted with the intention of creating a thorough review of the literature to the best of my ability although the primary focus of this work

is on microbially derived OM. The intention is to present a comprehensive review of the current literature on OM in icy environments and a comprehensive overview of the tools that could be used for furthering our understanding of it.

MICROBIAL ECOLOGY OF GLACIERS

Glacier Habitats

Glacier ice may be suitable for life wherever liquid water is found, even on a micron scale in the liquid-filled veins between ice crystals (Maccario et al., 2015). Cell abundance in glacier ice is associated with the concentration of sediment or dust particles. These particles can act as a substrate and source of nutrients which may supply active microbial communities (Stibal et al., 2012). Dust, prokaryotic cells and other organic and inorganic material are deposited onto the surfaces of glaciers by wind and precipitation. These materials may originate from lower latitudes or elevations and may have traveled long distances between continents (Chuvuchina et al., 2011).

The geographic location, sunlight regimes, and differences in the local environment can affect the organic and inorganic composition of glaciers (Chuvuchina et al., 2011). In turn, this has been associated with differences in the microbial community structure of supraglacial habitats (Edwards et al. 2013). The taxonomic groups most widely reported in snow and ices from polar and alpine glaciers are Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (Edwards et al., 2013, Shen et al., 2017), however, studies by Cameron et al. (2016) and Smith et al. (2018) have shown that even among supraglacial habitats on the same glacier or ice sheet there can be significant structural and functional diversity.

Glacier habitats can be divided into three general categories: supraglacial (located within a few meters of the glacier surface and including snowpack), subglacial (the basal ice and hydrological systems beneath glaciers), and englacial habitats (the vast zone between the other two regions which is characteristically solid ice). Figure 2 illustrates the breadth of these microbial habitats in glacier systems.

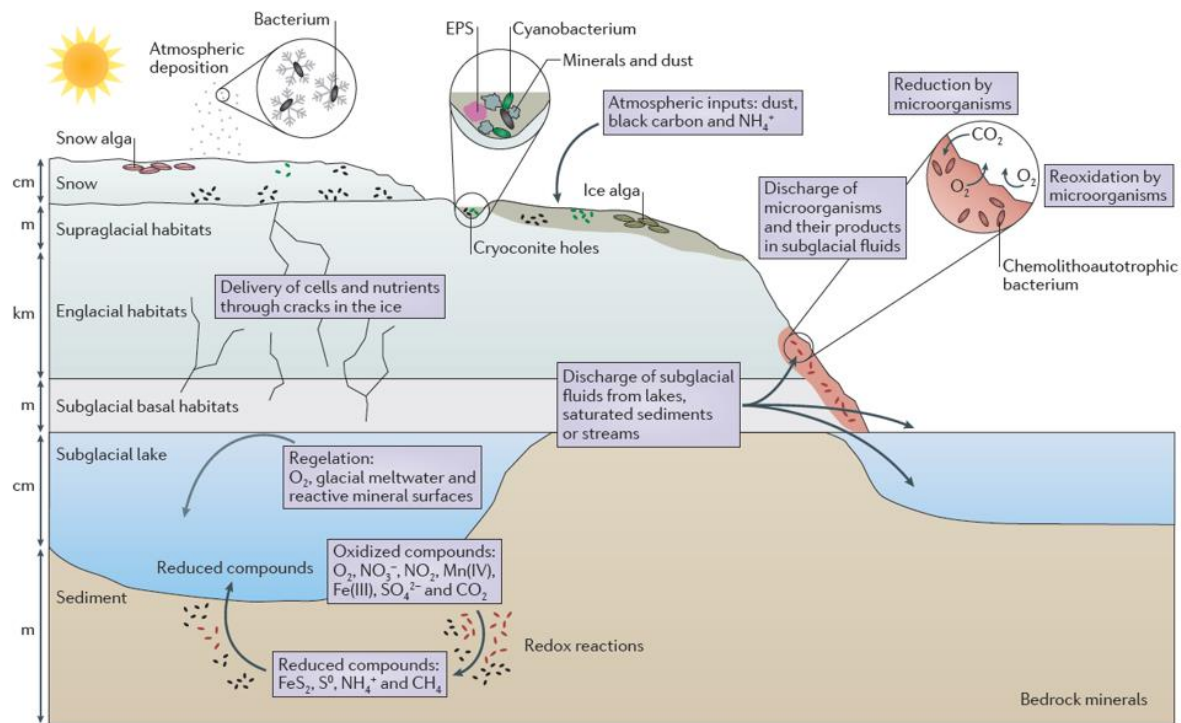


Figure 2. Representation of microbial habitats and microbial metabolism on glaciers and ice sheets from Boetius et al. (2015).

Consideration of the englacial zone as habitable is somewhat controversial since there has been no direct observation of active microbial communities in these ices. Work that has been done is largely theoretical or based on representative laboratory studies (Price, 2007; Bakersman

& Skidmore, 2011). This region is thought to play a negligible role in nutrient dynamics and there is some dispute as to whether the englacial zone should be considered ‘habitable’ (Hodson et al., 2008). However, this region makes up the bulk of a glacier’s volume and is thought to store a considerable amount of carbon. This zone is also the most analogous to what may be found on extraterrestrial icy worlds such as Europa (Garcia-Lopez & Cid, 2018). More studies are needed to assess the habitability of englacial ice.

Research on supraglacial and subglacial habitats has been much more robust. Microbial metabolism in subglacial environments is dominated by anoxic and lithotrophic pathways due to limited access to sunlight, isolation from the atmosphere and ample minerals present in the bedrock. Studies from Greenland and Antarctica have revealed that simpler, low-energy yielding metabolic processes such as methanogenesis, methane oxidation, and iron reduction are the primary means of energy for subglacial communities (Dieser et al., 2014; Nixon et al., 2017).

