Bacteria and their Role in Organic Carbon Dynamics in Antarctic Snow

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By

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Statement of the Candidate

As required under the University Ordinance OB.19.8. (vi), I hereby state that the present thesis titled "**Bacteria and their role in organic carbon dynamics in Antarctic snow**" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge, the present study is the first comprehensive work of its kind from the area mentioned. The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed.

Runa Antony

Date:

Certificate

As required under the University Ordinance OB.19.8. (viii), it is certified that the thesis titled "**Bacteria and their role in organic carbon dynamics in Antarctic snow**", submitted by **RUNA ANTONY** for the award of the degree of Doctor of Philosophy in Marine Science (Microbiology), is based on original studies carried out by her under my supervision. The thesis or any part of thesis has not been previously submitted for any other degree or diploma in any university or institution.

Dr. Shanta Achuthankutty

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(Co-Supervisor)

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List of abbreviations

ABM - Antarctic Bacterial Medium

AI_{mod} - Modified Aromaticity Index

AIS - Antarctic Ice Sheet

BC - Black Carbon

CFU - Colony Forming Units

CRAM - Carboxylic-rich Alicyclic Molecules

DBC - Dissolved Black Carbon

DBE - Double Bond Equivalent

DOC - Dissolved Organic Carbon

DOM – Dissolved Organic Matter

DON - Dissolved Organic Nitrogen

DOP - Dissolved Organic Phosphorous

DOS - Dissolved Organic Sulfur

ESI - Electrospray Ionisation

FTICR-MS - Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

HMW - High Molecular Weight

HySPLIT - Hybrid Single Particle Lagrangian Integrated Trajectory

ITS - Internal Transcribed Spacer

LMW - Low Molecular Weight

m asl – meters above sea level

MODIS - Moderate Resolution Imaging Spectroradiometer

MSA – Methanesulfonic acid

MW – Molecular Weight

NA - Nutrient Agar

$nssCa^{2+}$ - non-sea-salt Ca^{2+}

OS_C - Carbon Oxidation State

PCR – Polymerse Chain Reaction

rRNA - ribosomal ribonucleic acid

SEM - Scanning Electron Microscopy

SOA - Secondary Organic Aerosol

$ssNa^+$ - sea-salt Na^+

TOC – Total Organic Carbon

TSA - Tryptone Soy Agar

ZMA - Zobell Marine Agar

CHAPTER 1

General Introduction

1.1. Antarctica

Glaciers and ice sheets occupy 11% of Earth's surface area, majority of which comprises the Antarctic ice sheet (AIS), covering an estimated 14 million km^2. Ninety eight percent of Antarctica is covered by thick ice sheets that have an average thickness of 2.4 km (maximum 4.8 km) (Stonehouse, 2002) and contains about 90% of the world's ice and 70% of its fresh water (Fox and Cooper, 1994). Among the various habitats, snow has the maximum areal extent and overlays the majority of the Antarctic continent (Goodison et al., 1999). An overview of the major geographical features on the Antarctic continent is shown in Figure 1.1.

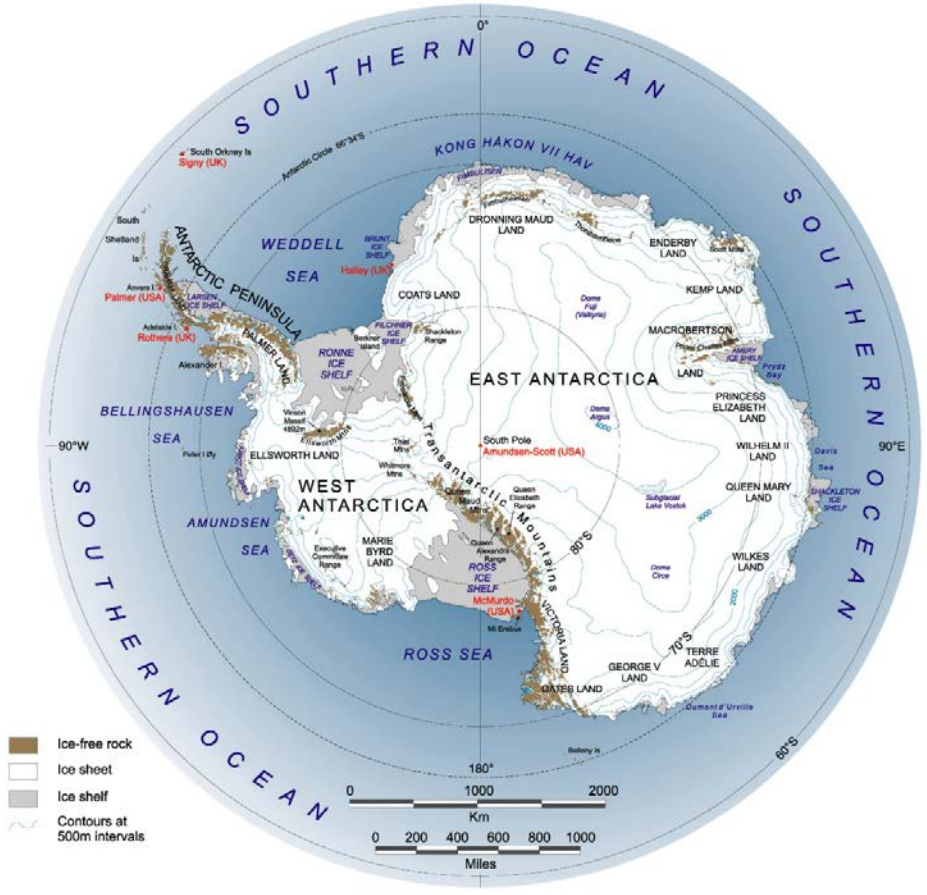

Figure 1.1. *Major geographical features on the Antarctic continent (Map extracted from http://lima.nasa.gov/pdf/A3_overview.pdf).*

The climate of Antarctica is very arid, and it contains the most extreme cold-desert regions on Earth. The interior plateaus of Antarctica experiences extremely low temperatures (lowest: −89°C) because of its high elevation, absence of cloud and water vapour in the atmosphere, and the isolation of the region from the relatively warm maritime air masses

found over the Southern Ocean (Turner et al., 2009). Additionally, seasonal depletion of stratospheric ozone, (Farman et al., 1985; Jones and Shanklin, 1995), means that the surface environment of Antarctica is exposed to elevated levels of solar UVB radiation (Lubin and Frederick, 1991; Madronich et al., 1998). The Antarctic region is therefore one of the most extreme environments for life on Earth, with combined stresses of low temperature, desiccation, fluctuating light levels from continuous light in the polar summer to continuous dark in the winter, elevated levels of UV radiation, high pressure (Siegert et al., 2001; Christner et al., 2006), variable oxygen concentrations (McKay et al., 2003; Hodgson et al., 2009), high salt concentrations (sea-ice and saline lakes), and limited availability of nutrients (Christner et al., 2006; Hodgson et al., 2009). The existence of such extremes means that the influence of the Antarctic region on both climate and oceans extends not only to its immediate area, but also into mid-latitude global systems. In fact, Antarctica plays a remarkable role in the global environmental system in terms of climate, global heat balance, oceanic circulation and marine nutrient cycling. Spatial and temporal variations in these systems are crucially important not only for an understanding of how the planet currently functions, but also for predicting the future changes (Doran et al., 2002).

1.2. Antarctic ice sheet as a reservoir of organic carbon

Tentative estimates of the prokaryotic carbon pool in the AIS are 4.4×10^{10} g (Priscu et al., 2008) and represents an important reservoir of global organic carbon. In addition, dissolved organic carbon levels (9.3×10^{15} g) within the AIS exceed those in all surface fresh waters (rivers and lakes) by 18-fold. Furthermore, ancient marine sediment deposits are believed to exist beneath the AIS, and may contain upwards of 21×10^{18} g of organic carbon (Wadham et al., 2012). If accurate, the magnitude of this organic carbon reservoir would be second only to the entire oceanic pool, estimated at ~39×10^{18} g (Batjes, 2014). These comparisons indicate that the Antarctic continent contain a globally relevant pool of dissolved and cellular organic carbon (Priscu et al., 2008) and are an integral component of the global carbon cycle. Snowpacks, through the various inorganic and organic compounds present in them and through the physical, photochemical and biological process occurring within them, can have a major impact on atmospheric chemistry and play an important role in a number of biogeochemical and climate processes (McNeill et al., 2012). For example, 1) organic materials present in the snow are often light-absorbing species, (Doherty et al., 2010; Hegg et al., 2010) contributing to ~ 20-50% of the light absorption in snowpacks

(Doherty et al., 2010). Light absorbing impurities decrease its reflectance (Doherty et al., 2010; Hegg et al., 2010), also known as albedo, increase its absorption of solar energy, and is a major contributor to warming (Flanner et al., 2007; McConnell et al., 2007) significantly amplifying regional climate change; 2) photochemistry of the snowpack organic compounds leads to volatile organic carbon production (Grannas et al., 2007) that impacts the surrounding environment in a number of ways: they control the oxidising capacity of the atmosphere (Perrier et al., 2002), contribute to the formation of secondary organic aerosol (Seinfeld and Pankow, 2003; Ervens and Volkamer, 2010) and produce humic-like substances in snow and ice (Guzman et al., 2007). Snowpack can act as a source for some gas phase species or a sink for others (Helmig et al. 2009); 3) Biological activity is another important, but as yet poorly constrained component of air-snow exchange processes.

While it is known that dissolved organic matter (DOM) on supraglacial surfaces (on the glacial surface) is highly reactive, resulting in the production of reactive gas phase species and free radicals that influence the oxidative capacity of the overlying atmosphere (Grannas et al., 2007), what is not known is how the resident microbial communities impact the composition and transformation of this material and its implications on supraglacial biogeochemistry. Increased understanding of glacier biogeochemistry is a priority, as snowpack cycling of DOM can be important on local, regional, and global scales (McNeill et al., 2012) and also because glacier environments are the most sensitive to climate warming. Freshwater fluxes from the AIS have been increasing dramatically over the last decade in response to a warming climate (Sheperd et al., 2012; Rignot et al., 2013) with consequent increase in the export of glacially derived DOM and microbes to downstream environments (Hood et al., 2015). This could affect downstream ecological functioning, as a substantial fraction of this DOM is labile and bioavailable (Hood et al., 2009; Singer et al., 2012). In particular, the release of DOM from glacier melt could stimulate microbial activity in glacial ecosystems and surface waters of the open ocean fed by glacial runoff (Hood et al., 2009; Singer et al., 2012). The presence of readily available carbon may also result in an increase in atmospheric CO_2 from DOM mineralisation by bacteria.

Despite its importance in biogeochemical cycling of carbon and global carbon dynamics at large, very little is known on the nature, distribution and sources of organic carbon in the snow, especially in Antarctica. While studies have indicated that marine aerosols are

enriched in particulate (e.g., small water-insoluble material and microorganisms) and dissolved organic matter (Leck and Bigg, 2005; Facchini et al., 2008), and may be transported to snow (Calace et al., 2005), no attempt has been made to elucidate the marine influence on organic carbon concentrations in snow. Further, the few studies on organic carbon in the East Antarctic region are fragmentary, being concentrated in the coastal areas near the Ross Sea and in the Dome C area (Lyons et al., 2007; Federer et al., 2008) and currently, little information exists on the spatial variation of organic carbon in other regions of Antarctica (Table 1.1). Consequently, the carbon dynamics underpinning these ecosystems remain poorly understood.

Table 1.1. *Organic carbon (OC) content measured in snow from various Antarctic sites.*

Sites, Elevation (m asl)*	Type of Sample	OC (ppbC) [N]**	Reference
South Pole (2850)	Surface snow	400 ± 130 [11]	Grannas et al., (2004)
Victoria Land	Snow pit	< 96 [36]	Lyons et al., (2007)
Concordia (3250)	Surface snow	14–27 [2]	Legrand et al., (2013)
Concordia (3250)	Snow pit	10–150 [24]	Legrand et al., (2013)

***** *m asl – meters above sea level, **N - the number of analysed samples.*

These observations highlight the need for quantifying and also identifying the sources, distribution and reactivity of organic carbon in snow for a better understanding of the regional and global carbon cycle. One specific challenge in quantifying organic carbon in snow is the analytical limitations in detecting and measuring them at trace quantities. Consequently, information on the composition, sources, reactivity and the processes by which DOM is transformed in Antarctic supraglacial environments are severely limited. In particular, the East Antarctic ice sheet, which encompasses about 90% of the ice in Antarctica, remains poorly studied. This is especially critical now as the ongoing loss of ice from the AIS (Sheperd et al., 2012; Rignot et al., 2013) has the potential to shift the timing and magnitude of glacial run off and export of DOM (Hood et al., 2015) to downstream environments.

1.3. Microbial abundance and diversity

Antarctic environment has been considered for a long time to be devoid of life or serving merely as repositories for wind-transported microorganisms trapped in the ice (Cowan and Tow, 2004). However, the increasing number of recent studies on the microbial ecology and diversity of various Antarctic ecosystems has been changing this view. This cold desert is a habitat dominated by microbial populations that possess unique adaptations to counter the environmental constraints (Ray et al., 1998; Feller and Gerday, 2003; Chintalapati et al., 2004; Chattopadhyay, 2006; Shivaji et al., 2007; Antony et al., 2012a; Shivaji et al., 2013). Antarctic snow has been found to harbour microbial communities (for example Carpenter et al., 2000; Yan et al., 2012; Antony et al., 2012b; Lopatina et al., 2013; Michaud et al., 2014) as abundant as $10^2 \times 10^3$ cells mL^{-1} (Carpenter et al., 2000; Michaud et al., 2014). Tentative estimates of the microbial numbers in the AIS are ~4×10^{24} (Priscu et al., 2008). This enormous microbial biomass represents a considerable reservoir of microbial diversity.

Although still limited, studies have shown that Antarctic environments harbor abundant, active and diverse microorganisms across a range of habitats such as ice (Christner et al., 2001; Antony et al., 2012a; Shtarkman et al., 2013; Shivaji et al., 2013), snow cover (Yan et al., 2012; Antony et al., 2012b; Lopatina et al., 2013), sea ice (Bowman et al., 1997), debris-rich basal ice (portion of an ice body that is in contact and interacts with the glacial bed; Doyle et al., 2013; Montross et al., 2014), supraglacial lakes (Laybourn-Parry and Pearce, 2007), subglacial lakes (Christner et al., 2006, 2014), soils (Bottos et al., 2014) and even in rock debris (Wynn-Williams and Edwards, 2000). Studies on microbial diversity in Antarctic snow using small subunit 16S ribosomal RNA (rRNA) gene-sequencing techniques, have revealed microbial assemblages dominated by Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Verrucomicrobia, and Cyanobacteria, (Yan et al., 2012; Michaud et al., 2014). The abundance and community composition of microbes detected in snow from various Antarctic sites are shown in Table 1.2.

Snowpack ecosystems are believed to be seeded through the atmospheric transportation of bacteria from local (Larose et al., 2010; Harding et al., 2011) as well as more distant sources (Harding et al., 2011) by wind, clouds and precipitation (Amato et al., 2007; Knowlton et al., 2013). Marine aerosols formed during bubble bursting at water–air interfaces, are enriched in marine microorganisms (Leck and Bigg, 2005; Facchini et al.,

2008) and are another important mechanism for the transportation of microorganisms to the snow (Antony et al., 2012a; Yan et al., 2012). Once deposited on the snow surface, these microbes may form permanent metabolising communities adapted to the specific snow environment and integrate into ecological processes in that environment (Xiang et al., 2009; Larose et al., 2010; Harding et al., 2011; Hell et al., 2013).

Table 1.2. *The abundance and community composition of microbes detected in snow from various Antarctic sites.*

Site	Sample Type	Abundance	Diversity	Method	Reference
Zhongshan Station to Dome A	Snow core (1 m)	0.008-0.32 CFU mL^{-1}	Firmicutes, Actinobacteria, Proteobacteria (α, γ) and Bacteroidetes.	16S RNA gene sequencing of culturable bacteria	Yan et al., 2012
Russian Antarctic stations	Surface snow	1-46 × 10^3 cells mL^{-1}	Proteobacteria (α, β , γ, δ), Bacteroidetes, Actinobacteria, Verrucomicrobiae and Firmicutes	16S RNA gene sequencing of culturable bacteria and 16S RNA gene clone library sequencing	Lopatina et al., 2013
Dome C	Surface snow	2.5±0.5-4.3±2.2×10^2 cells mL^{-1}	Actinobacteria, Bacteroidetes, Cyanobacteria, Proteobacteria (α, β , γ, ε), Verrucomicrobia, Fusobacteria, Planctomycete, Lentisphaerae, Firmicutes, Chlorobi, Tenericutes, Acidobacteria, Spirochaetes, and Chloroflexi	16S RNA gene clone library sequencing and 16S RNA gene pyrosequencing.	Michaud et al., 2014
South pole	Surface snow	2-50×10^2 cells mL^{-1}	Deinococcus-Thermus, Bacteroidetes, and Betaproteobacteria	16S RNA gene clone library sequencing	Carpenter et al., 2000
Yatude Valley, Langhovde	Red snow	5.8-32×10^2 cells mL^{-1} (red cells)	Bacteroidedes, Betaproteobacteria, Heterokontophyta and Chlorophyta	16S RNA gene clone library sequencing and PCR-DGGE analysis of eukaryotic SSU rRNA	Fujii et al., 2010

Snow associated microbial communities have been found to be metabolically active under *in situ* conditions in Antarctica (Carpenter et al., 2000). Recently, molecular approaches have identified specific functional genes, such as genes involved in nitrogen cycling, sulfur metabolism, and carbon and nutrient recycling in microbial communities inhabiting snow and ice (Simon et al., 2009; Larose et al., 2013; Shtarkman et al., 2014). Resident microbial communities are also believed to actively affect snow ecosystems by impacting nutrient dynamics, albedo and the hydrochemistry of snow (Hodson et al., 2005, 2008; Telling et al., 2011; Larose et al., 2013). Thus, the Antarctic region not only constitutes the most extensive glacial microbial habitat on Earth but also forms an integral part of the global climate system with important linkages and feedbacks (Goodison et al., 1999).