Supraglacial environments are dynamic relative to the other glacier regions with high inputs of aeolian materials and transport of nutrients facilitated by liquid water in supraglacial streams and melt ponds during warmer months. Due to high solar radiation, supraglacial microbial metabolism is dominated by



Figure 3. Photograph of a refrozen cryoconite hole cored from the Commonwealth Glacier, Antarctica.

photosynthesis (Hodson et al., 2008; Williamson et al., 2019).

Cryoconite holes are unique supraglacial features derived from a combination of surficial sediment and biological material (Figure 3). When heated by solar radiation, these surface particles begin to sink into the ice and form cylindrical pits in the glacier surface. The production of biofilms adheres the sediment particles in a layer and warmth from radiation absorption may leave pockets of liquid water above the sediment layer. Compared to the surrounding ice, cryoconite holes become rich with life and organic materials including photosynthetic bacteria, exopolymeric substances (EPS), humic substances, and sometimes small multicellular organisms such as rotifers and tardigrads (Cook et al. 2015; Smith et al., 2016). These surface features are significant contributors to carbon and nitrogen cycling on glacier surfaces (Foreman et al., 2007; Telling et al., 2012).

Adaptation to life in ice

Adaptation for survival in these cold environments is not limited to adaptation to low temperatures. Once cells are immured in ice, they are often subjected to other environmental stressors such as poor nutrient availability, osmotic pressure, aridity, and fluxes in pH. Additionally, glaciers and ice sheets are located either at high latitude or high elevation, consequently their surfaces are often exposed to significant UV radiation. These extreme conditions require microbial cell adaptations which can affect both their intracellular and extracellular milieus.

These adaptations can include spore formation or thickening cell walls. Some of the components of EPS, like that in cryoconite holes, may aid in survival in icy environments. Ice-binding proteins can be attached to cellular membranes or secreted into the extracellular environment. These proteins inhibit the recrystallization of ice thereby protecting cell membranes from rupturing during freeze thaw cycles (Mangiagalli et al., 2017). In response to high radiation some microorganisms will increase expression of pigments colloquially called sun-screen pigments. These pigments can cloak cells from lethal DNA damage caused by solar irradiation. Shen et al. (2017) showed that the percentage of cultivated colonies from the Tibetan Plateau which expressed pigmentation was positively correlated with increased depth. This suggests exposure to repeat fluxes in radiation selects for microorganisms capable of producing these pigments.

GLACIAL ORGANIC MATTER

Chemical Composition

Discussions of OM can be complicated by the absence of a universal consensus on naming conventions. For instance, aquatic OM is often filtered before analysis to be divided into dissolved and particulate fractions. However, the qualifications for what is ‘dissolved’ versus what is ‘particulate’ is ambiguous and dependent on the filtration process chosen by the analyst (Repeta, 2014). For the purposes of this review the term organic matter (OM) will be used broadly to encompass all organics regardless of size but excluding microorganisms and viruses.

The smaller molecular components of OM include simple monomers such as vitamins and fatty acids, larger polymers such as proteins and carbohydrate, and very complex carbon compounds such as black carbon. Examples of larger components that may contribute to OM are complex humic substances, pollen grains, and plant fragments. Some of the previously mentioned proteins, pigments, and extracellular exudates involved in cellular cold adaptation may contribute to OM, potentially creating a composition unique to icy environments. Figure 4 depicts the various components that may contribute to a pool of natural OM and its cycling in glaciers and ice sheets.

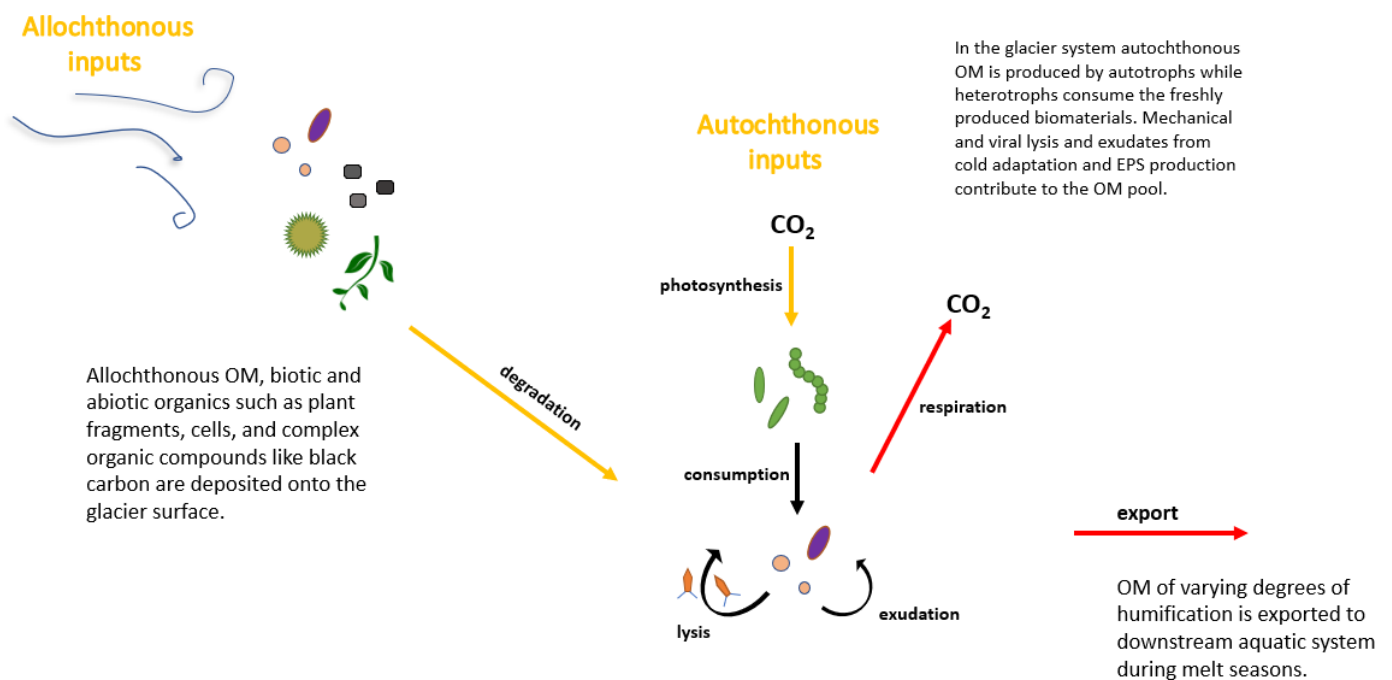


Figure 4. Depiction of components and cycling of carbon in glacial systems. Yellow arrows indicate sources, red arrows represent outputs and black arrows represent englacial transformations. of OM.

When discussing OM and how it interacts with microbial communities, it is often said to be either labile, semi-labile, or recalcitrant. Compared to marine and freshwater ecosystems, OM in glacial ices is reportedly more labile (D'Andrilli et al., 2015) and therefore more susceptible to transformation by microbial metabolic activity. The carbon pool in the Antarctic ice sheet is thought to be especially labile due to its relative geographic isolation from aeolian inputs and considerable autochthonous production of OM via microbial photosynthesis (Smith et al., 2017). Determining the lability of OM can be challenging, but it is an important predictor of its fate in biogeochemical cycles.

Supraglacial OM cycling

The source of OM in glacier environments is still a subject of debate. Radiocarbon dating has suggested that glacier OM is ancient (Hood et al., 2009) and the results from Stubbins et al. (2012) indicated that the source of this ancient material is the anthropogenic combustion of fossil fuels. Surprisingly, these studies also found the OM exported to downstream ecosystems to be highly labile, which is contradictory to what has been seen in studies of age and bioreactivity from other natural environments (Raymond & Bauer, 2001).

In addition, studies of primary production on glaciers in Antarctica and Greenland have demonstrated that microbial metabolism is a major source of labile OM (Smith et al., 2017, Musilova et al., 2017). The work by Smith et al. (2017) showed the amount of carbon fixed from CO₂ by phototrophs from an Antarctic supraglacial stream was approximately four times the

amount consumed by glacial heterotrophy implicating the glacier surface as a net sink for atmospheric CO₂ and an exporter of labile OM.

It is unclear whether the radiocarbon aging contradicts these more recent studies of primary production and more experimentation is needed. It is possible that this aged OM was originally produced by ancient microbial communities by processes like those observed by Smith et al. 2017. It seems most likely that there are multiple carbon sources and the contributions of each is dependent on the glacier's unique environment. Future analysis of OM from a larger sample size of glaciers and larger transects are needed to distinguish among spatial and temporal differences to create a more refined understanding of carbon sources.

Our understanding of OM transformation on glacier surfaces is also in its infancy. In contrast, marine microbial processes have been extensively reviewed (Jiao et al., 2010). The 'biological pump' and 'microbial loop' describing the microbial processes involved in carbon fixation and sequestration is a known model for marine systems. Similar biological processes have been described on glacial surfaces, however no model currently exists. Unlike the biological pump in the ocean which shunts a large portion of recalcitrant and particulate organic matter to the deep ocean, the glacial biological pump likely shunts low molecular weight and highly labile matter to downstream environments.

METHODS OF DETECTION AND CHARACTERIZATION

For those interested in probing the nature and composition of organics in icy environments, there are several techniques for collecting character data in the lab and in the field.

The most commonly used methods for determining the chemical character of OM are based on either the molecular formulae or the optical properties of the mixture. Methods widely used for molecular-level analysis include high-resolution Mass Spectroscopy (MS) and Nuclear Magnetic Resonance (NMR). These techniques give detailed structural information about the elemental and molecular components which can be used to deconvolute heterogeneous mixtures such as natural OM. Concurrently, optical techniques are another popular method for analyzing OM chemistry based on the optical signatures from the fluorescent portion of OM. The following discussion is organized around brief introductions to techniques for investigating OM quality that were found in the literature, and examples which demonstrate how they can be used for studies of icy systems.

Mass Spectroscopy

High-resolution mass spectroscopy is one of the preeminent techniques for chemical characterization of natural OM. If working with solid ice, the sample must first be melted, followed by a chemical or physical fractionation procedure such as solid-phase extraction (SPE) or ultrafiltration, SPE being the popular choice for aquatic and ice samples (Repeta, 2014; D'Andrilli et al., 2017; Antony et al., 2014). Fractionations are performed in order to isolate the OM from the sample, and although these processes are standard practice, they have been suspected to alter the character of OM (Repeta, 2014). Fractionation procedures can also be relatively complex and time-consuming, making sample preparation a potential drawback to techniques which require it.

Following fractionation, the material is usually injected into the spectrometer. At this point the molecules of the material undergo ionization by an electron beam before being passed through a magnetic field. This process bends the ions based on the strength of their charge and the spectrum is collected by a detector. This is a very simplified explanation and Biemann (2014) is recommended for a more detailed review of MS.