1.4. Supraglacial carbon cycling

Carbon fluxes through microbial communities on the supraglacial surface show that they may be important in carbon cycling through the production and consumption of DOM (Skidmore et al., 2000; Anesio et al., 2009; Hodson et al., 2010; Yallop et al., 2012). Modeling studies indicate that combined respiration and photosynthesis fluxes in supraglacial cryoconite (water-filled cylindrical pits on surface ice) ecosystems are 10^1–10^2 Gg C a^{-1} (Hodson et al., 2010). Microbially mediated processes impart a significant effect upon the composition and abundance of nutrients in supraglacial environments (Hodson et al., 2008). Active heterotrophic microbial communities associated with snow surfaces could make use of the available DOM as a source of energy and carbon (Amato et al., 2007, Antony et al., 2012b), and produce organic metabolites with vastly different properties. Organic material from microbial sources may react with radical compounds in snow (e.g., OH), thus serving as radical sinks. By-products of these reactions can result in the generation of additional organic compounds in snow (Grannas et al., 2004). Hence, microorganisms can potentially modify the snow chemistry through biochemical transformations in snow. This transformation of organic matter by snow processes may impact our understanding of the carbon biogeochemical cycle at high latitudes. Yet, very little is known about the microbial turnover of carbon in Antarctic supraglacial environments and the fate of organic carbon reservoirs on the ice sheet. As such, data on the origin, composition, metabolic potential and ecological role of snow microbial communities in the vast and climate sensitive landscape of the AIS are sparse.

Hence, a detailed study was carried out with the following major objectives:

- To determine the concentration and molecular composition of the organic carbon in Antarctic snow.
- To determine bacterial abundance and diversity in Antarctic snow.
- To understand the marine influence on organic carbon composition and bacterial community structure in snow.
- To understand the role of bacterial community in organic carbon dynamics.

In this doctoral thesis, high sensitivity measurements of total organic carbon along with ionic, dust and microbiological components were systematically carried out along a 180 km traverse perpendicular to the coast in the East Antarctic region for a better understanding of the concentration and spatial distribution of organic carbon as a function of increasing elevation and distance from the coast. In addition, ultrahigh resolution mass spectrometry was used to elucidate molecular details of DOM in Antarctic snow. The molecular details of DOM obtained was utilised to determine the composition of specific molecules within DOM, as well as to identify the compositional differences among pools of DOM over a relatively large area of Antarctica, which include both coastal and the interior East Antarctic plateau. This allowed for novel insights on the character of supraglacial DOM. The abundance and diversity of microbial communities associated with the Antarctic snow was determined using culture-based and culture independent studies. *In situ* experiments through a combination of field studies and ultrahigh resolution mass spectrometry, gave insights into the interaction between microbes and individual molecules within DOM and its transformation through microbial communities on the AIS surface.

CHAPTER 2

Spatial variability and sources of organic carbon in Antarctic snow

2.1. Introduction

Dissolved organic carbon plays a vital role in ecosystem carbon cycling owing to its role as an energy and carbon source for microbes. Studies on organic carbon in the Antarctic snow are few in number, thus making it one of the least understood fractions in the snow. Until now, apart from methanesulfonate for which systematic investigations have been conducted to reconstruct past marine biogenic emissions of dimethyl sulfide (Legrand et al., 1991; Wolff et al., 2006), the existing studies dealing with organics in ice focus only on formaldehyde (Staffelbach et al., 1991; Hutterli et al., 2003), monocarboxylates like formate and acetate (Legrand et al., 1995), C_2-C_5 dicarboxylates (Legrand et al., 2007), and long chain (C_{14}-C_{22}) carboxylates (Kawamura et al., 1996). Recent works have added more information on the distribution of humic substances in the Antarctic snow (Calace et al., 2005; Cincinelli et al., 2005). Apart from these studies dealing with individual organic species, only a small data set exists on the concentrations of total organic carbon (TOC) in the snow from Antarctica. These include data from snow collected from the Talos Dome (Federer et al., 2008), Southern Victoria Land (Lyons et al., 2007), and South Pole (Grannas et al., 2004).

Organic carbon estimates for the Antarctic region indicate that the AIS hold a significant pool (9.29 Pg C) of the world's reserve of cellular and dissolved organic carbon (collectively referred here to as the total organic carbon) (Priscu et al., 2008). These estimates, however, are tentative as these are based on limited data of organic carbon concentrations available for this region. Active microbial communities associated with the snow (Christner et al., 2000; Carpenter et al., 2000; Christner et al., 2001; Larose et al., 2013) could make use of the available organic carbon substrates (Amato et al., 2007; Antony et al., 2012b), thereby impacting the biogeochemistry of these environments (Stibal et al., 2012). Additionally, it has been shown that the organic carbon in snow undergoes photochemical reactions, thereby releasing reactive gas-phase species to the overlying atmosphere (Sumner et al., 1999; Guimbaud et al., 2002; Grannas et al., 2004). Though limited, these data imply that Antarctic snow contain an organic carbon reservoir that must be considered seriously while addressing the issues concerning global carbon dynamics.

Despite its importance in air-snow exchange processes, biogeochemical cycling of carbon

and global carbon dynamics at large, very little is known on the distribution and sources of organic carbon in snow, especially in the Antarctic region. The East Antarctic ice sheet, which encompasses about 90% of the ice in Antarctica, remains poorly studied. Consequently, there are major gaps in quantifying the organic carbon and also identifying its sources in the Antarctic snow. In the present study, high sensitivity measurements of TOC along with ionic composition, dust and microbiological components were systematically carried out along a transect perpendicular to the coast in the East Antarctic region. This work for the first time provides information on the spatial variations of TOC concentrations and its sources in the Antarctic snow from the coast to inland along the Princess Elizabeth Land in the East Antarctic region.

2.2. Methodology

2.2.1. Sampling

The sampling locations are along a traverse extending from the coastal area of Larsemann Hills to ~180 km inland in the Princess Elizabeth Land region in East Antarctica (Fig. 2.1).

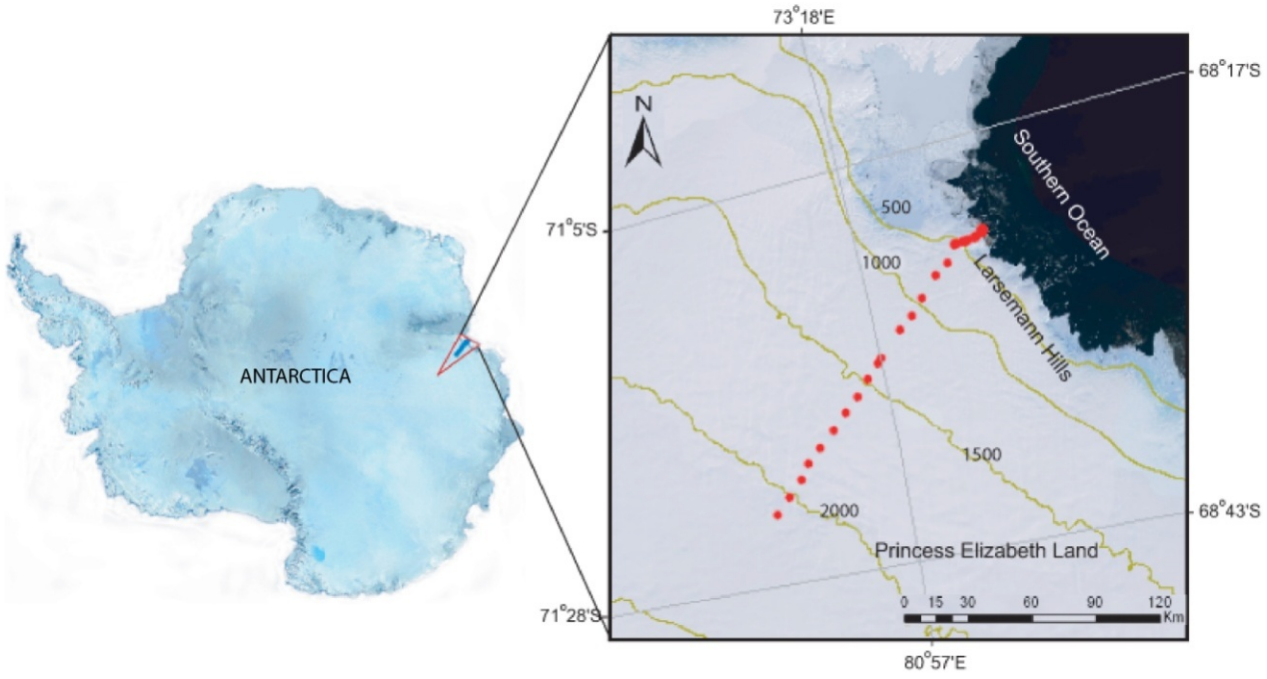

Figure 2.1. *Map showing snow sampling locations (red circles) along the Princess Elizabeth Land transect.*

Thirty eight surface snow samples (~10 cm deep) were collected using a pre-cleaned polypropylene scoop from near sea level to about 2210 m above sea level (m asl). The

geographical location, distance from coast and elevation of each sampling point are given in Table 2.1.

Table 2.1. *Details of the surface snow sampling locations and elevation along the Princess Elizabeth Land transect (* m asl – meters above sea level).*

Sr. No	Sampling location		Elevation (m asl)*	Distance from coast (km)
	Latitude	Longitude		
1	69˚24'13"S	76˚11'17"E	3	0
2	69˚24'19"S	76˚11'10"E	5	0.3
3	69˚24'16"S	76˚11'42"E	3	0.5
4	69˚24'34"S	76˚11'9"E	9	1.1
5	69˚24'36"S	76˚11'13"E	11	1.2
6	69˚24'43"S	76˚11'43"E	12	1.6
7	69˚24'44"S	76˚12'44"E	12	1.6
8	69˚24'44"S	76˚12'44"E	3	2.0
9	69˚24'48"S	76˚12'48"E	8	2.1
10	69˚24'52"S	76˚13'52"E	11	2.5
11	69˚25'24"S	76˚13'24"E	65	2.9
12	69˚26'25"S	76˚14'25"E	201	5.1
13	69˚26'45"S	76˚15'45"E	225	5.9
14	69˚27'6"S	76˚15'6"E	248	5.9
15	69˚29'5"S	76˚16'5"E	396	9.4
16	69˚28'44"S	76˚16'44"E	378	9.6
17	69˚29'24"S	76˚16'24"E	413	10.4
18	69˚30'10"S	76˚16'10"E	443	11.5
19	69˚29'49"S	76˚16'49"E	429	11.6
20	69˚30'34"S	76˚16'34"E	458	12.6
21	69˚32'2"S	76˚16'2"E	509	14.6
22	69˚33'S	76˚18'E	300	20.0
23	69˚36'S	76˚28'E	792	30.0
24	69˚40'S	76˚35'E	975	40.0
25	69˚45'S	76˚49'E	1113	50.0
26	69˚49'S	77˚E	1234	60.0
27	69˚53'S	77˚08'E	1280	70.0
28	70˚S	77˚25'E	1494	80.0
29	70˚01'S	77˚29'E	1509	90.0
30	70˚05'S	77˚39'E	1585	100.0
31	70˚09'S	77˚50'E	1631	110.0
32	70˚13'S	78˚E	1722	120.0
33	70˚17'S	78˚11'E	1768	130.0
34	70˚22'S	78˚22'E	1875	140.0
35	70˚26'S	78˚32'E	1920	150.0
36	70˚29'S	78˚43'E	1987	160.0
37	70˚33'S	78˚54'E	2118	170.0
38	70˚37'S	79˚05'E	2210	180.0

The samples were stored in well-sealed sterile whirl-pack bags. Necessary precautions were taken, such as sterile, long-hand, powder-free nitrile gloves and face masks, during

sampling to reduce the risk of contamination. Also, in order to avoid contamination due to vehicular movement of the sampling team, sampling was done 50 m upwind from the landing site at each location. Samples were kept frozen at -20°C in dark condition until the analysis in the laboratory at NCAOR.

2.2.2. Precautions taken during sample handling and processing

As high-sensitivity analysis was involved, stringent precautions were taken to minimise carbon contamination from the environment. Only glass containers and vials were used during preparation of standard solutions, sample processing and analysis so as to avoid problems related with organic carbon contamination, known to occur in the case of using plastic wares. All glass wares were soaked in 0.5% ultrapure nitric acid for 48 hr, rinsed thoroughly with ultrapure (Milli-Q Element) water, and combusted at 450°C for 5 hr. Only freshly purified ultrapure water was used during every stage of the analysis. Ultrapure water was collected directly into combusted glass bottles, leaving no headspace and immediately sealed tight. In order to determine the magnitude of contamination from the sample bag material, the outer layer of snow sample that was in contact with the material of the bag as well as snow from the center of the bag that was at no time in contact with the sample bag material was analysed. The average TOC concentration in snow from the outer region in contact with the bag was about 100 μg L^{-1} higher than from the inner region which was not in contact with the bag. Therefore, the snow from the inner region was only used for analysis.

2.2.3. Analysis

2.2.3.1. Total Organic Carbon

Snow samples were transferred to acid-cleaned and pre-combusted (450°C, 5 hr) screw-capped glass bottles under a laminar-flow bench housed in a -15°C cold room processing facility. Care was taken to ensure that the bottles were filled leaving no head space and tightly sealed in order to minimise contamination from the atmosphere. Samples were allowed to melt in the dark in a class-100 clean room and analysed immediately for TOC using a high-sensitivity TOC analyser (Shimadzu TOC-V_{CPH}). In order to check for possible contamination during sample handling and melting, blanks comprising ultrapure water were processed in a similar manner as that of the samples. Since prolonged storage of samples can lead to increase in organic carbon concentrations, only two samples were

melted at a time and analysed within 20 min. Measurements were carried out in triplicate by the non purgeable organic carbon method (Findlay et al., 2010). In this method, samples are automatically drawn into the syringe which is followed by auto addition of 2 M ultrapure HCl to lower the pH to ~2 and hydrocarbon free high purity N_2 sparged at a flow rate of 150 mL min^{-1} to eliminate inorganic carbon. The sample is then introduced into the quartz combustion tube containing the high-sensitivity platinum catalyst (0.5% platinum on quartz wool) and heated to 680°C. During the high-temperature catalytic oxidation, the remaining carbon (organic carbon) in the sample is converted to CO_2 and swept along with the carrier gas through a dehumidifier and halogen scrubber and finally into a non dispersive infrared detector cell for quantification. Standards and additional blanks comprising fresh, ultrapure water were run intermittently between samples to check for contamination during sample analysis. During TOC measurements, samples were contained within well sealed, combusted glass vials from where the instrument automatically withdrew sample by piercing through the vial seal. The samples were therefore never exposed to the atmosphere during the course of analysis, thus avoiding CO_2 contamination from the air. Calibration was carried out using potassium hydrogen phthalate as the standard. All standard solutions for generation of calibration curve were analysed immediately after preparation to avoid concentration changes as a result of storage. Replicate analysis of the standard yielded a precision better than 7%. Relative standard deviation of the measurements was <5%. The concentration of TOC is expressed as μg L^{-1}.