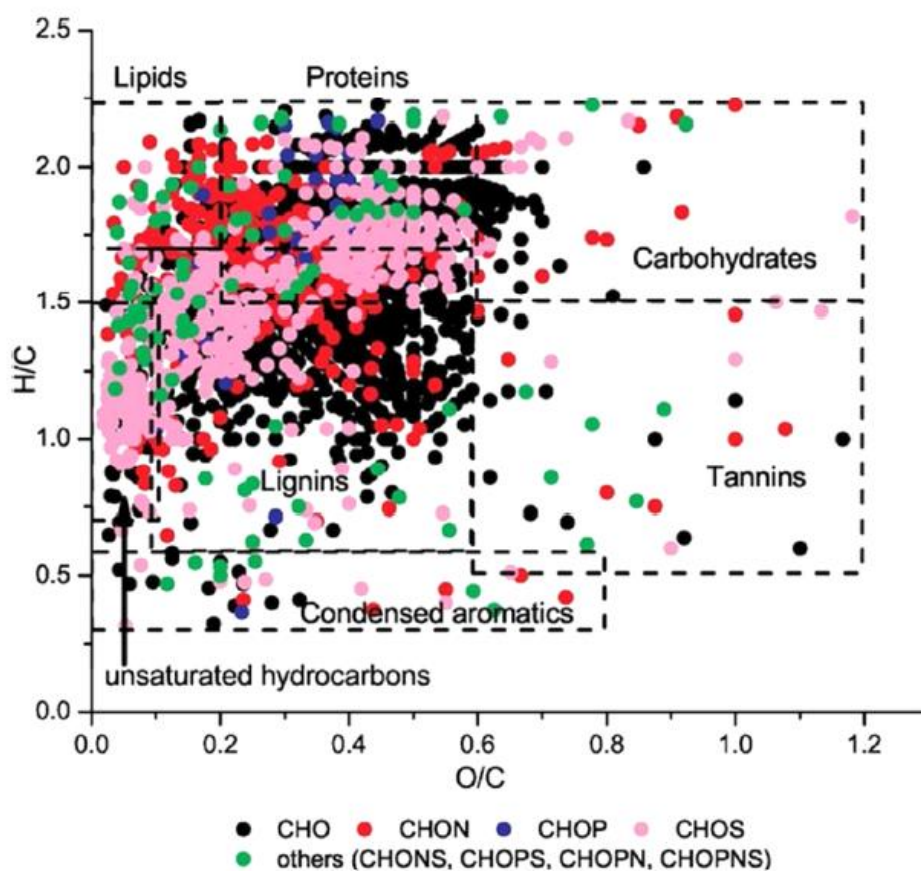


Figure 5. The van Krevelen diagram is the primary method for visualizing MS data of natural OM. This plot shows the molecular formulae obtained from a sample of OM from Antarctic snow (from Antony et al., 2017).

Mass spectra are used to identify the chemical formulae for the molecules present in a given sample. For the analysis of OM, molecules are often divided into seven categories of natural compounds defined by their atomic ratios: lipids, proteins, carbohydrates, lignins, tannins, unsaturated hydrocarbons, and condensed aromatics.

The most common plot for visualizing mass spectra of OM is the van Kevelan diagram with the hydrogen:oxygen (H/O) atomic ratio plotted as a function of the oxygen:carbon (O/C) atomic ratio. Figure 5 shows a representative van Kevelan diagram of OM from Antarctic surface snow collected by Antony et al. (2018). In this study, the researchers employed the use of MS to measure the initial composition of OM in Antarctic snow. They performed a series of irradiation experiments on samples with and without the addition of microorganisms and used MS to measure the effects on OM transformation. The results showed that there were both photochemical and microbial-driven transformations that occur separately and in combination creating molecularly diverse pools of organic material. Studies such as this which probe the source and transformations of OM are one of the more popular uses of mass spectroscopy.

Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance is another common method for determining the molecular structure of OM in natural environments based on the environment (chemical bonding) of either the hydrogen or carbon atoms in the sample. Like MS it is also an excellent tool for assessing the source and transformations of OM. Compared to MS, NMR often provides a more detailed molecular analysis and it has the added benefits of being both quantitative and non-destructive.

However, it is much less commonly used than MS for studies of glacial OM. This may be due to the lower sensitivity of NMR compared to MS and the much higher costs of purchasing, operation, and maintenance.

Unlike mass spectroscopy, NMR analysis of OM does not require fractionation to isolate the OM from the sample. Instead, sample preparation generally calls for filtration before analysis. There has been some dispute as to whether this step may alter the OM character (Maske & Garciamendoza, 1994). For their work on Antarctic glacial ice, Pautler et al. (2011) determined that the filtration process did not seem to alter the NMR spectra. For a more detailed review of the technical aspects of NMR and its applications for environmental studies the review by Simpson et al. (2018) is recommended.

In Pautler et al. (2012) NMR spectra were used for a detailed molecular-level comparison of cryoconite material from three different Arctic and Antarctic glaciers (Figure 6). The prominence of protein and fatty acid signals, as well as an absence of signal from plant-derived biomarkers in the Antarctic cryoconite OM indicated there are differences in OM composition dependent on the glacier's geographic location. These results agree with other studies suggesting that Antarctica's relative isolation from intercontinental aeolian inputs results in primarily microbially-derived organic matter.

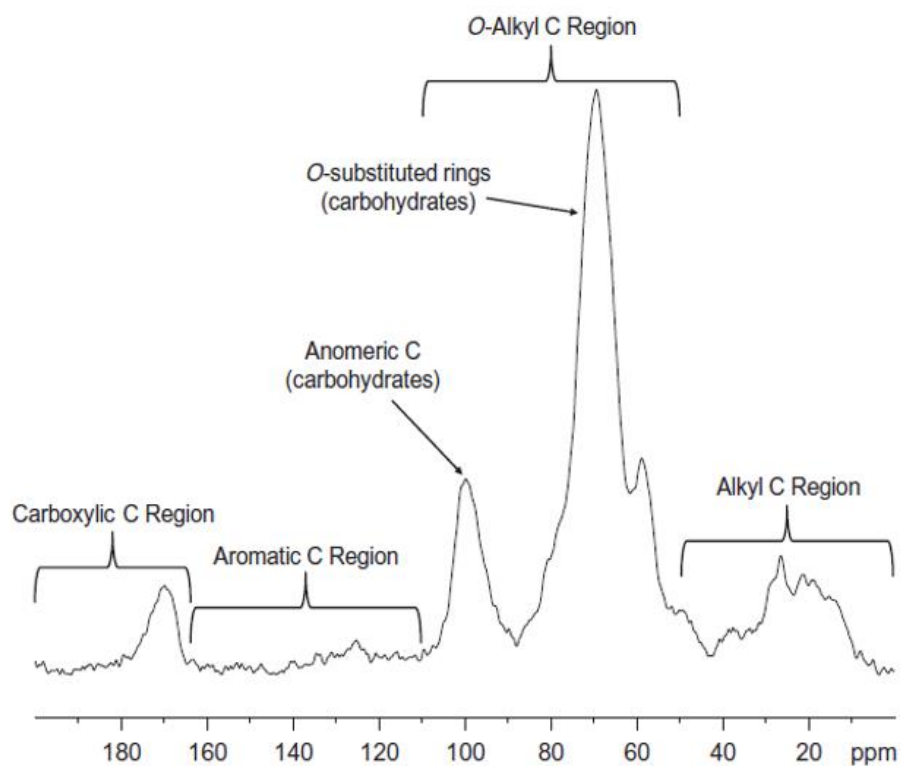


Figure 6. NMR spectrum of a sample of cryoconite organic matter (<0.2 nm) from Pautler et al. (2012). This spectrum shows the major carbon functional groups. NMR spectra are commonly displayed without a y-axis label and the x-axis showing the chemical shift of the peaks relative to the peak of a standard such as tetramethylsilane (TMS).

Fluorescence Spectroscopy

Fluorescence spectroscopy is an alternative approach to defining the character of OM by analyzing the portion of the mixture which is optically active. Although fluorescent chromatophoric OM makes up only a portion of the analyte, its optical properties have been

linked to its underlying biochemistry (Stedmon et al., 2003). Emission Excitation Matrices (EEMs) have been especially successful for inferring the bioreactivity of OM in icy and aquatic systems and for determining its source and transformation history (D'Andrilli et al., 2015; McKnight et. al, 2001; Barker et al., 2010). The popularity of fluorescent techniques may be attributed to how quick and cost effective it is to use them. Minimal sample preparation is required if the samples are optically dilute, thus the low cost, high sampling rate leads to greater spatiotemporal coverage. When measuring OM in solid ice samples, they must first be melted. As these samples are typically optically dilute there are no inner filter effects to consider and filtration and dilution are optional.

Multivariate parallel factor (PARAFAC) is the primary analysis method for identifying fluorescent components and constructing excitation emission matrices. Fluorescence data is broken down into three parts: excitation (the wavelength at which the molecule or functional group absorbs photons), emission (the wavelength of the emitted light), and the relative intensity (usually measured on the Z axis). Coble et al. (1996) identified five major fluorescent peaks in marine dissolved OM. This metric is still the most commonly used reference for deconvoluting dissolved OM fluorescence and although this metric was first developed for the identification of fluorophores in aquatic ecosystems, it has since been applied to the analysis of OM fluorescence from many natural environments.

Table 1 lists each of these five components and their respective excitation and emission wavelengths. Fluorophores A and C are associated with humic-like fluorescence and indicative of more recalcitrant compounds. Fluorophores B and T identify more labile protein-like compounds, and the M fluorophore is associated with marine humic-like fluorescence. The

lability of samples from supraglacial environments has been determined by the high intensities of peaks B and T reported in the literature (Pautler et al., 2012; Smith et al., 2017). Diagnostic indices have also been created to quantify important ecological attributes from fluorescence signals. For instance, the Fluorescence Index may be used to determine if the OM is terrestrially or microbially derived, with a higher number (>1.7) indicating microbial production (McKnight et al., 2001; McKnight & Cory, 2005). The Freshness Index, described by Parlanti et al. (2000), can also be derived from fluorescence data, and its value indicates the contribution of recently produced OM to the carbon pool. Furthermore, the Humification Index may be used as an indicator of the level of humification of the OM in a sample (Ohno et al., 2002). For a more in-depth review of EEMs Fellman et al. (2010) is recommended and Murphy et al. (2013) for more information regarding PARAFAC analysis.

Table 1. Fluorescent components of organic matter as described by Coble (1996). The wavelengths of excitation (Ex.) and Emission (Em.) are given for each component in nanometers.

Fluorophore	Ex. λ (nm)	Em. λ (nm)	Indication
A	260	380-460	Humic-like, recalcitrant
B	275	310	Protein-like, labile
T	275	340	Protein-like, labile
M	312	380-420	Marine humic-like, Recalcitrant
C	350	420-480	Humic-like, recalcitrant

Laser-induced deep ultraviolet (DUV) fluorescence is another optical method which has been used to identify microbial cells, complex degradation products, and other organic material (Bhartia et al., 2010; Shkolyar et al., 2018; Eshelman et al., 2019). Like EEMs, this technique relies on the natural fluorescence of OM but has higher sensitivity due to the use of higher excitation energy. Bhartia et al. 2010 demonstrated DUV fluorescence microscopy can be used

to image a single cell, and Eshelman et al. 2019 showed that stand-off applications can detect down to ~ 2 micromolar concentration of free amino acids or less than 300 cells. Additionally, it is not necessary to melt an ice sample to acquire spectral information, leaving the spatial context of the organic material intact within the ice matrix. Figure 7 demonstrates spatially resolved fluorescence data collected in lab prepared ice samples.