To determine the detection limit, fresh ultrapure water was injected 12 times and the standard deviation was recorded. The detection limit, defined as the TOC concentration that gives a signal equivalent to 3 times the standard deviation of noise was then determined to be 6.2 μg L^{-1}. For accurate and reliable measurements of total organic carbon, the blank associated with the method was critically evaluated and accounted for during data analysis. The Shimadzu TOC-V_{CPH} instrument has the unique ability to generate 'carbon-free' water by passing ultrapure water several times through the catalyst bed at 680°C and analyse the CO_2 peaks obtained by re-injecting this water through the catalyst without exposure to the atmosphere. It is therefore possible to determine independently, the carbon contamination obtained from the instrument as well as the reagent acid and ultrapure water. The instrument blank value determined using the automated blank checking programme of the Shimadzu TOC-V_{CPH} was found to be

equivalent to about 20 μg C L^{-1}. The instrument blank value was subtracted from the sample peak values while the TOC concentration of the snow samples was calculated. The average carbon contamination in the acidified water used for preparation of the calibration standards was found to be 6.6 μg L^{-1}. The magnitude of carbon contamination from the reagent acid was tested by analysing ultrapure water with increasing additions of acid. Reagent acid accounted for 80% of the carbon contamination in the acidified ultrapure water. The carbon content in ultrapure water used for preparation of the calibration standards and in the reagent acid was taken into account while the calibration curve was generated and while the TOC concentration of the snow samples was calculated. Analysis of ultrapure water blanks to check for contamination during sample processing, melting and sample analysis showed that changes in organic carbon concentration were <2 μg L^{-1}, suggesting that minimum contamination occurred during handling of samples in the laboratory and during the melting process in the clean room.

2.2.3.2. Inorganic ions

Among the primary aerosols, the concentration of Na^+ is directly related to proximity to the sea and is considered to be the most conservative ionic proxy for sea spray in coastal Antarctica (Traversi et al., 2004; Kärkäs et al., 2005). Ca^{+2} is sourced from continental dust and sea spray but is predominantly of crustal origin (Legrand and Mayewski, 1997). Thus, in order to determine crustal as well as marine contributions to snow composition, chemical analysis of surface snow samples were carried out. All sub-sampling equipment and sample containers were pre-cleaned by rinsing several times with ultrapure water, soaking for at least 24 hr, followed by rinsing with fresh ultrapure water and drying in a laminar-flow bench. Samples were melted immediately prior to the analysis in a class-100 clean room facility. Na^+ and Ca^{+2} were measured in the melted snow samples using ion chromatography (Thamban et al., 2010). Measurements were carried out using a Dionex DX-2500 ion chromatography system with IonPac CS17 column with methanesulfonic acid as eluent at a flow rate of 1.0 mL min^{-1} and an IonPac CG17 Guard column with a CSRS-ULTRA Cation Self Regenerating Suppressor. The sample injection volume was 100 μL. Calibration was done using IV (Inorganic Ventures) high-purity standards. The concentration of ions is expressed as μg L^{-1}. The detection limit was less than 2 μg L^{-1} for both Na^+ and Ca^{+2}. Reference standards and random samples were analysed routinely to estimate the analytical precision, which was better than 5% for both ions.

2.2.3.3. Dust particles

All sub-sampling equipment and sample containers were pre-cleaned by rinsing several times with ultrapure water, soaking for at least 24 hr, followed by rinsing with fresh ultrapure water and drying in a laminar-flow bench. Samples were melted immediately prior to the analysis in a class-100 clean room facility. Dust particle concentration and grain size measurements were carried out using a Multisizer 4 Coulter Counter (Beckman Coulter), placed in a class-100 clean room following Wu et al., (2009). Analysis of dust particles of size between 1 and 25 μm diameter was carried out using a 50 μm diameter aperture. Size calibration was achieved using certified standards of latex beads of 5 μm radius and a precision better than 5% was obtained. Measurements were carried out in triplicates and relative standard deviation of most measurements was <10%. Blanks of 0.2 μm filtered ultrapure water treated in the same way as the samples yielded very low and stable background particle counts. Particle mass concentration in μg L^{-1} was calculated from the volume, assuming a mean particle density of 2.6 g cm^{-3} (Sugimae, 1984).

2.2.3.4. Cell carbon

For estimation of cell carbon, microbial cell numbers were determined by the DAPI (4', 6-diamino-2-phenylindole) method described in Porter and Feig, (1980). Snow samples were melted in sterile containers in a laminar flow bench, stained with the fluorescent dye 4',6-diamino-2-phenylindole (DAPI) at a final concentration of 5 μg mL^{-1} (for 5 min) and immediately filtered through black 0.2 μm Nuclepore track-etch membrane under low vacuum (~2 in. Hg). Both autofluorescing and DAPI stained non-autofluorescing cells were counted by epifluorescence microscopy (Olympus BX53) at 1000X magnification using blue, green and UV excitation. Twenty fields were counted on each filter. Bacterial abundance is expressed as numbers mL^{-1} of snow melt. The cell numbers were converted into cell carbon assuming a carbon content of 11 fg for bacteria (Priscu et al., 2008) and 20 fg for picoplankton (Meador et al., 2010). Cell carbon is expressed as ng L^{-1}.

2.2.3.5. Scanning Electron Microscopy

For scanning electron microscopy (SEM) samples were concentrated onto sterile 0.22 μm pore-size Nuclepore filters. After air drying, the dried filter paper was firmly fixed on metal stubs using a conductive adhesive and then coated with a film of platinum using

JEOL JFC-1600 auto fine coater. The cellular morphology was observed using a JEOL JSM-6360 scanning electron microscope.

2.3. Results and Discussion

TOC concentrations in the surface snow samples ranged from 88±4 to 928 ±21 μg L^{-1} with a mean concentration of 259 μg L^{-1}. TOC values decreased with increasing distance from the coast. Up to about 10 km from the coast, the values ranged from 132±9 to 928±21 μg L^{-1} (mean 354 μg L^{-1}), while beyond this distance, the range was comparatively narrow (88±4 to 271±6 μg L^{-1}, mean 182 μg L^{-1}). Statistical analysis (t - test) performed on TOC values obtained from the coastal (up to 10 km) and inland (beyond 10 km) regions showed that the TOC values in coastal samples were significantly higher ($p < 0.001$) than that in the inland samples. Thus, the organic carbon content exhibited considerable spatial variation between the coast and the interior regions (Fig. 2.2).

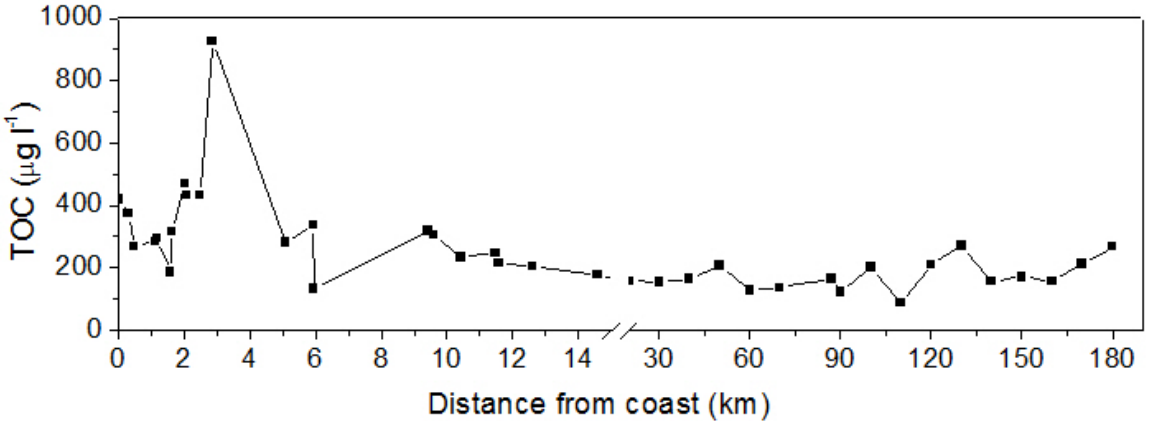

Figure 2.2. *Spatial distribution of Total Organic Carbon (TOC) [Note the break in the scale of X-axis].*

Na^+ concentrations varied from 36 to 20126 μg L^{-1} (mean 5004 μg L^{-1}) and showed a spatial trend similar to that of TOC with significantly higher ($p < 0.001$) values at the coastal sites (Fig. 2.3).

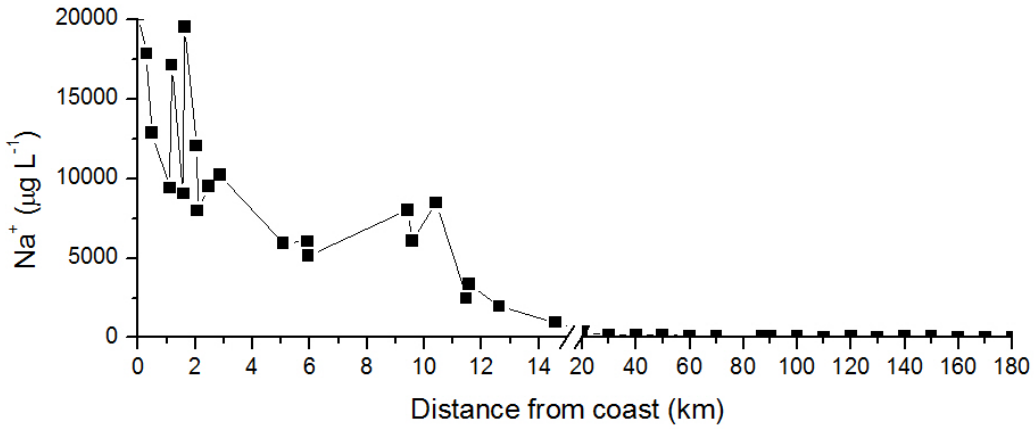

Figure 2.3*. Spatial distribution of* Na^+ (*Note the break in the scale of X-axis*).

Total cell numbers in snow ranged from 9.43×10^3 to 9.27×10^4 cells mL^{-1} accounting for 104-1351 ng carbon L^{-1} (mean 365 ng C L^{-1}) with no significant difference between the coastal and inland regions. Dust concentration ranged from 141-3500 µg L^{-1} and did not show any trend in spatial distribution from coast to inland. Average concentrations of TOC, cell carbon, Na^+ and dust at each sampling site in the Princess Elizabeth Land transect are given in Table 2.2.

Earlier studies have reported the presence of substantial amounts of organic carbon in the snow in different regions of Antarctica (Grannas et al., 2004; Lyons et al., 2007). The values obtained from the inland region of Princess Elizabeth Land are comparable with the TOC concentrations (<96-281 µg L^{-1}) in the Antarctic snow pit samples collected from Southern Victoria Land from sites >10 km from coast (Lyons et al., 2007), but were lower than that of the South Pole snow (mean 400±130 µg L^{-1}) (Grannas et al., 2004). TOC concentrations in an ice core from Talos Dome (East Antarctica) ranged from 80 to 360 µg L^{-1} (Federer et al., 2008) and these values are also comparable with the present data. Similarly, TOC concentrations of 30 to 700 µg L^{-1} at remote northern high latitude sites such as Alert in Canada and Greenland (Twickler et al., 1986; Grannas et al., 2004) are also consistent with the present data.

Table 2.2. *Average concentrations of TOC, cell carbon, Na^+ and dust at each sampling site in the Princess Elizabeth Land transect.*

Distance from coast (km)	TOC (μg L^{-1})	Cell carbon (ng L^{-1})	Na^+ (μg L^{-1})	Dust (μg L^{-1})
0	420	841	20126	646
0.3	375	593	17668	2390
0.5	268	341	12724	540
1.1	285	323	9338	2409
1.2	296	125	16975	1851
1.6	187	233	9003	551
1.6	316	177	19304	1803
2.0	469	306	12049	845
2.1	434	253	7874	500
2.5	434	474	9384	490
2.9	928	821	10158	1639
5.1	282	672	5852	748
5.9	337	172	5978	227
5.9	132	128	5093	237
9.4	318	137	7899	3500
9.6	307	1351	6046	543
10.4	235	248	8382	3120
11.5	246	121	2472	1036
11.6	215	104	3335	2227
12.6	203	423	2010	767
14.6	178	341	1033	222
20.0	158	178	348	466
30.0	156	264	176	334
40.0	164	229	163	1443
50.0	207	134	173	303
60.0	128	175	110	513
70.0	137	399	125	372
80.0	166	224	92	544
90.0	123	212	123	141
100.0	202	181	65	158
110.0	88	506	53	393
120.0	210	519	64	296
130.0	271	367	47	208
140.0	158	1020	73	308
150.0	171	475	66	251
160.0	158	354	36	297
170.0	214	190	53	337
180.0	269	281	44	157

The TOC profile closely resembled that of Na^+ and also exhibited a strong positive correlation ($p < 0.001$). Since Na^+ is considered to be the most conservative ionic proxy for sea spray in coastal Antarctica (Traversi et al., 2004; Kärkäs et al., 2005), the strong correlation with TOC suggests that the sea spray might have contributed to the organic carbon load in these samples. Although Na^+ in the Antarctic snow is predominantly from marine source, it may also have a continental dust source (Röthlisberger et al., 2002). Therefore, in order to evaluate the sea-spray contribution of Na^+, the sea-salt Na^+ ($ssNa^+$) fraction in the samples was estimated. It is well established that in Antarctica, although Ca^{+2} can have inputs from crustal sources as well as from sea-spray, it is of a predominantly crustal origin (Kreutz and Mayewski, 1999; Thamban et al., 2010), especially in interior Antarctica and in areas of locally exposed bedrock. Therefore, $ssNa^+$ was estimated by subtracting the crustal contribution (calculated on the basis of the Ca^{2+} content) from the measured total Na^+ concentration following Röthlisberger et al., (2002) using the following two equations, assuming that the mean Ca^{2+}/Na^+ ratio for marine aerosols (R_m) to be 0.038 and for the average crust (R_t) to be 1.78 (Bowen, 1979).

$$nssCa^{2+} = Ca^{2+} - (R_m * ssNa^+)$$

$$ssNa^+ = Na^+ - (nssCa^{2+}/R_t)$$

where, $nssCa^{2+}$ is the non-sea-salt Ca^{2+}, $ssNa^+$ is the sea salt Na^+, while Ca^{2+} and Na^+ are the measured concentrations of these ions in snow. The $ssNa^+$ fraction of the total Na^+ was found to be >85% for most sites, indicating that a substantial portion of the Na^+ in these snow samples was derived from sea-spray. The strong correlation ($p < 0.001$) obtained between TOC and $ssNa^+$ suggests that TOC probably has a source similar to that of Na^+. Previous studies have shown that 77% of the submicrometer-sized fraction of marine aerosols are composed of organic carbon, largely comprising microorganisms, small water insoluble particles, exopolymeric material, and phytoplankton exudates (Leck and Bigg, 2005; Facchini et al., 2008). Similarly, a study by Calace et al., (2005) on humic acids in Antarctic snow has shown the importance of marine aerosols in the transport of organic matter in Antarctic snow. The elevated TOC values in the coastal region indicate that proximity to the sea has an important influence on TOC concentrations in snow. TOC showed a statistically significant inverse relationship both with distance from the sea ($r = -0.39$, $n = 38$) and altitude ($r = -0.49$) (Fig. 2.4a,b).

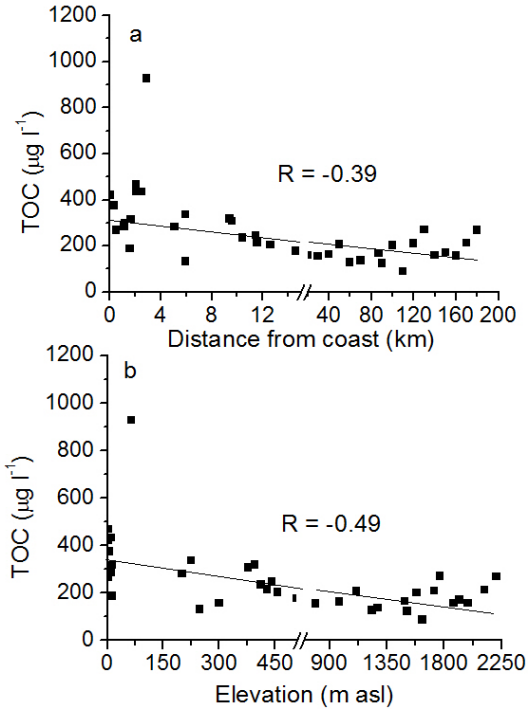

Figure 2.4. *Correlation of total organic carbon with (a) distance from the coast and (b) elevation, in the Princess Elizabeth Land region* (*Note the break in the scale of X-axis*).

Earlier studies have shown that distance from the coast is one of the most important factors influencing the spatial distribution of the sea-salt ions and components originating from marine biogenic activity (Kärkäs et al., 2005). While TOC concentrations decreased with increasing distance from the coast, with significantly lower values (mean 182 µg L^{-1}) in the inland sites compared to that in the coastal sites (mean 354 µg L^{-1}), the values remained fairly constant beyond 20 km inland. This is unlike $ssNa^+$ concentrations, which decreased systematically beyond a distance of 20 km. Similarly, when plotted as a function of altitude (Fig. 2.4b), TOC concentrations decreased with increasing elevation from near sea level to about 500 m asl and then remained fairly constant up to 2210 m asl. Comparing the trends of TOC and $ssNa^+$, it appears that, in the inland sites, the reduced organic carbon input from sea-spray is partially counterbalanced by the presence of alternative sources. It is also possible that organic carbon may be transported to longer distances from the coast than $ssNa^+$, as organic carbon in marine aerosols is mainly concentrated in the submicrometer size fraction and have higher mobility and life span (Saltzman et al., 1996; Calace et al., 2005; Fattori et al., 2005). Jaenicke et al., (1980) calculated the tropospheric residence time of aerosols as a function of size and showed that aerosol particles that had the longest residence time were those ranging in size from a few tenths of a micrometer to

a few micrometers. Aerosol particles in this size range remain airborne for more than one week, travelling long distances. In contrast, sea-salt ions such as Na^+ are concentrated primarily in the coarser fraction (Saltzman et al., 1996; Fattori et al., 2005) and therefore get transported shorter distances. Although organic carbon concentrations in the snow appear to be dominated by marine influence, especially in the coastal areas, as suggested earlier, the possibility of other sources of organic carbon cannot be ruled out.