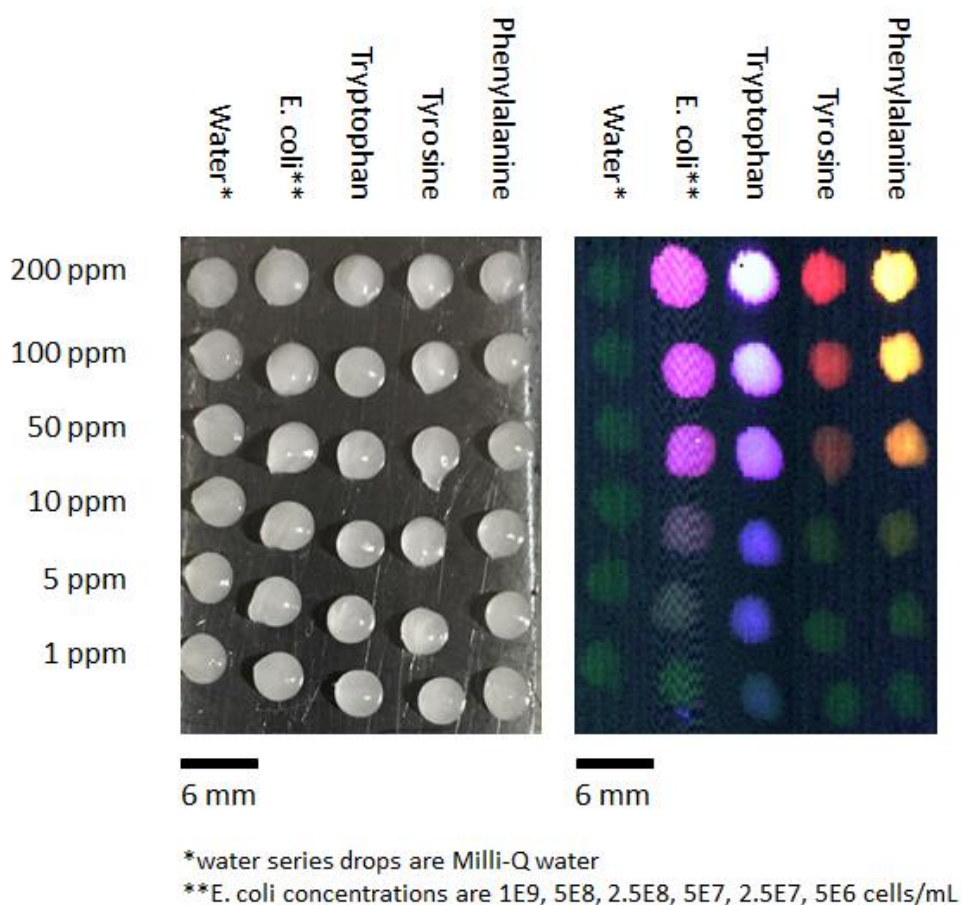


Figure 7. Dilution series of *E. coli* and aromatic amino acid solutions were frozen in water droplets (supercooled and flash-frozen) onto an aluminum plate. DUV fluorescence spectrometer was used to create a spectral map of the fluorescent material on the plate (from Mellerowicz et al., 2018). The differences in false color are associated with differences in the fluorescence (wavelengths) emitted from the different solutions.

Raman Spectroscopy

Raman spectroscopy is a proven tool for nondestructive chemical characterization of organic and inorganic substances. The techniques' high molecular specificity has motivated its application across disciplines. It is commonly used in paleogeology, forensics, and material sciences. More recently Raman spectroscopy has been applied to the study of active biological systems, but the ecologically relevant qualities of OM Raman spectra have yet to be established. As a method for identifying biosignatures Raman spectroscopy has largely been used to examine ancient carbonaceous material in the fossil record. Raman spectroscopy has also been used to differentiate among microbial species, distinguish whole prokaryotic cells from free floating biomolecules, and detect organics at <0.1 wt% concentration on Mars analog surfaces (Sil et al., 2017; Sapers et. al, 2019;Abbey et al., 2018).

Raman spectroscopy introduces monochromatic light to a sample, triggering a change in the vibrational state of the molecules and creating inelastic scattering of photons (Raman scattering). This type of light scattering is a rare occurrence and until recent advancements in efficient laser sources and optics this method was considered low sensitivity. The use of a UV laser source (~244-266 nm wavelength) may induce resonance or pre-resonance vibration of aromatic rings. This decreases the limit of detection particularly for biomolecules such as aromatic amino acids, and proteins which contain them.

In ice core analyses Raman spectroscopy has been used to effectively detect trace gases and minerals within the inter-grain boundaries of ice crystals with negligible melting or

transformation of the ice crystal lattice (Barletta et al., 2012). Additionally, Green Raman Spectroscopy (~542 nm laser source) has proven to be an excellent tool from identification of pigments including carotenoids, the photoprotective pigments often expressed in cold-adapted microorganisms in glacier habitats (Jehlicka et al., 2016; Amaretti et al., 2014; Shen et al., 2018).

Field Instrumentation

Progressive improvements have advanced these technologies and, in some cases, adapted them for field instrumentation. Access to polar and high alpine field sites is often limited by sunlight hours and severe weather making time for data collection in the field highly valuable. Remote sensing and field instrumentation are ideal for extending the sampling season and removing the need for costly shipping of samples back to the lab for further analysis. Shipping and handling of ice can introduce contamination and variations in temperature (even those temperatures below freezing) can alter the crystal structure of ice.

The NASA Jet Propulsion laboratory in Pasadena, CA has developed an instrument called the Wireline Analysis Tool for Subsurface Observation of Northern ice sheets (WATSON) (Figure 8). This DUV spectrometer collects 2-dimensional fluorescence maps (excitation = 248 nm, emission = 275-450 nm) from inside the englacial zone and is configured for attachment to an ice drill built by Honeybee Robotics (Eshelman et al., 2019). This instrument collects spectral maps which can be used to measure fluorescence without altering its native spatial distribution. This fluorescence spectrometer could also be coupled with DUV Raman capabilities similar to

the SHERLOC instrument which is on the payload for the Mars 2020 rover. Benchtop applications of this technology have demonstrated it is able to differentiate between amino acids and whole cells (Sapers et al. 2019). The combined DUV fluorescence and Raman spectroscopy and compact design would make WATSON a uniquely valuable tool for detecting and characterizing OM in terrestrial and extraterrestrial ices.

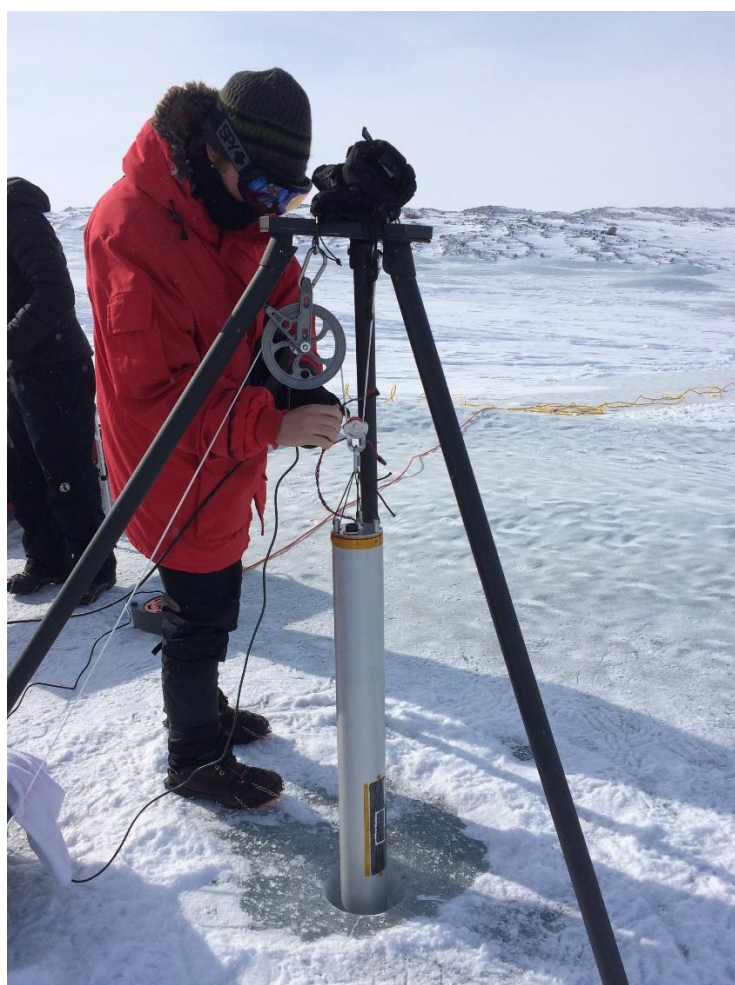


Figure 8. Photograph of WATSON prototype being deployed into a borehole on the Southwestern edge of the Greenland ice sheet. WATSON collected fluorescence maps and measured solar radiation to a depth of ~3 meters.

DISCUSSION

Table 2 summarizes the techniques for characterizing OM which have been discussed in this review. No individual technique is superior to another, rather the right tool depends on the goals of the analyst and the characteristics of the sample and study environment. It is often necessary to employ the use of multiple techniques to create a holistic analysis of OM in a natural system. However, when working with ice cores, particularly with deep ice core samples, it may be challenging to collect a large enough sample of OM to successfully use NMR for characterization.

Sample preparation is an important element to consider when selecting an analytical method and when comparing data across different techniques. It is also apparent from reviewing the literature that the discrepancies between what is considered ‘dissolved’ is more than just semantics. It can complicate comparisons of data between studies. For example, Pautler et al. (2012) used a 0.2 μm filter before collecting NMR spectra of ‘dissolved’ OM. As the authors intended, this will remove whole cells, but it is also smaller than what is typically used for analysis of ‘dissolved’ organics in many other studies (typically filtrate < 0.45 to 0.7 μm). Therefore, care should be taken when drawing conclusions between studies as they may be comparing different constituents of the OM pool.

Tools which use bulk sample measurements (fluorescence and Raman spectroscopy) have the benefit of minimizing the chance of altering the chemical structure of OM due to sample preparation. The trade-off with bulk measurement techniques which rely on natural fluorescence is that the analyst will only collect information on the optically active portion of the OM pool.