Snow harbours diverse kinds of microorganisms, such as bacteria, fungi, algae, diatoms, and viruses (Hodson et al., 2008) which represent a substantial reservoir of organic matter (Priscu et al., 2004). Thus, organic carbon concentrations in snow may also be influenced by local biogenic sources such as the indigenous microbial communities inhabiting the snow. Microscopic examination of snow samples revealed the presence of bacteria and diverse consortia of microalgae, which included the pico- and nanoplankton (Fig. 2.5).

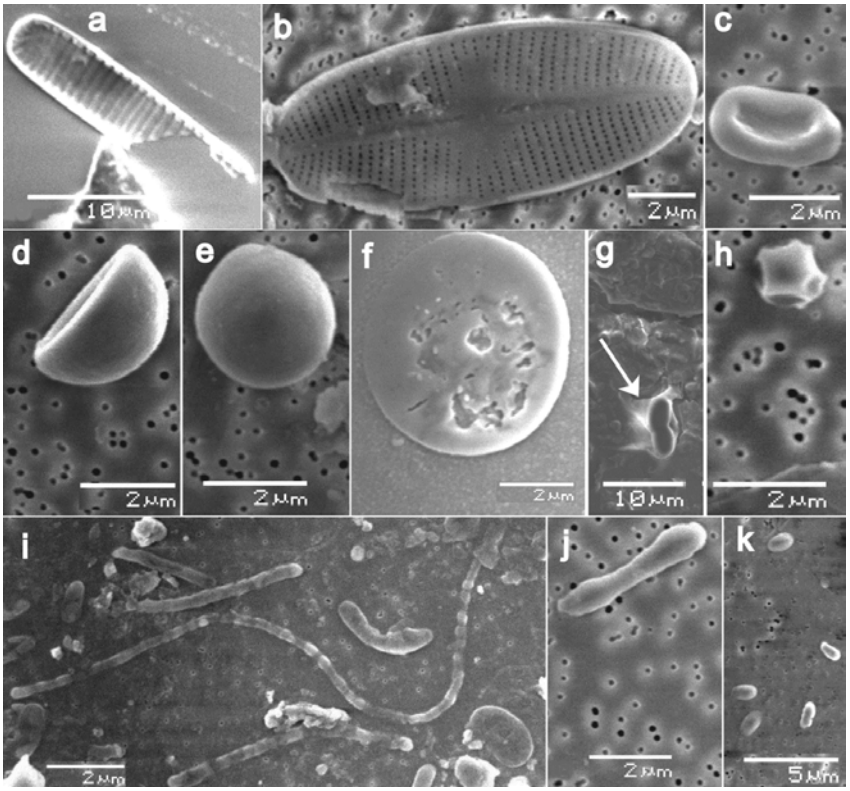

Figure 2.5. *SEM images showing the microbial diversity in snow: (a, b) diatoms, (c-g) pico-like single-celled microalgae (note the exopolymer secretion around the cells in g, indicated by the arrow), (h) unidentified microbe, (i) cyano-like filamentous microbe, (j) dividing bacteria, and (k) short rod-shaped bacteria.*

About 55% of the samples comprised picoplankton with concentrations ranging from 3.93 × 10^2 to 5.5 × 10^4 cells mL^{-1} and formed up to 71% of the total cells (9.43 × 10^3 to 9.27 × 10^4 cells mL^{-1}). Both bacteria and picoplankton together accounted for 104-1351 ng carbon L^{-1} (mean 365 ng C L^{-1}). However, the fraction of microbial cell carbon may be an underestimate, as the contribution of nanoplankton-derived carbon to the total carbon was not determined in this study. Although the cellular carbon pool accounted for only a small fraction of the TOC, these values when used to compute the microbial cell carbon for the AIS (3.01×10^7 km^3) was equal to about 11 × 10^{12} g C.

This estimated cellular carbon pool is about 1 order higher than that of all the Earth's surface fresh waters combined (Whitman et al., 1998) and is higher than that reported for ice sheets by Priscu et al., (2008). The higher estimate obtained from this study may be due to the fact that (1) microbial abundance data that were used in this study to compute cellular carbon in the ice sheets were higher than that used by Priscu et al., (2008) and (2) the cellular carbon estimates of East Antarctic ice sheet include both bacterial and picoplankton-derived carbon, while only bacterial carbon was accounted for by Priscu et al., (2008). Nevertheless, the crude estimates from both studies reflect the immense pool of cellular carbon held in the AIS.

In addition to the microbial cell carbon, the by-product of their metabolic activity could also be a significant source of organic carbon in snow. Previous studies have shown that microorganisms are metabolically active at subzero temperatures (Carpenter et al., 2000; Christner, 2002a; Amato et al., 2009). While microbial activity can alter and degrade dissolved organic matter, it can simultaneously release newly synthesized dissolved organic species (Ogawa et al., 2001). SEM examination showed that the microalgae in some of the samples produced exopolymeric materials (Fig. 2.5g). Exopolymers are primarily composed of polysaccharides, amino acids, amino sugars, glycoproteins etc., and are important sources of autochthonous organic compounds (Decho, 1990). The photo-autotrophic pico- and nanoplankton can fix atmospheric CO_2 into organic matter, thereby adding carbon to supraglacial systems. Measurements of *in situ* microbial primary production and community respiration have suggested that supraglacial environments are largely autotrophic systems (Tranter et al., 2004; Anesio et al., 2009). Microbially derived fulvic acids detected in supraglacial samples have been attributed to primary productivity of algae and bacteria on the glacial surface (Lafrenière and Sharp, 2004; Barker et al., 2006). Also, the molecular level characterisation of dissolved organic matter in the

Antarctic and Greenland glacial ice has revealed that it is predominantly composed of compounds originating from *in situ* microbial processes (Bhatia et al., 2010; Pautler et al., 2011). Thus, the photosynthetic and metabolic activity of these microorganisms together with the release of microbial exudates and exopolymeric substances could add to the total organic carbon content in the snow samples.

Organic carbon and microbial cells in Antarctic snow could also have an allochthonous source through wind-borne mineral particles deposited on the snow surface (Stibal et al., 2008; Price et al., 2009). These dust particles are mainly of crustal origin coming from areas of locally exposed bed-rock or are transported long distance from the continents. In order to elucidate the possibility of dust-borne organic matter deposition in snow, the relation between TOC and dust particles was determined. Total dust concentrations in this study ranged from 141 to 3500 μg L^{-1} (Table 2.2). TOC did not show any relation with dust concentrations. The present study thus suggests that the crustal contribution to organic carbon load in the Antarctic snow may not be significant.

While this study provides important insights into the spatial distribution and possible sources of organic matter in the Antarctic snow, further knowledge on the nature, composition, and reactivity of supraglacial DOM can be obtained only through a detailed investigation at the molecular level. Therefore in Chapter - 3, the results of the molecular studies on DOM are presented which would give novel insights on the nature of organic matter associated with the East Antarctic ice sheet.

CHAPTER 3

Molecular-level characterisation of dissolved organic matter

3.1. Introduction

Snowpack cycling of DOM can be important on local, regional, and global scales (McNeill et al., 2012). However, the extent to which supraglacial DOM participate in photochemical processes or affect the functioning of resident microbial communities, will largely depend on the chemical nature and composition of the DOM. Despite the importance of supraglacial DOM in biogeochemical processes and evidence that it is potentially labile (Lafrenière and Sharp, 2004; Barker et al., 2006; Hood et al., 2009), knowledge of its cycling and chemical composition is poor, with any net shifts in size, function and composition largely obscured by current analytical limitations. While bulk DOM abundance studies are useful as first order investigations, they offer little information regarding the origin, reactivity and bioavailability of the supraglacial DOM pools. Until recently, characterisation of supraglacial DOM has been hindered by methodologies in which only bulk parameters were ascribed or for which a significant pool of the DOM resided outside the analytical window.

The advent of Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS) coupled to an ionisation source such as Electrospray Ionisation (ESI) provides an opportunity to study a larger portion of the DOM pool, and to characterise the reactivity of specific molecules in biogeochemical processes. ESI is a 'soft' (low-fragmentation) ionisation technique that ionises the polar molecules with acidic and basic functional groups within a complex mixture and produces largely intact ions (positively or negatively charged) from analyte molecules over a wide mass range ($10 < m/z < 3000$). When coupled to a mass spectrometer, such as FTICR-MS which is capable of ultrahigh mass resolution (>500,000) and mass accuracy (error <0.5 ppm), thousands of individual molecular species in a complex DOM mixture can be simultaneously and accurately resolved at each nominal mass (Kujawinski, 2002; Grannas et al., 2006; Bhatia et al., 2010; Stubbins et al., 2010). The ultrahigh mass accuracy and resolving power of FTICR-MS, is the key to this technique as it enables the assignment of elemental formulas solely from the mass measurement - a major breakthrough for the characterisation of DOM and its molecular messages (Kujawinski, 2002; Kujawinski et al., 2004; Stenson et al., 2003). Although observed ion intensities may be biased by the matrix in the ionisation source, FTICR-MS has revolutionised our ability to analyse natural organic matter by providing detailed molecular information for DOM. The molecular details obtained can be utilised to determine the composition of specific molecules within DOM, as well as to identify the

compositional differences among pools of DOM and thus allow for novel insights on the character of DOM. The potential of FTICR-MS to directly identify the individual compounds that comprise the highly complex DOM pool is currently unrivalled. Recently, FTICR-MS has been utilised to characterise DOM in non-polar glaciers (Grannas et al., 2006; Stubbins et al., 2012a) and the Greenland ice sheet (Bhatia et al., 2010). Information on the composition, sources, reactivity and the potential biogeochemical role of DOM in Antarctic supraglacial environments are severely limited. Thus, molecular-level characterisation of DOM associated with the AIS will be a critical aspect to help understand its significance to regional and global scale carbon dynamics both now and in a warming climate.

In this chapter, the nature of carbon pools associated with the coastal as well as the interior plateau region of East Antarctica were investigated using ESI-FTICR-MS. Presently, the molecular information on glacial DOM has been obtained from either negative (ESI−) (Bhatia et al., 2010; Singer et al., 2012; Stubbins et al., 2012a) or positive (ESI +) (Grannas et al., 2006) ion mode, but not both together. Recent work has shown that ESI+ and ESI− target different groups of compounds, resulting in different mass spectra for the same sample (Rostad et al., 2004; Hockaday et al., 2009). Therefore, in this study, both ESI− and ESI+ were used together with 12 Tesla FTICR-MS to increase the analytical window and to allow for enhanced characterisation of the molecular components of DOM in Antarctic surface snow. By establishing the baseline values of the type of organic carbon present in the Antarctic supraglacial environment, this study serves as the foundation for broader investigations into the potential biogeochemical role of glacial DOM in supraglacial carbon cycling and the impact of increased melt water runoff from the ice sheet to surrounding marine environments.

3.2. Methodology

3.2.1. Sample Preparation and FTICR Mass Spectrometry

For FTICR-MS analysis, snow samples (approximately 100−150 mL) were melted in sealed amber coloured glass bottles that were pre-cleaned by soaking in a 1 M HCl bath for at least 3 hr, followed by rinsing with ultrapure water several times, and combustion at 475 °C for 4 hr. The steps of the DOM extraction procedure are presented in Figure 3.1. Samples were allowed to melt at ambient temperature (Fig 3.1a) and extracted by solid phase extraction using 100 mg PPL cartridges (Agilent Bond Elut-PPL; Fig. 3.1b),

following Dittmar et al. (2008). Cartridges were preconditioned with at least 2 cartridge volumes of LC-MS grade methanol and then rinsed with 3 volumes of 0.01 M HCl (trace-metal grade). Each snow sample previously acidified to pH 2 was applied to a fresh cartridge and rinsed with 3 cartridge volumes of 0.01 M HCl (trace-metal grade). Sorbents were dried under N_2 gas (Fig 3.1c) and the adsorbed organic matter was immediately eluted off with 1.5 mL of LC-MS grade methanol. Samples were analysed using a Bruker Daltonics 12 T Apex Qe FTICR-MS (Fig. 3.1d), housed at the College of Sciences Major Instrumentation Cluster (COSMIC) at Old Dominion University, Virginia (USA).

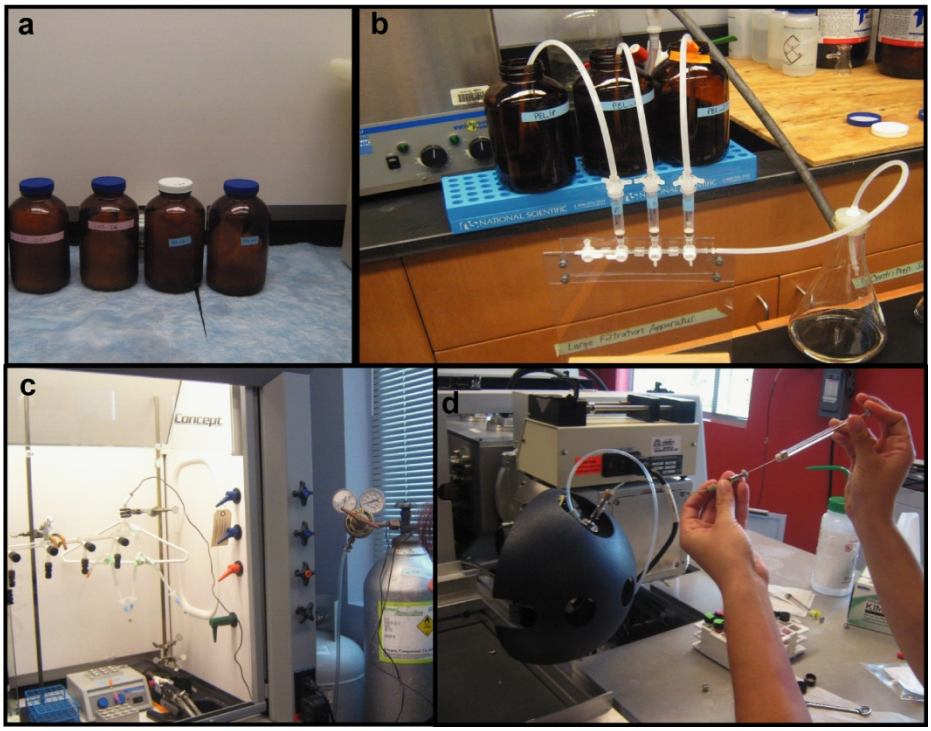

Figure 3.1. *Solid phase extraction and analysis of DOM from snow samples.*

For both positive and negative ion modes, samples were infused continuously into the Apollo II ESI ion source at a rate of 120 µL hr^{-1}. In order to increase ionisation efficiencies, snow samples were amended with ammonium hydroxide/formic acid for negative/positive ion modes, respectively, to produce a final sample composition of 50:50 (v/v) methanol:water with either 0.1% NH_4OH or 0.1% HCOOH. Before analysing each sample, a solvent blank using the same composition as that of the sample was analysed to ensure that no carry over from previous analyses contaminated the sample's spectra. These blank analyses confirmed that there was no contamination from previous samples. Procedural blanks comprising ultrapure water processed in the same manner as that of the snow samples (i.e., acidified and PPL extracted) were also analysed. The capillary

temperature was set at 200°C. For negative ion mode, the ESI shield voltage and capillary voltage were optimized to 3.4 kV and 4.1 kV, respectively, while for positive ion mode the respective voltage was 3.8 kV and 4.6 kV. Ions were accumulated in the hexapole for 3 s before being transferred to the ICR cell, where 300 scans were collected and co-added in broadband mode using $200 < m/z < 1200$.

3.2.2. Calibration and elemental formula assignments

Prior to data analysis, the instrument was externally calibrated with a polyethylene glycol standard and internally calibrated with fatty acids and other naturally present homologous series detected within the sample (Sleighter et al., 2008). The accurate m/z values were determined to 5 decimal places. Only m/z values with a signal-to-noise (S/N) above 3 were considered. The individual sample and solvent blank peak lists were aligned, and all peaks found in the solvent blanks were removed from the appropriate master list. These blank-corrected master peak lists in each sample were used in further data treatment. Procedural blanks were relatively free of interfering compounds, and none of the blank peaks were significant peaks in the sample mass spectra. In order to ensure reproducibility of the acquired mass spectra, all samples (only negative ion mode) were analysed in duplicate. Peak detection reproducibility was evaluated by calculating the percentage of common peaks in replicate analyses by comparing peak lists at $S/N \geq 3$ and correcting that threshold with peaks of $S/N \geq 2.5$ (Sleighter et al., 2012). The average percentage of common peaks between replicates was 71% (range of 55−78%). Formulas were then assigned to peaks that were common between the replicates using a molecular formula calculator developed at the National High Magnetic Field Laboratory in Tallahassee, Florida, (Molecular Formula Calc v.1.0 NHMFL, 1998) that generated formula matches for ESI− and ESI+ modes using the criteria $C_{5–50}$, $H_{5–100}$, $N_{0–5}$, $O_{1–30}$, $S_{0–2}$, $P_{0–2}$, and $C_{5–50}$, $H_{5–100}$, $N_{0–5}$, $O_{0–30}$, $S_{0–2}$, $Na_{0–1}$, respectively. About 61−83% of the total number of peaks resolved in each sample in negative and positive ion modes following blank correction were assigned molecular formulas (excluding contributions from ^{13}C isotopes). The majority of assigned formulas agreed with the measured masses within an error of ≤ 0.5 ppm.

After exact elemental formulas had been assigned, molecules were categorised by compound class using various chemical metrics. Double bond equivalent (DBE) values were calculated as $DBE = 1 + C - 0.5H + 0.5N + 0.5P$ (Stubbins et al., 2010). Molecular formulas with $DBE/C < 0.3$ and $H/C \geq 1$ were unambiguously assigned as aliphatics

(Stubbins et al., 2010). The modified aromaticity index (AI_{mod}), which is a measure of the probable aromaticity for a given molecular formula assuming that half of the oxygen atoms are doubly bound and half are present as σ bonds was calculated as: AI_{mod} = (1 + C - 0.5O - S - 0.5H)/(C - 0.5O - S - N - P) following Koch and Dittmar, (2006). Formulas with AI_{mod} ≥0.5 and <0.67 were assigned as aromatics, while formulas with AI_{mod} ≥0.67 were assigned as condensed aromatics (Koch and Dittmar, 2006). The following compound classes were defined based on Hockaday et al., (2009) and Ohno et al., (2010): lipids (O/C = 0−0.2, H/C = 1.7−2.2), proteins (O/C = 0.2−0.6, H/C = 1.5−2.2, N/C ≥0.05), lignin (O/C = 0.1−0.6, H/C = 0.6−1.7, AI_{mod} <0.67), carbohydrates (O/C = 0.6−1.2, H/C = 1.5−2.2), tannins (O/C = 0.6−1.2, H/C = 0.5−1.5, AI_{mod} <0.67), and unsaturated hydrocarbons (O/C = 0−0.1, H/C = 0.7−1.5). Carboxylic-rich alicyclic molecules (CRAM) were defined as having DBE/C = 0.30−0.68; DBE/H = 0.20−0.95; DBE/O = 0.77−1.75 (Hertkorn et al., 2006). Ionisation efficiencies vary for different types of molecules, in both positive and negative ionisation modes. As such, the combination of these ionisation modes result in a greater representation of the molecular species that are present in the isolated DOM. However, it should be noted that certain types of molecules have poor ionisation efficiencies in both modes (for example, hydrocarbons). Thus, in all cases, when the relative abundances of molecular species identified were compared, it was in comparison with the total number of species identified and should not be interpreted as a quantitative assessment relative to the entire DOM pool.

3.2.3. HySPLIT Model

In order to assess long range atmospheric transport of organic matter to Antarctica, air mass back-trajectories were calculated using the Hybrid Single Particle Lagrangian Integrated Trajectory (HySPLIT) transport and dispersion model from the NOAA Air Resources Laboratory (Draxler et al., 2012). The surface snow samples in this study represented the early spring and summer snowfall events, based on the snow accumulation rates estimated at the sampling sites (Mahalinganathan et al., 2012). First, NASA's Modern Era Retrospective Re-Analysis observational data were utilised for precipitation between Oct 2008 and Jan 2009 in order to determine precipitation events representative of these snow samples. Back-trajectories were then calculated using the HySPLIT model for the days that recorded precipitation, in order to determine the source regions for air masses arriving at Princess Elizabeth Land. Ten day back-trajectories at 6-hr intervals were

calculated at an altitude of 1500 m above ground level for three different sites (middle and end members) located at 69°29′S, 76°10′E; 70°05′S, 77°39′E; and 70°37′S, 79°05′E.

3.2.4. Analysis of methanesulfonic acid

Methanesulfonic acid (MSA) is a common secondary organic aerosol component in marine aerosols that results from the atmospheric oxidation of dimethyl sulfide and represents an unequivocal indicator of marine biogenic activity (Charlson et al., 1987). Thus, in order to determine marine aerosol contributions to DOM composition in the snow, chemical analysis of surface snow samples were carried out. All sub-sampling equipment and sample containers were pre-cleaned by rinsing several times with ultrapure water, soaking for at least 24 hr, followed by rinsing with fresh ultra pure water and drying in a laminar-flow bench. Samples were melted immediately prior to the analysis in a class-100 clean room facility. Measurement of MSA ($CH_3SO_3^-$) was carried out using Dionex ICS 2000 using IonPac AS11-HC (4 mm) column with potassium hydroxide (KOH) at 1.2 mL min^{-1} as eluent and an IonPac AG11-HC Guard column, with an AERS-ULTRA (4 mm) Anion Electrolytically Regenerating Suppressor. The sample injection volume for analysis was 1000 µl. Calibration was done using IV (Inorganic Ventures) high-purity standards. The detection limit was less than 2 µg L^{-1}. Reference standards and random samples were analysed routinely to estimate the analytical precision, which was better than 5%.

3.3. Results and Discussion

The resolving power (the exact mass of the peak divided by the full width at the half maximum of that peak) of FTICR-MS across the 200–800 m/z range exceeded 600,000, enabling the assignment of exact molecular formulas to thousands of peaks in the DOM mass spectra and demonstrated the high level of molecular complexity inherent to DOM. Over 10,900 molecular formulas were identified in the snow samples. The assigned molecular formulas were represented in van Krevelen diagrams which plot molar H/C vs O/C of each distinct empirical formula, aligning formulas in regions that can be attributed to proteins, lipids, amino sugars, carbohydrates, lignins, tannins, unsaturated hydrocarbons, and condensed aromatics (Fig. 3.2). The presence of formulas that are characteristic of amino sugars, lipids, and proteins in these samples correlated well with previous work identifying these as biologically derived components of DOM in glaciers and ice sheets (Grannas et al., 2004; Bhatia et al., 2010; Pautler et al., 2011). In addition,

the presence of microbially derived material corresponded with the occurrence of bacteria and microalgae in these samples.

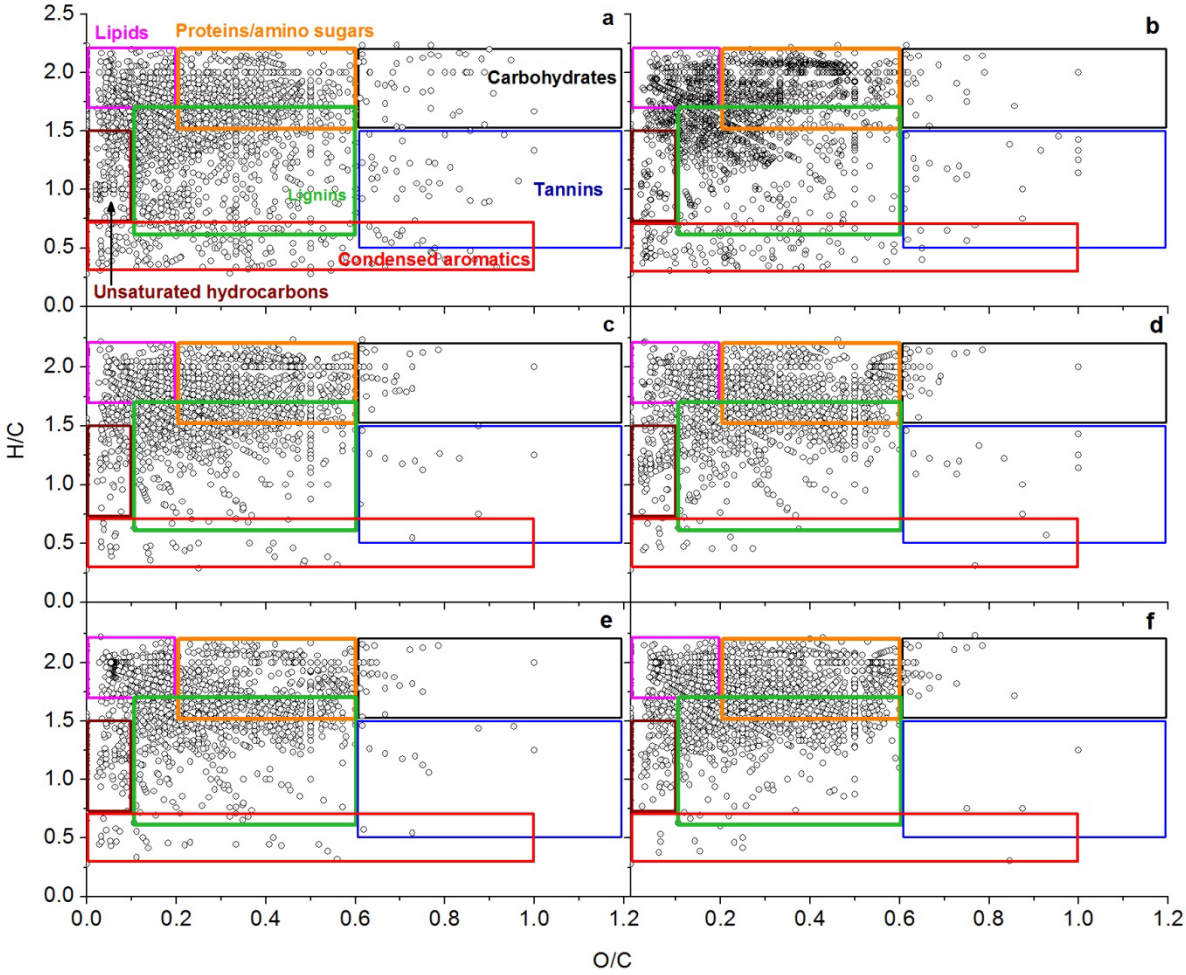

Figure 3.2. *van Krevelen plots for molecular formulas assigned to the negative and positive ion mode FTICR mass spectral peaks. Major biochemical compound classes detected in surface snow samples collected at sites located at (a) 10 km, (b) 20 km, (c) 50 km, (d) 80 km, (e) 130 km, and (f) 180 km from the coast are shown. Boxes overlain on the plots indicate biomolecular compound classes. Regions in which compounds share common elemental ratios (overlapping boxes) are distinguished by nitrogen content or aromaticity index.*

Proteinaceous substances in DOM have been interpreted as evidence for *in situ* microbial activity and/or freshly produced DOM (Barker et al., 2010). The predominance of microbial products contributing to more than half the identified molecular species indicates that most of the identified supraglacial DOM components most likely were originated from *in situ* microbial activities. The detection of protein- and lipid-derived molecules in all

snow samples in this study and in other glacial snow, ice, and meltwater samples (Grannas et al., 2004; Bhatia et al., 2010; Barker et al., 2010) is indicative of the ubiquitous presence of these materials in glacial surfaces. These compounds could represent a potential source of carbon and nutrients that can be transported to downstream aquatic ecosystems in glacier meltwater (Barker et al., 2010; Singer et al., 2012). Active microbial communities associated with snow and ice sheet surfaces (Christner et al., 2000; Carpenter et al., 2000; Christner et al., 2001; Larose et al., 2013) could also make use of the supraglacial organic carbon. Organic carbon produced by resident autotrophic communities (autochthonous organic carbon) is the dominant substrate on glacier surfaces (Bhatia et al., 2010; Anesio et al., 2010; Pautler et al., 2011) and are readily mineralised by supraglacial heterotrophs (Foreman et al., 2007; Anesio et al., 2010). Ancient and anthropogenic aerosol organic matter found on glacier surfaces is biologically reactive (Hood et al., 2009; Stubbins et al., 2012a). The general density of the elemental data in the region of low O/C (<0.6) and high H/C (>1.7) reflects the contribution of aliphatic compounds, possibly derived from algal detritus and/or microbial biomass (Schmidt et al., 2009) to the DOM composition in these samples. Aliphatic molecules (DBE/C < 0.3; H/C ≥ 1) (Stubbins et al., 2010), likely from microbial and algal biomass, comprised between 650 and 989 peaks per sample, constituting about 37−52% of the total formulas assigned for each sample (Table 3.1).

Table 3.1. *Details of molecular formulas identified in surface snow samples. The number of molecular formulas reported here are from positive and negative ion mode ESI-FTICR-MS formula assignments combined.*

Distance of sampling site from coast (km)	Total number of formulas assigned	Number of FTICR-MS formulas		
		Aliphatic molecules of algal and microbial origin	Terrestrial organic material (lignin and tannin)	Condensed aromatics
10	2612	977 (37%)	947 (36%)	225 (9%)
20	1600	739 (46%)	522 (33%)	145 (9%)
50	1695	831 (49%)	627 (37%)	46 (3%)
80	1411	650 (46%)	537 (38%)	25 (2%)
130	1702	865 (51%)	629 (37%)	40 (2%)
180	1889	989 (52%)	660 (35%)	21 (1%)

Distant allochthonous organic carbon, such as black carbon from anthropogenic sources and terrestrially derived cellulose and lignin, are also likely utilised by some heterotrophic bacteria (Margesin et al., 2002; Norwood et al., 2013). Thus, carbon flow and transformation through supraglacial microbial communities may have an impact on the biogeochemistry of glacier and ice sheet environments (Stibal et al., 2012). In addition to a predominantly autochthonous microbial source, there is a terrestrial component within all the snow samples, as indicated by the presence of tannin-like and a high density of lignin-like formulas (Fig. 3.2, Table 3.1). Between 498 and 904 lignin-like formulas were detected. In general, while major biogeochemical compound classes have characteristic H/C and/or O/C molar ratios, and thus appear in specific regions of the van Krevelen plot (Kim et al., 2003), it should be noted that these regions do not strictly represent all similar molecules, but provide guidelines for identifying compounds of similar composition. Recently, an overlap of the lignin region with carboxylic-rich alicyclic molecules (CRAM), a group of compounds previously identified as a component of freshwater (Lam et al., 2007) and deep ocean DOM, has been reported (Hertkorn et al., 2006; Sleighter and Hatcher, 2008). Here, it was seen that about one tenth to one-third of the lignin-like peaks overlapped with the region associated with CRAM-like molecules (DBE/C = 0.30−0.68; DBE/H = 0.20−0.95; DBE/O = 0.77−1.75) (Hertkorn et al., 2006) and could perhaps be attributed to CRAM. The lignin-like molecular signatures for terrestrial inputs obtained in this study were similar to those observed for terrestrially derived DOM deposited in the Greenland ice sheet and other non polar glaciers (Grannas et al., 2004; Bhatia et al., 2010; Singer et al., 2012). Such ubiquitous signatures of vascular plant derived material have not been reported earlier from Antarctic surface snow. These findings of terrestrially derived DOM in snow are counter to the prevalent view that organic carbon in Antarctica is hardly influenced by terrestrial sources (Lyons et al., 2007). The presence of vascular plant-derived materials is intriguing, as the East Antarctic ice sheet has no known source of vascular plants and is remotely located from any direct sources of plant material. The most plausible explanation for lignin and tannin incorporation into the snowpack is through long range atmospheric transport and deposition of organic matter originating from other continents. The 10-day back trajectory plots show that the air masses arriving at the Princess Elizabeth Land region of East Antarctica on 5 October and 21 November 2008 passed over southern South America. This could indicate a South American source for organic carbon in the East Antarctic ice sheet (Fig. 3.3a,c).

While distant aerosol transport and deposition of terrestrial carbon have been reported in snowpack and ice cores in remote locations such as Summit, Greenland (Grannas et al., 2004) and Franz Josef Land, Russia (Grannas et al., 2006) that are far removed from any vegetation sources, no terrestrial DOM signatures have been reported for the AIS.