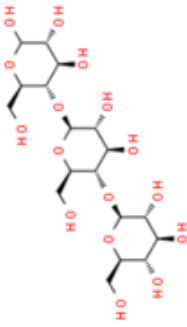
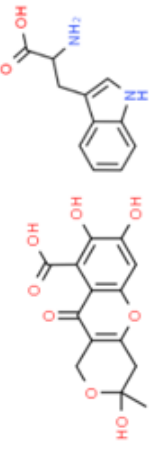
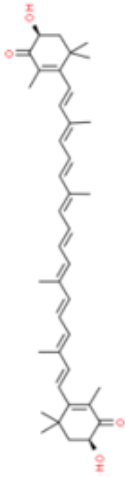
The techniques requiring OM isolation steps such as NMR and high-resolution MS have the benefit of providing detailed molecular information for the entire analyte.

Mass Spectroscopy remains a standard for molecular characterization of OM. The development and coupling of MS to Fourier Transform Ion Cyclotron Resonance (FTICR) has increased resolution and accuracy in determining OM formulae (D'Andrilli et al. 2015). This improved sensitivity and the lower operational costs compared to NMR make MS ideal for performing high resolution molecular-level analysis of OM in glacial environments.

Similar to MS, fluorescence spectroscopy could be considered the standard for determining the optical properties of OM. EEMs and diagnostic indices such as FI continue to be reliable indicators of OM composition and source. Although the excitation range used for constructing EEMs encompasses the DUV spectrum, the use of laser spot energy sources gives laser-induced DUV fluorescence a higher sensitivity which may be useful for detection of OM in low biomass ice samples. Further development of fluorescence spectrometers and their use in tandem with other analytical techniques has exciting implications for future studies of glacial OM.

Raman spectroscopy is a relatively new technique for analyzing biological and organic materials. Increased sensitivity and molecular specificity, particularly for relevant biomolecules such as pigments and aromatics, make Raman spectroscopy an exciting new tool for characterizing OM in glacial systems. However, currently no ecological indices for Raman of OM exist. Thus, there is potential to expand the use of Raman spectra for identifying new metrics of characterizing the biochemical properties of OM and more research is needed to discern the full potential of Raman spectroscopy techniques for ice core analysis.

Table 2. Summary of techniques reviewed for chemical characterization of OM in glaciers and ice sheets. Molecular structures were found in the ChemSpider Database provided by the Royal Society of Chemistry (www.chemspider.com).

Technique	Description	Chemical targets	Sample quantity	Example data products
Mass Spectroscopy	Molecular-level analysis particularly useful for characterizing aliphatic portion of OM compared to other techniques. Pros include detailed analyses of chemical formulae and high throughput. Cons include sample preparation may bias analysis.	 Molecular structure of all compounds in the fraction analyzed. Particularly useful for analysis of hydrophobic components and low molecular weight proteins.	<1ppm of fractionated material	Collect the atomic ratios in a fraction of OM. Identification of lipid fraction and comparison of aliphatic vs aromatic portions. The molecular lability boundary (MLB) can be used to estimate the lability of OM (D'Andrilli et al, 2015)
Nuclear Magnetic Resonance	Molecular-level analysis which is determined by the spin states of the hydrogen atoms (proton NMR) or stable isotopes of carbon (^{13}C NMR) within the sample. Pros include nondestructive analysis and potentially quantitative analysis. Cons include comparatively low sensitivity and high cost.	 Atomic-level analysis of all compounds in the fraction analyzed. Particularly useful for analysis of high-molecular weight polysaccharides and amino sugars.	Down to 1mg, 50-100 mg recommended	Highly detailed molecular analysis of OM that can be used for identifying the molecular structure of all compounds in the filtrate that is analyzed.
Fluorescence Spectroscopy	Optical technique which relies on the natural fluorescence emitted by optically active portion of OM. Pros include low cost, nondestructive measurements and no sample prep required. Cons include lower resolution chemical analysis and limited to optically active OM.	 Optically active portion of chromatophoric OM. Particularly useful for characterization of humic substances. UV fluorescence suited for identification of aromatic compounds such as proteins.	Down to ~2μM concentration	Excitation Emission Matrices (EEMs) and identification of fluorescent components. Fluorescence Index (FI) (Cory and McKnight, 2005) to determine if microbially or terrestrially derived. Freshness Index (Ohno, 2002) which can indicate microbial transformation.
Raman Spectroscopy	Optical technique which measures the Raman scattering of molecules after excitation either at 532nm (Green Raman) or 244-266nm (UV Raman). Pros include nondestructive measurements, no sample prep required, and molecular specificity. Cons include relatively low sensitivity and non-quantitative.	 Aromatic compounds, pigments, and complex, disordered carbon compounds in OM.	Down to 1-10 mg	Molecularly specific spectra for distinguishing organic and inorganic compounds.

CONCLUSION

Reported values for the total abundance of prokaryotic cells in the Earth's glaciers and estimates of the total amount of organic carbon stored in the Earth's cryosphere are extrapolative in nature. Given the poor accessibility of polar and high alpine climates, these estimates are based on limited sample sizes, small transects, and predominately based on measurements collected during warmer summer seasons. For these reasons, more studies of glacial systems are needed to obtain an unbiased understanding of the ecological role of glaciers and glacial microbial communities.

Advancements in technologies can address the unique issues which arise when studying OM in polar and alpine glaciers and this review has outlined some of their most promising applications. Low biomass samples, the structural complexity of OM, and limited access to field locations are factors that make the study of OM in polar and high alpine environments challenging. However, the technological advancements described here, and future studies are opening new windows to the field of microbial ecology and nutrient cycling in glaciers and ice sheets. Additionally, the ability of sensitive technology such as WATSON to be deployed into ice boreholes offers a fresh way to investigate the potential habitability of the englacial zone, and adaptation of these technologies into field instrumentation demonstrates their potential usefulness for detecting and characterizing organic compounds on icy worlds in our solar system.

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