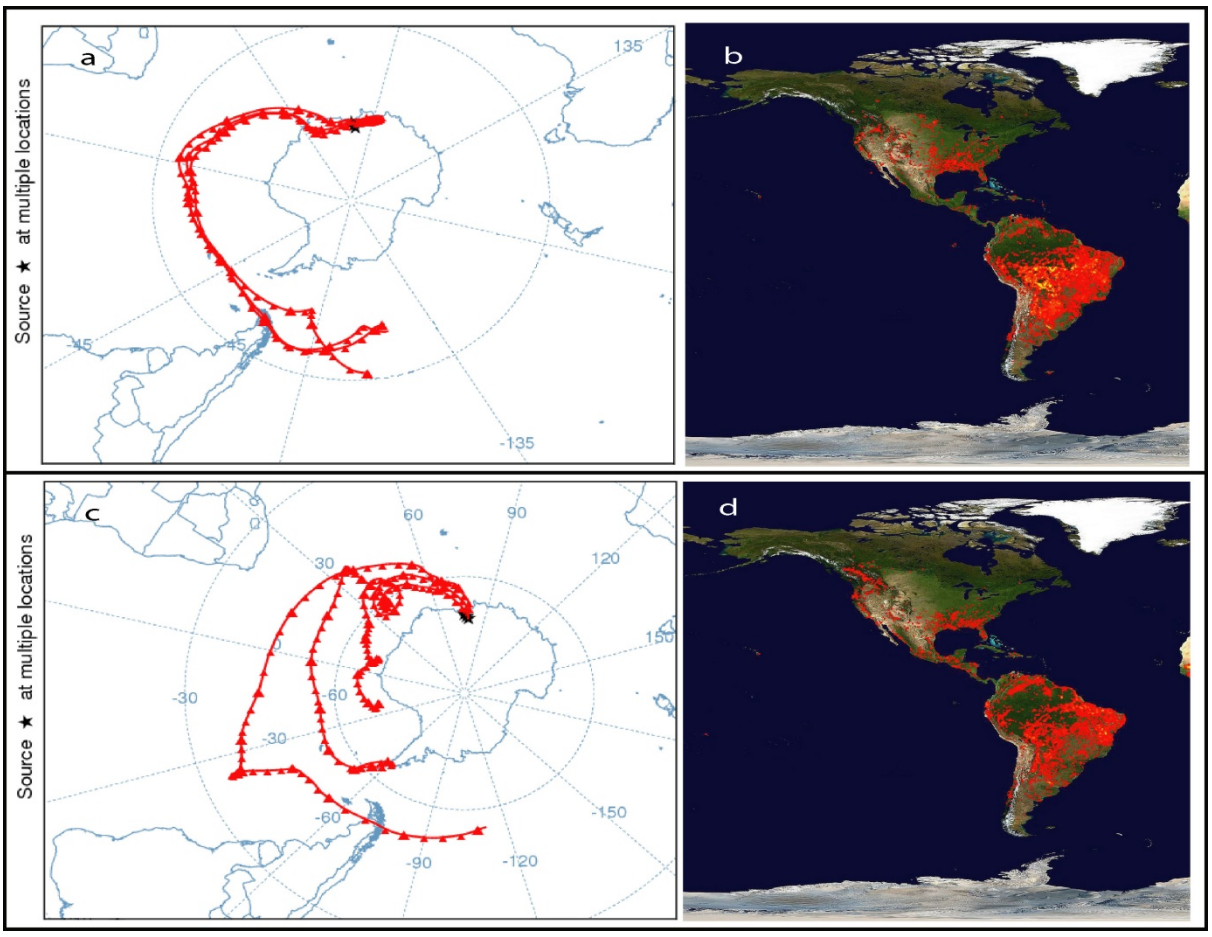

Figure 3.3. *Air mass transport to East Antarctica from South America. HySPLIT modeled 10- day back-trajectories for air masses arriving to the Princess Elizabeth Land region of East Antarctica on (a) 5 October 2008 and (c) 21 November 2008. Back-trajectories were calculated for one mid- and two end members of the sampling transect. Moderate resolution imaging spectroradiometer (MODIS) onboard NASA's Terra and Aqua satellites (http://earthdata.nasa.gov/lance/rapid-response) shows wild fires on the South American continent during the same time frame when air masses passed over the continent, i.e., about 10 days before arriving to East Antarctica on (b) 5 October 2008 and d) 21 November 2008.*

This study shows for the first time a strong contribution of terrestrially derived organic compounds to the DOM present on the surface of the AIS. Because the terrestrial DOM in

glacial ecosystems is in a form that is highly bioavailable to heterotrophs (Hood et al., 2009) and terrestrial DOM is a significant driver of bacterial production in aquatic systems (Pollard and Ducklow, 2011), the present findings suggest the possibility that glacial discharge to the ocean could potentially alter carbon dynamics in the coastal oceans. As bacterial production and community composition are particularly sensitive to the quantity and quality of DOM in natural environments (Judd et al., 2006), the extent to which DOM derived from such vascular plants could impact the activity and composition of microbial communities residing on the surface of the ice sheets and in marine ecosystems that receive their runoff is not known.

The modified aromaticity index (AI_{mod}), a parameter used for the identification of aromatic ($AI_{mod} \geq 0.5$ and <0.67) and condensed aromatic structures ($AI_{mod} \geq 0.67$) based on the assigned molecular formulas (Koch and Dittmar, 2006), shows that the snow samples contained between 21 and 225 distinct formulas of hydrogen deficient condensed aromatic constituents characteristic of dissolved black carbon (DBC)-like material. These account for up to 9% of the total identified molecular formulas per sample (Table 3.1 and Fig. 3.4). Because condensed aromatics are formed from thermal alteration of organic carbon during biomass and fossil fuel burning, they are indicators of the presence of previously combusted organic carbon in these snow samples. About 39−71% of the condensed aromatic formulas had low O/C ($\leq$0.2). Such low values were previously shown to be associated with DBC originating from wild fires and soils containing charred residues (Schmidt et al., 1999). Because back-trajectory analysis indicates long distance atmospheric transport of organic carbon from South America on 5 October and 21 November 2008, Moderate Resolution Imaging Spectroradiometer (MODIS) data from the Aqua and Terra satellites were used to locate active vegetation fires in South America during this period. Real-time imagery from the MODIS Rapid Response system showed biomass burning events on the South American continent during the time that the air-masses passed over it (i.e., approximately 10 days before arriving at East Antarctica) (Fig. 3.3b,d). This is in agreement with the condensed aromatic formulas detected in the study area that could be attributed to biomass burning. These results indicate that forest fires are likely contributors to DBC detected in the AIS and provide evidence supporting the earlier indications of biomass burning emissions from South America reaching the Antarctic continent (Fiebig et al., 2009).

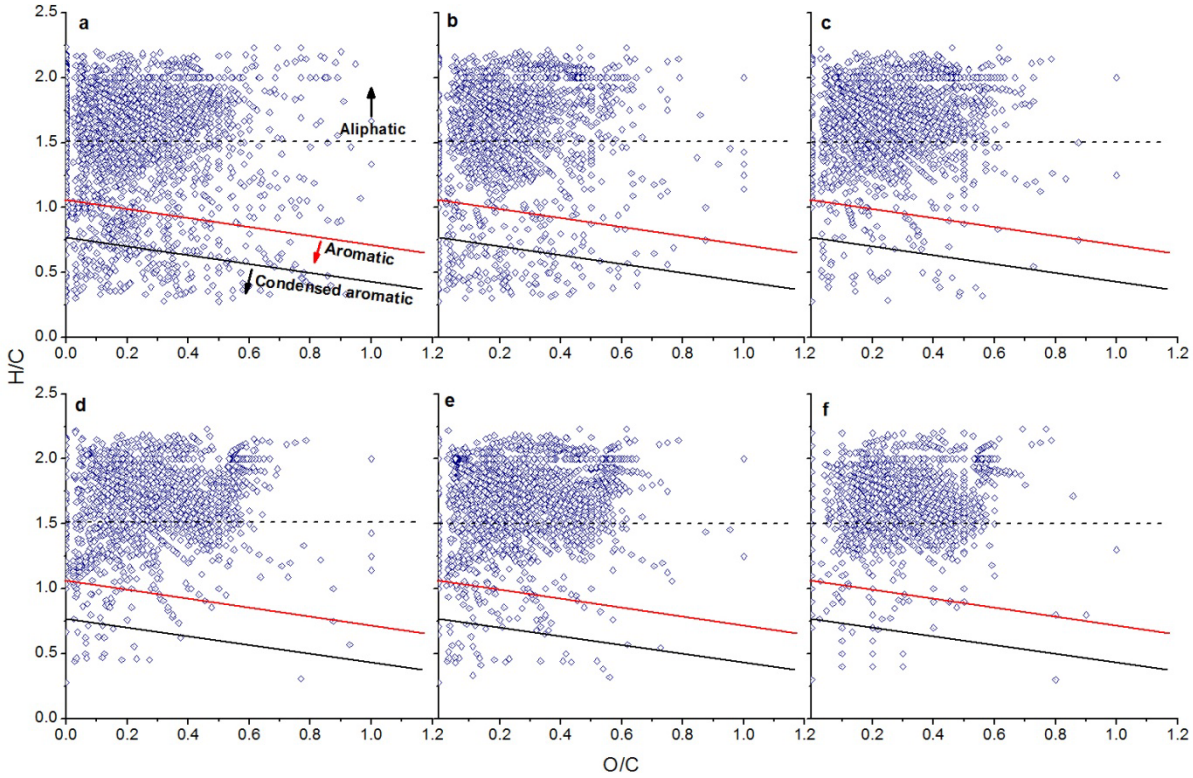

Figure 3.4. *van Krevelen plot showing the distribution of aliphatic (H/C ≥1, DBE/C <0.3), aromatic (AI_{mod} = ≥0.5 and <0.67), and condensed aromatic (O/C = 0-1, H/C = 0.3-0.7, AI_{mod} ≥ 0.67) molecular formulas identified in surface snow samples collected from a site located a) 10 km, b) 20 km, c) 50 km, d) 80 km, e) 130 km and f) 180 km from the coast.*

The present data also identified aromatic compounds with high O/C values (>0.4). Condensed aromatic ring structures extensively substituted with oxygen-containing functional groups have been previously linked with microbially mediated oxidation of dissolved black carbon (DBC, Dittmar and Koch, 2006), with humic acid leachates of soil charcoal (Hockaday et al., 2006), and with highly aromatic humic acids from regularly charred agricultural soils (Kramer et al., 2004). Thus, it is possible that these compounds, which constituted 6−28% of the total identified condensed aromatic formulas in the snow, could be modified DBC or DBC derived from soil humic acids. Microbial degradation of black carbon results in its oxidation to water-soluble species (Hockaday et al., 2006), enabling them to be potentially distributed throughout the ocean through glacial discharge. The cold Antarctic surface waters have been found to be slightly depleted in thermogenic DOM (Dittmar and Paeng, 2009), as these are also one of the regions least impacted by continental runoff and human activity. Thus, the identification of DBC in supraglacial environments (Bhatia et al., 2010; Stubbins et al., 2012a) and the rapid rise in climatically

driven ice loss from polar ice sheets indicate that the polar ice sheets need to be considered as potentially important sources of DBC to the oceanic DOM pool. There remain several blind spots in our understanding of DBC export from polar ice sheets and their cycling in recipient systems. The importance of glacial ecosystems as sources of DBC has neither been assessed in a quantitative way, nor the environmental consequences of increased transport of DBC from glacial discharge to marine systems. However, as the dissolved fraction of black carbon can be quite labile (Norwood et al., 2013) and could further accelerate the decomposition of bulk DOM (Masiello and Louchouarn, 2013), it is expected that this may be reflected in changes in DOM cycling and associated impacts on aquatic food webs.

Aromatic and condensed aromatics together constituted up to 18% of the total molecular formulas identified in the surface snow samples. These compounds have been previously found to be predominantly photolabile (Stubbins et al., 2010). The photochemical transformations of such compounds in snow may result in the production and release of photochemically active compounds that can significantly impact the composition of the overlying atmosphere (Grannas et al., 2007). Observational and modeling data in both polar regions (Arctic and Antarctic) show that photochemically mediated emissions from snow are fundamental to driving both local as well as regional boundary layer chemistry (Grannas et al., 2007) and that snowpack cycling of DOM can be important on local, regional, and global scales (McNeill et al., 2012). The presence of numerous aromatic and condensed aromatic compounds among the identified formulas in the surface snow samples suggests that they could potentially be important in snow photochemistry and air−snow exchange processes. Moreover, the detection of microbially modified and photolabile organic compounds indicates that a portion of the supraglacial DOM is probably delivered to the marine ecosystem after photochemical or biological degradation.

Numerous lines of evidence suggest that a number of compounds detected in the snow samples are from secondary processing in the atmosphere. Firstly, over 575 CHO-only compounds (formula consisting of only carbon, hydrogen, and oxygen atoms) have molecular formulas consistent with those detected in aerosol water-soluble organic carbon (Mazzoleni et al., 2012) and in secondary organic aerosols (SOAs) formed by ozonolysis of organic seed aerosols like α-pinene and limonene (Kundu et al., 2012). Secondly, consistent with the report of SOA products of CHO compounds formed by the esterification reactions involving a mass increase of $C_3H_4O_2$ (Mazzoleni et al., 2010), 67

individual oligomer series of two compounds separated by multiples of $C_3H_4O_2$ were identified within the CHO subset of assigned molecular formulas in the snow samples. These oligomer series accounted for up to 10% of the total CHO formula assignments for each sample. Thirdly, a significant number of formulas assigned in this work have high numbers of oxygen and nitrogen atoms consisting of NO_3−NO_9, N_2O_5−N_2O_9, and N_3O_8 subgroups, which is consistent with what has been previously observed in fog water-soluble organic matter (Mazzoleni et al., 2010). These subgroups comprised about 35−67% of the total CHON-only formula assignments. Fourthly, over 61% of the S-containing formulas (CHOS) have high O/S ratios (4−15) and exhibited a high degree of oxidation, consistent with the presence of sulfate functionality (Altieri et al., 2009). This agrees with previous studies that reported organosulfates in atmospheric aerosols (Reemtsma et al., 2006; Surratt et al., 2007). Fifthly, several of the CHONS compounds have sufficient oxygen (≥7 O) in their formula that would allow the addition of S as a sulfate group and N as a nitrate group (Altieri et al., 2009), forming nitrooxy-organosulfates that have previously been determined as components of SOAs (Reemtsma et al., 2006). Thus, ESI-FTICR-MS analysis of the snow samples points to the presence of organic species whose empirical formulas are similar to those found in atmospheric aerosols and fog, providing evidence for secondary processing in the atmosphere from volatile organic carbon precursors. Accordingly, it appears that snowpack DOM composition is influenced by the variability in deposited atmospheric species derived from SOAs.

While CHO-only compounds were the major category of assigned formulas followed by CHOS, CHONS, CHON, and others (CHOP, CHOPNS), in more inland samples, CHOS and CHONS compounds progressively decreased from 16% to 8% and 20% to 4%, respectively (Fig. 3.5). Another trend observed was that the DOM was chemically more diverse in samples closest to the coast, with over 1600 formulas that were unique to the coastal end member of the sampling transect and not present in any other sample. In contrast, the inland snow samples had only 240 formulas that were uniquely observed, while all others were commonly detected in the other surface snow samples. The high diversity of molecular formulas in the coastal samples points to the possibility of the ocean being an important source of DOM to these sites. This is consistent with the findings that the coastal region of the Princess Elizabeth Land receives a substantial input of sea spray aerosols and sea spray associated organic matter. Earlier studies have shown higher

contributions of N and S containing formulas in marine DOM (Sleighter and Hatcher, 2008; Kujawinski et al., 2009) and enrichment of CHON and CHOS compounds in sea-spray aerosols (Schmitt-Kopplin et al., 2012). These results correspond well with the increased heteroatom functionality observed in the coastal snow samples. Also, the potential organosulfate and nitrooxyorganosulfate SOA components in snow decreased systematically from coast to inland (Fig. 3.6a, b), suggesting that they may have originated from the oxidation of biogenic volatile organic carbons emitted from the ocean.

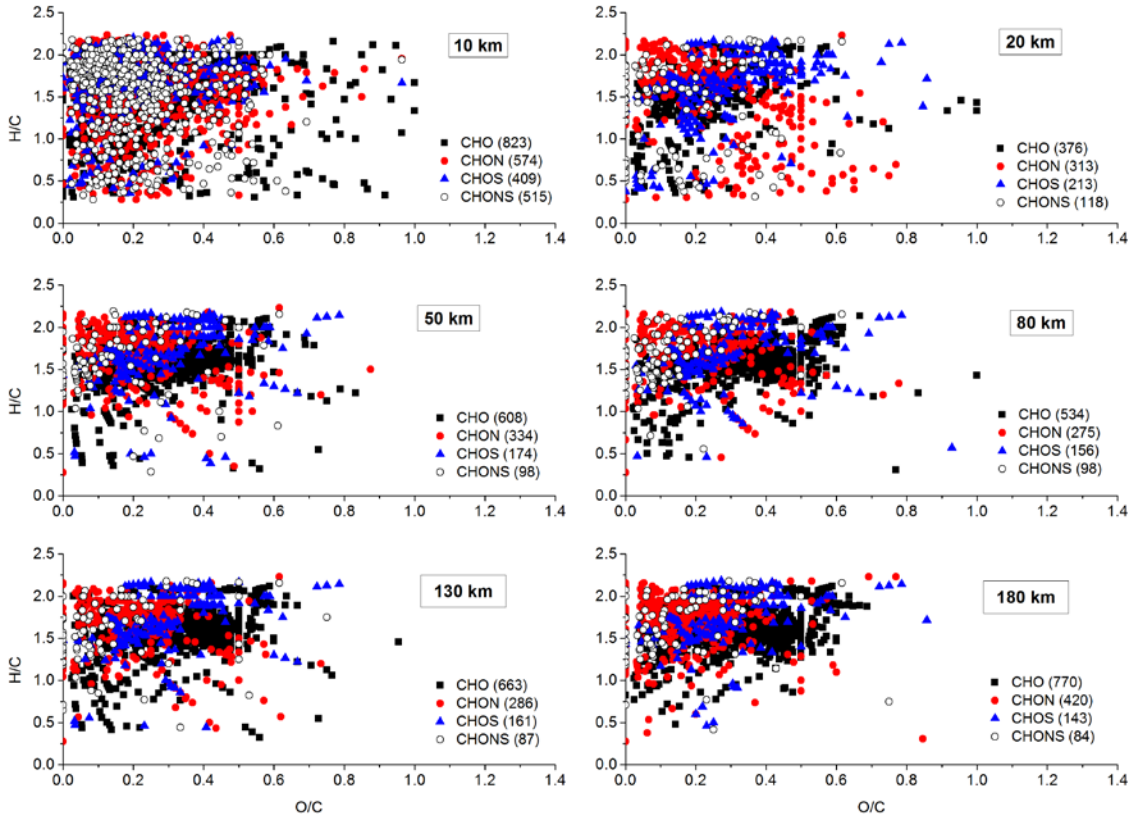

Figure 3.5. *van Krevelen diagram of the distribution of CHO, CHON, CHOS, and CHONS along the Princess Elizabeth Land transect as a function of increasing distance from the coast. Values in parentheses indicate the number of identified molecular formulas representing each compound class.*

This hypothesis is supported by high concentration methanesulfonic acid measurements in the coastal snow samples (Fig. 3.6c). Methanesulfonic acid is a common SOA component in marine aerosols that results from the atmospheric oxidation of dimethylsulfide (Charlson et al., 1987). Thus, the ocean could be an important source of both primary and secondary organic aerosols to the snowpack. Finally, comparison of the molecular formulas identified in the snow samples collected at various sites along this 180 km long

transect revealed that only a small fraction of the molecular species (<280), constituting about 3% of the total formulas, were common to all the samples. The fact that microbially derived material was the most prevalent among the identified molecular formulas and that there was very little overlap in formulas between samples along the transect suggests possible localised differences in bacterial composition and metabolic processes. Bacterial concentrations as well as community composition in this region have been reported to be distinctly different from coast to inland (Yan et al., 2012). These results, together with other findings that describe the presence of abundant microbial communities (Carpenter et al., 2000; Larose et al., 2013; Hell et al., 2013) and microbially derived DOM in supraglacial environments (Grannas et al., 2004; Bhatia et al., 2010; Pautler et al., 2011), suggest that microbial processes could be responsible for the great diversity of organic species observed and may control the molecular composition of DOM in different glacial environments.

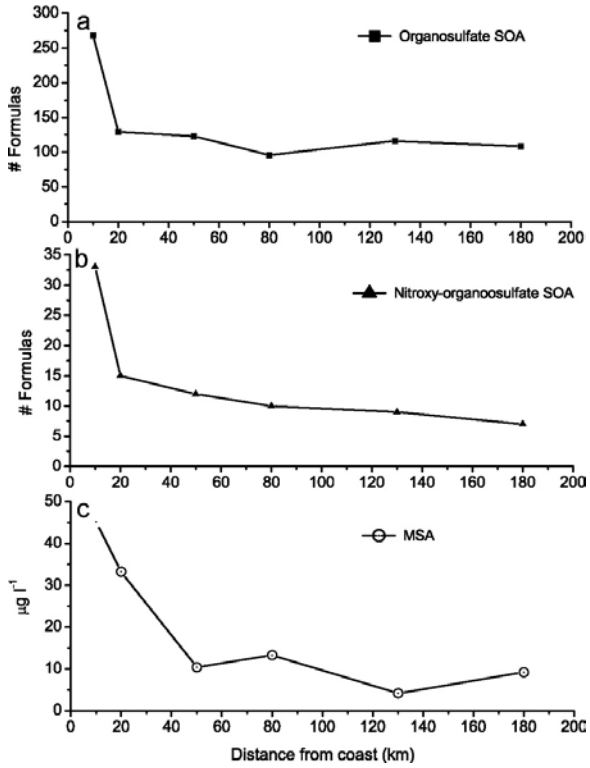

Figure 3.6. *Spatial variation of the number of molecular formulas of potential a) organosulfate secondary organic aerosol (SOA), b) nitrooxy-organosulfate SOA and c) methanesulfonic acid (MSA) concentrations detected in the snow samples.*

As both the ESI− and ESI+ modes were used to characterise this DOM, the molecular species ionised by both the modes was assessed. A comparison of the molecular formulas

showed that only 1−11% of the formulas was common to ESI− and ESI+. CHO compounds were dominantly detected in ESI− (3062 formulas) compared to ESI+ (712 formulas). However, a greater number of aromatic, condensed aromatic, and N containing formulas were observed in the ESI+ (378, 344, and 2551 formulas, respectively) compared to ESI− (146, 158, and 1467 formulas, respectively). Therefore, formulas detected in both modes complemented each other and the use of both provided a more complete characterisation of DOM.

This study suggests that Antarctic snowpack have a very diverse and reactive carbon pool associated with them and provides evidence that autochthonous DOM production through *in situ* microbial processes is prevalent in the snowpack. The biogeochemical significance of inputs of such glacier derived microbial DOM depends on the magnitude of DOM flux from the ice sheets. Over the past decade, the AIS have been disintegrating at an alarming rate of 87 Gt yr^{-1} (Sheperd et al., 2012). On the basis of previous measurements of DOM in the AIS (Christner et al., 2006; Priscu et al., 2008), it is estimated that an annual loss of 87 Gt of ice from the continent would result in the release of ~32 Gg (0.32×10^{11} g) of carbon annually to the coastal waters of the Southern Ocean. In comparison, annually rivers transport approximately 0.21×10^{15} g of carbon from continents to the ocean in dissolved form (Ludwig et al., 1996). However, it should be noted that in the present calculation of the magnitude of carbon flux from the AIS, the ice loss from basal melting (1325 ± 235 Gt $year^{-1}$) (Rignot et al., 2013) was not taken into account, implying that the present estimate is highly conservative and comprises a lower limit of the actual fluxes. Nevertheless, this estimate suggests that the AIS is a quantitatively important source of organic carbon to marine ecosystems.

The C/N ratio of DOM can give a broad perspective on the bioavailability of DOM. Generally, C/N ratio shows an inverse correlation with DOM lability (Hunt et al., 2000; Wiegner et al., 2004). DOM with low C/N ratios and low aromatic carbon content is generally more reactive to microbial metabolism (McKnight et al., 1994) and correlates with higher bacterial growth efficiencies (Kroer, 1993; Amon and Benner, 1996; Fellman et al., 2008). In this study, 22−39% of the total identified formulas had low C/N ratios (≤20) which is typical of more labile materials (Hunt et al., 2000). The biodegradable percent of the total supraglacial DOM formulas were similar to those reported from glacial runoff in the Gulf of Alaska (range: 23−66%) (Hood et al., 2009), but lower than those reported from the Alpine glaciers (mean: 59 ± 20%, Singer et al., 2012). A fraction of the

terrestrial DOM formulas (14−33%) also exhibited low C/N, suggesting that supraglacial terrestrial DOM is potentially labile. This is consistent with previous studies that the terrestrial organic matter was largely bioavailable and readily degraded by heterotrophic bacteria (Hedges et al., 1997; Raymond and Bauer, 2001). While the effect of these autochthonous and distant allochthonous organic carbon sources on the carbon balance in recipient downstream ecosystems remains unclear, the present study has expanded the view that the Antarctic snowpack is an important source of potentially labile terrestrial and microbial DOM to downstream ecosystems. Further, given the vast expanse of glaciers and ice sheets covering approximately 10% of the Earth's surface and the immense quantity and diversity of organic carbon present in these systems (Priscu et al., 2008), the role of glaciers and ice sheets in regional and global scale biogeochemical cycles and carbon dynamics cannot be overlooked. Understanding the nature and composition of organic carbon in polar ice sheets is especially important now, as the polar regions are getting exposed to a period of rapid climatic change.

CHAPTER 4

Microbial communities associated with snow in the East Antarctic Ice sheet

4.1. Introduction

Snow environments are characterised by permanently low temperatures, low nutrient composition, desiccation, and high levels of ultraviolet radiation (Miteva, 2008). Although the snowpack is a critical component of the cryosphere, the concept of the snowpack as an ecosystem itself remains largely unexplored and its ecological role has probably been underestimated (Larose et al., 2013). Our knowledge of microbial abundance in supraglacial ecosystems is sketchy as compared to other ecosystems (Whitman et al., 1998) because it is based on small number of samples only from accessible sites. Limited available data indicate that bacteria are present in snow in significant amounts ranging from 10^2 to 10^3 cells mL^{-1} of snow melt (Carpenter et al., 2000; Fujii et al., 2010; Lopatina et al., 2013; Michaud et al., 2014). Thus, the contribution of snow ecosystems to global bacterial abundance may be significant as 35% of the Earth's surface is covered by snow (Miteva, 2008).

Studies on microbial communities within the Antarctic snowpack reveal that these permanently frozen environments harbour diverse, viable microbial populations that represent almost all the major phylogenetic groups (Lopatina et al., 2013; Fujii et al., 2010; Yan et al., 2012; Michaud et al., 2014; Carpenter et al., 2000). *In situ* evidence of bacterial DNA and protein synthesis in snow has been recorded at subzero temperatures of -12 to -17°C (Carpenter et al., 2000). Recently, molecular approaches have identified specific functional genes, such as genes involved in nitrogen cycling, sulfur metabolism, carbon and nutrient recycling in microbial communities inhabiting snow and ice (Simon et al., 2009; Larose et al., 2013; Shtarkman et al., 2013). Resident microbial communities are now believed to be actively impacting the nutrient dynamics, albedo and hydrochemistry of snow ecosystems (Larose et al., 2013).

Although these studies demonstrate that diverse microorganisms are present in the snow that are potentially active and may be involved in various biological processes, the data on microbial abundance in snowpack, the dominant supraglacial environment in terms of volume and area, and the factors that control it are severely limited. As a result, current large-scale estimates of microbial biomass (estimated between 10^{25} and 10^{29} cells) in icy habitats are empirical and span many orders of magnitude (Irvine-Fynn and Edwards, 2014). In addition, details concerning activity and metabolic capabilities of snow microbial communities remain scarce. In the light of the limited understanding of the

Antarctic snowpack microbial communities that may prove significant in terms of spatial extent, biogeochemical cycling and sensitivity to a changing climate, a greater insight into the distribution and dynamics of snowpack communities is needed. In this study, the diversity and likely sources of microbes in the snow from the East Antarctic ice sheet have been examined using culture-based and culture independent methods.

4.2. Methodology

4.2.1. Culture-dependent microbial diversity

4.2.1.1. Sample processing

For microbiological studies, 3 locations from the coastal to interior plateau region were selected to get an overall representation of the sampling transect. Snow samples were collected from near the coast (coastal), 80 km (midland), and 180 km (inland) away from the coast along a transect of East Antarctica. Processing of the snow samples was carried out in a sterile laminar flow in the −15°C processing room under clean conditions. Sterile, powder free gloves, UV-sterilised clean full body suits, and face mask were used during samples handling. Snow was sub sampled using a sterile spatula and allowed to melt in sterile containers at 4°C. To check for bacterial contamination in the laboratory, a control sample consisting of sterile ultrapure water was processed along with the sample.

4.2.1.2. Enrichment and colony isolation

To obtain a broad range of microbes, several media were used based on previous reports of successful recovery and isolation of stressed bacteria from polar ice samples (Christner et al., 2000; Reddy et al., 2000; Amato et al., 2007). Enrichments were carried out by inoculating 1 mL aliquot of the melted snow sample into 9 mL of 1, 10 and 25% (w/v) media such as tryptone soy broth, nutrient broth and Zobell marine broth, supplemented with NaCl and $MgCl_2$ to obtain original strength salt concentration. Additionally, low nutrient media like Antarctic bacterial medium, R2A and R3A media were also used. The composition of these media is given in the Appendix. Liquid enrichments were incubated at 4 ± 0.1°C and 20 ± 0.5°C. Suitable controls were also maintained for each type of medium. The tubes were visually observed for growth at regular intervals. On observation of growth, 0.1 mL was plated onto the corresponding solid medium for isolation of colonies. Un-inoculated controls were also plated on respective solid medium.

Morphologically distinct colonies were picked up and streaked onto the same medium to obtain single, pure colonies. The purity of the colonies was verified by gram staining before sub culturing.

4.2.1.3. DNA isolation, 16S rRNA gene /ITS region amplification and sequencing

Eighty two pure isolates were selected for 16S rRNA gene/ITS region sequencing based on differences in colony morphology, pigmentation, and media of isolation. Genomic DNA was isolated from each culture using UltraClean microbial DNA isolation kit (MoBio), according to manufacturer's recommendation. DNA concentration was measured using nanodrop and found to be between 180 and 300 µg ml^{-1}. The extracted genomic DNA was amplified for 16S rRNA gene corresponding to nucleotides 27–1492 of the *Escherichia coli* 16S rDNA and for the internal transcribed spacer (ITS) regions of fungal rDNA. The primers used are listed in Table 4.1.

Table 4.1. *Oligonucleotide primers used for amplification.*

Description	Primer sequence (5′-3′)	Reference
27F	AGAGTTTGATCCTGGCTCAG	Lane, (1991)
1492R	GGTTACCTTGTTACGACTT	Lane, (1991)
ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al., (1990)
ITS4	TCCTCCGCTTATTGATATGC	White et al., (1990)

Each Polymerse chain reaction (PCR) mixture (50 µl) contained 2 µl of the DNA extract, 15 pmol of each primer and 25 µl 2X Taq PCR Master Mix (400 µM of each dNTP, 1.25 U Taq DNA polymerase, 3 mM $MgCl_2$). Amplification was carried out using a Bio-Rad C-1000 thermal cycler. After an initial denaturation at 95°C for 2 min, 30 cycles of amplification consisting of: 95°C for 30 sec, 55°C for 1 min, and 72°C for 1.5 min; final extension at 72°C for 10 min, were performed for primer pair 27F/1492R and 30 cycles of: 95°C for 30 sec, 50°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min, were performed for primer pair ITS5/ITS4. The PCR products obtained were examined by agarose gel electrophoresis. The amplified products were purified using the UltraClean PCR Clean-Up Kit (MoBio) and sequenced using an Avant 3100 gene analyser (Applied Biosystems). The 16S rRNA sequences obtained (~ 1 Kb and covering the V3-V5 region) and the ITS region of fungal 18S rRNA genes ~ 0.5 kb) were assembled and edited using DNA Baser Sequence Assembler v4 (Heracle Software, Germany), before

sequence alignment. Taxonomic assignments were performed using EzTaxon-E (Kim et al., 2012). Phylogenetic tree was constructed using the Neighbor-Joining method. Bootstrap analysis was performed for 1000 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004).

The threshold of 98.7 to 99% similarity was used for the operational definition of species (Stackebrandt and Ebers, 2006). Consequently, phylotypes showing ≥ 98.7% 16S rRNA gene sequence similarity with a type strain were considered as belonging to that species. For cases where an identity of < 98.7% with a validated species was found then these phylotypes were classified as potentially new species within the identified genus whereas those with < 95% with the phylogenetically closest type strain were not assigned to a particular genus and were classified as potential new genera (Sentausa and Fournier, 2013).

4.2.1.4. Nucleotide accession numbers

The bacterial and fungal DNA sequences were deposited in the GenBank database under the accession numbers, KP299176 to KP299240 and KP299241 to KP299256, respectively.

4.2.2. Culture-independent microbial diversity

4.2.2.1. Environmental genomic DNA Extraction, amplification of 16S /18S rRNA gene and clone library construction

Melted snow samples (~445 mL) were filtered through a 0.2 μm Isopore filter (Millipore), and DNA was extracted from the collected particulates using the MoBio PowerWater DNA extraction kit, as per the manufacturer's instructions. The genomic DNA obtained was quantified using the PicoGreen (Molecular Probes Inc.) dsDNA quantitation method following manufacturer's instructions and found to range from 14 to 18 ng. A control consisting of 0.2 μm filtered and autoclaved MilliQ water was processed the same way as the samples and included in molecular amplification procedures to monitor for contamination at each experimental step. Amplicons for bacteria, fungi and Archaea were generated using the following primer combinations: 27F (bacterial 16S rDNA) with 1492R or 1525R (bacterial and archaeal 16S rDNA); 63F (bacterial 16S rDNA)/1387R (bacterial 16S rDNA); 515F (universal 16S rDNA primer)/1392R (universal 16S rDNA primer);

UA571F (archaeal 16S rDNA)/UA1406R (archaeal 16S rDNA); UA751F (archaeal 16S rDNA)/UA1204R (archaeal 16S rDNA); NS1(fungal 18S rDNA)/NS8(fungal 18S rDNA). Details of primers used for DNA amplification are provided in Table 4.2. Reaction mixtures (50 µl) consisted of 25 µl 2X Taq PCR Master Mix (400 µM of each dNTP, 0.05 U Taq DNA polymerase/µl, 3 mM $MgCl_2$), 15 pmol of each primer, and 2 µl of template.

Table 4.2. *Oligonucleotide primers used in the study*

Description	Primer sequence (5′-3′)	Reference
1525R	AAGGAGGTGWTCCARCC	Lane, (1991)
1387R	GGGCGGWGTGTACAAGGC	Marchesi et al., (1998)
63F	CAGGCCTAACACATGCAAGTC	Marchesi et al., (1998)
515F	GTGCCAGCMGCCGCGGTAA	Turner et al., (1999)
1392R	ACGGGCGGTGTGTRCA	Lane, (1991)
UA571F	GCYTAAAGSRICCGTAGC	Baker and Cowan, (2004)
UA1406R	ACGGGCGGTGWGTRCAA	Baker and Cowan, (2004)
UA751F	CCGACGGTGAGRGRYGAA	Baker and Cowan, (2004)
UA1204R	TTMGGGGCATRCIKACCT	Baker and Cowan, (2004)
NS1	GTAGTCATATGCTTGTCTC	White et al., (1990)
NS8	TCCGCAGGTTCACCTACGGA	White et al., (1990)

PCR mixture was subjected to an initial denaturation at 95°C for 2 min, followed by 30 cycles of amplification by denaturing for 30 sec at 95° C, annealing for 1 min at 46.2-55°C, and extension for 1.5 min at 72°C followed by a final extension for 10 min at 72°C. No bands were detected in the first PCR step with fungus and Archaea specific primers. Thus, an aliquot (2 µl) of the resulting product from the first PCR using UA571F/UA1406R and NS1/NS8 was used as the template in a second PCR, with UA751F/UA1204R and NS8/NS19, respectively. Samples from each PCR reaction were evaluated by electrophoresis through 1% agarose gels and purified using UltraClean PCR cleanup kit (MoBio). Amplicons were not generated from material collected from the procedural blank. This was also the case when sensitive two-stage nested amplification strategies were used.

A TOPO TA cloning kit (Invitrogen) was used for cloning the amplified products. Individual DNA molecules from the PCR sample were ligated into the pCR4-TOPO vector (Invitrogen) containing the *ccd*B gene for positive selection, only permitting growth of plasmid vectors with recombinants. The ligation reaction included: 4 µl of PCR product, 1 µl of salt solution (200 mM NaCl, 10 mM $MgCl_2$) and 1 µl of vector. The ligation reaction

was carried out following the manufacturer's directions. One Shot TOP10 competent *E. coli* cells were transformed with the ligation reaction. Inserts of the predicted size were confirmed by PCR with primers that annealed to the flanking T3 (5´-ATTAACCCTCACTAAAGGGA-3´) and T7 (5´-TAATACGACTCACTATAGGG-3´) regions of the vector. Around 70 clones each were obtained from coastal, midland and inland sites. Each clone was cultured in Luria Bertani medium (Appendix) amended with 100 µg mL^{-1} of ampicillin, and plasmid DNA was purified using the Quick plasmid MiniPrep kit (Invitrogen), quantified on a Nano spectrophotometer and sequenced on a 3130xl Genetic Analyser (Life Technologies). The sequences were checked for the presence of any contaminating vector sequences using the Vecscreen program of NCBI and sequencing reads were manually trimmed to remove flanking vector and primer sequences using the software Sequencher V4.10.1 (GeneCodes Corporation, Ann Arbor, MI, USA). The DECIPHER tool http://decipher.cee.wisc.edu/ FindChimeras.html) was used to identify the potential chimeric sequences and were discarded from the analysis.

4.2.2.2. Nucleotide accession numbers

The clone sequences with GenBank accession numbers KR339062-KR339141 for bacteria; KR131431-KR131443 for eukarya and KR090706-KR090740 for Archaea were deposited in the GenBank database.

4.2.3. Shannon-Wiener diversity index

Bacterial diversity within the samples was compared using the Shannon-Wiener diversity index (H′) which takes into account both number of microbial groups and the evenness, or relative distribution of the groups present (Edwards et al., 2001). This index was calculated using the equation:

$$H' = \sum_{i=1}^{s} (P_i)\ (log_e\ Pi)$$

where *s* is the number of species in the sample and P_i is the proportion of species *i* in the sample.

4.3. Results and Discussion

4.3.1. Enrichment and isolated strains

Reviving bacteria from an oligotrophic environment necessitates the use of properly

balanced nutrients in moderate amounts in the culture medium or some substances and/or certain conditions need to be provided for growth and reproduction. Alternatively or additionally, they may need physical stimulation, such as brief incubation at a temperature above that of the natural habitat (Poindexter, 2009) as observed during attempts to revive and recover bacteria from oligotrophic habitats such as snow and ice (Christner et al., 2000; Zhang et al., 2006; Antony et al., 2012a). Incubation in liquid media has been found to enhance the recovery and isolation of bacteria from frozen environments that might have been damaged or persisted in a dormant/low metabolic state (Miteva et al., 2004). In the present study, growth was observed within 2-30 days of incubation at 20°C and 10-60 days of incubation at 4°C in liquid media. Colonies appeared within 3–8 days of incubation on plating of the enriched broth on respective agar media. No colonies were found on solid media from the un-inoculated controls. Overall, the incubation at 20°C yielded more number of colonies than that at 4°C, irrespective of the media, which is consistent with previous reports of recovery of bacteria from snow/ice (Christner et al., 2000; Zhang et al., 2006; Antony et al., 2012a).

The enrichment technique was found suitable for isolating bacteria present in low numbers in ice core samples (Zhang et al., 2001; Christner, 2002b), as well as to increase the number and diversity of recovered isolates (Amato et al., 2007; Elster et al., 2007). Consistent with the previous reports of recovery of bacteria from the Antarctic snow/ice (Christner et al., 2000; Christner et al., 2001; Reddy et al., 2004; Amato et al., 2007; Srinivas et al., 2009; Antony et al., 2012a; Doyle et al., 2013; Shivaji et al., 2013), the dilute Nutrient Agar (NA), Tryptone Soy Agar (TSA), Zobell Marine Agar (ZMA), Antarctic Bacterial Medium (ABM) and R2A used in the present study were also found suitable for isolation of microorganisms from snow samples. Although the isolates were from snow, a much colder environment, 68% of these isolates grew at 20°C, indicating that they were more likely psychrotolerant than psychrophilic. Bacteria isolated from the Antarctic ice and other cold habitats have been reported to grow at optimal levels at temperatures higher than that of the natural environment (Steven et al., 2006; Veerapaneni, 2009). In general, all growth media used and temperatures maintained, gave access to some taxa that were only found under these conditions (Appendix, Table S1).

Calculations based on the average colony forming units (CFU) per milliliter showed that only a small fraction of the microbes were culturable. The culturable fraction ranged from

0.02-8.8 CFU mL^{-1} and falls within the range of 0.008 to 16 CFU mL^{-1} reported for the Antarctic snow and ice samples (Hardfield et al., 1992; Dancer et al., 1997; Christner et al. 2000; Pearce, 2012; Yan et al., 2012; Shivaji et al., 2013). In general, about 0.006% of cells (~2×10^4 cells mL^{-1}) in the snow samples were culturable and the cultivability at 20°C was about 3 times higher than that at 4°C. These results are consistent with that of Coutard et al., (2007) who reported cells that were viable but non-culturable at 4°C became culturable after a temperature up-shift to 20°C.

A total of 82 different isolates were recovered and the microscopic examination revealed that 17 strains were yeasts. It was also observed that majority of the bacterial isolates were non-spore formers. The recovery of more number of non-spore forming bacteria from snow suggests that these bacteria were able to survive and maintain themselves in cold and desiccated conditions. These findings are also in agreement with that of other studies which showed that most of the bacteria detected in polar ice core samples (Shafaat and Ponce, 2006; Antony et al., 2012a; Shivaji et al., 2013), including the ice collected at a depth of upto 2750 m (Abyzov et al., 1999) were non-spore formers. The prevalence of non-spore forming bacteria in these environments has been attributed to the fact that DNA in non-spore forming bacteria degrades far more slowly due to maintenance of metabolic activity and DNA repair than that in endospore formers (Johnson et al., 2007). Therefore, it appears that continuous metabolic activity with DNA repair may be a more successful strategy for longevity than spore formation. About 38% of the bacterial strains isolated were pigmented, of which majority were found in the midland, followed by coastal and inland sites (Table 4.3).

Table 4.3. *Bacteria isolated from surface snow at the three sampling sites.*

Sampling site	No of isolates	Temperature of isolation		Pigmentation (%)
		4°C	20°C	
Coastal	33	24%	76%	24%
Midland	24	46%	54%	83%
Inland	25	32%	68%	12%

Occurrence of a high percentage of pigmented bacteria concurs with the high frequency of pigmented bacteria obtained from Antarctic ice and is consistent with the role of pigments providing protection from solar irradiation (Arrage et al., 1993) during atmospheric transport and on the surface of the ice sheet (Christner et al., 2000; Foght et al., 2004;

Zhang et al., 2008). Pigmentation is also believed to play a role in adaptation to cold environments by modulating membrane fluidity in bacteria (Chattopadhyay et al., 1997; Jagannadham et al., 2000).

4.3.2. Microbial diversity from culture-based approach

The 82 isolates were phylogenetically related to 4 major lineages of the domain bacteria: Proteobacteria (includes α-, β-, and γ-Proteobacteria), Actinobacteria, Firmicutes and Bacteroidetes, and also 1 major lineage of the domain eukaryota: Basidiomycota (Fig. 4.1).

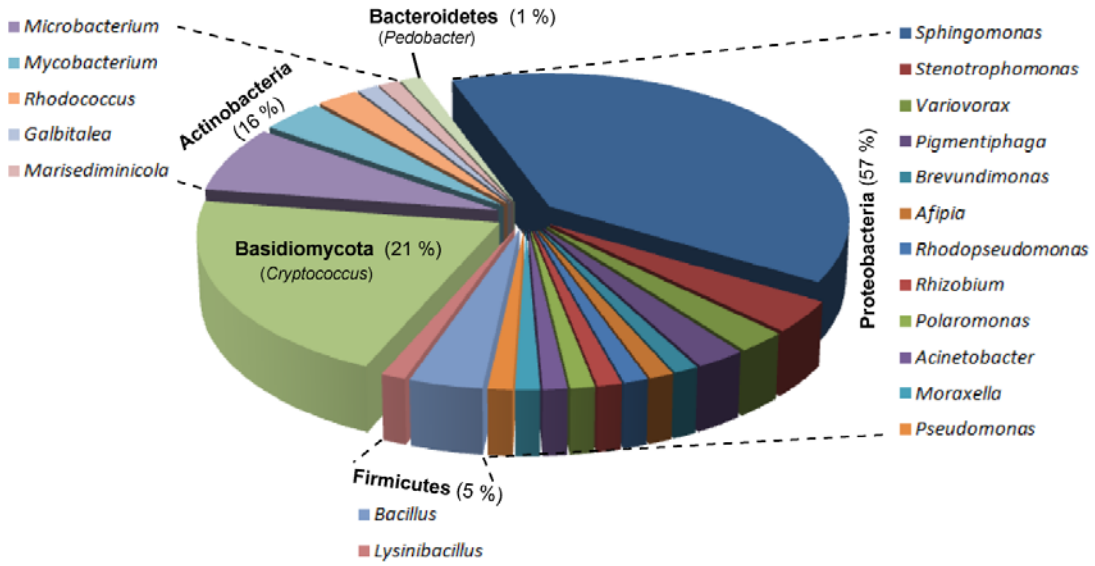

Figure 4.1. *Taxonomic distribution of the cultured microbial communities.*

A total of 21 different genera were identified in the present study. For 2 phylotypes (L1j and P18Cd), the phylogenetic analysis did not allow reliable assignment to an existing genus because their 16S rRNA gene sequence similarities were < 95 % with all related genera. So, these were classified as potential new genera. About 40.2% of the isolates belonged to phylotypes identified as validated species (Table 4.4). Details of the morphological characteristics and identification of the 82 isolates are given in the Appendix (Table S1). The majority of isolates (59.7%) were identified as potentially new species in the existing genera. Because of the limited resolving power of the 16S rRNA gene (Fox et al., 1992), for the phylotypes classified as potentially new taxa, further characterisation by a polyphasic approach will be necessary for a decisive identification at the species level. Interestingly, some isolates that showed >98.7% 16S rRNA gene sequence similarity to a given species comprised several representative isolates with

different morphologies and pigments, indicating considerable genetic variation. This feature has also been previously observed in the bacterial isolates recovered from polar and non-polar glacial ice (Christner et al., 2000). Thus, further analyses that are more discriminative will be needed to verify whether these phylotypes represent more than one species.

Table 4.4. *List of species identified from snow at the three sampling sites.*

Sampling Site	Nearest Neighbour	No of isolates	Similarity %
Coast	*Cryptococcus victoriae* CBS 8915	1	100.0
	Sphingomonas glacialis C16y(T)	1	99.5
	*Microbacterium oxyda*ns DSM 20578(T)	1	99.5
	Microbacterium pumilum KV-488(T)	1	99.5
	Lysinibacillus macroides LMG 18474(T)	1	99.3
	*Microbacterium hatanon*is JCM 14558(T)	1	99.0
	Rhodococcus erythropolis DSM 43066(T)	1	99.0
	Stenotrophomonas rhizophila e-p10(T)	1	99.0
	Afipia broomeae ATCC 49717(T)	1	98.7
Midland	*Sphingomonas aquatilis* JSS7(T)	7	98.7-99.7
	Sphingomonas glacialis C16y(T)	4	98.8-99.6
	Sphingomonas melonis DAPP-PG 224(T)	1	99.4
Inland	*Cryptococcus victoriae* strain P41A001	9	99.0
	Brevundimonas olei MJ15(T)	1	98.9
	Microbacterium xylanilyticum S3-E(T)	1	99.0
	Moraxella osloensis NCTC 10465(T)	1	98.8

The Proteobacteria-related sequences were the predominant phylum (47 in total; 57% of all isolates) and comprised 12 genera: *Brevundimonas, Afipia, Rhizobium, Sphingomonas, Pigmentiphaga, Polaromonas, Variovorax, Acinetobacter, Moraxella, Pseudomonas, Rhodopseudomonas,* and *Stenotrophomonas*. Among these, *Sphingomonas* related phylotypes were the most abundant ones and comprised 39% of the identified sequences. The Actinobacteria were represented by 5 genera: *Mycobacterium, Rhodococcus, Galbitalea, Marisediminicola* and *Microbacterium*. The Firmicutes were represented by 2 genera: *Bacillus* and *Lysinibacillus*, whereas the Bacteroidetes related sequence belonged to the genera *Pedobacter*. The detected bacterial genera in this study were similar to those reported in snow, ice and other habitats from Antarctica, Greenland and non-polar icy regions (Table 4.5).

Similarly, all 17 fungal isolates identified were affiliated to the genera *Cryptococcus,* and showed the closest similarity to *Cryptococcus victoriae* which was first isolated from

South Victoria Land, Antarctica (Montes et al., 1999). The isolation of *Cryptococcus* sp. in the present study is consistent with the isolation of viable *Cryptococcus* yeasts from snow and ice in Antarctica (D'Elia et al., 2009; Buzzini et al., 2012), Arctic (Butinar et al., 2007), and other non polar glaciers (Uetake et al., 2011; Turchetti et al., 2015). *Cryptococcus* spp. appear to be the predominant yeast in polar regions (Zalar and Gunde-Cimerman, 2014) with the broadest adaptive potential and the widest distribution across various polar niches (Zalar and Gunde-Cimerman, 2014).

Table 4.5. *Frequently isolated microbial genera from polar and non-polar icy habitats.*

Genera*	Isolation site	Reference
Sphingomonas, Stenotrophomonas, Bacillus, Microbacterium, Moraxella, Varivorax, Mycobacterium, Brevundimonas, Pseudomonas, Polaromonas, Afipia, Acinetobacter, Rhizobium, Cryptococcus	Snow/ice from Greenland, Antarctica and non-polar icy regions	Christner et al., 2000; Christner et al., 2001; Christner et al., 2003; Abyzov et al., 2004; Segawa et al., 2005; D'Elia et al., 2009; Miteva et al., 2009; González-Toril, 2009; Simon et al., 2009; Segawa et al., 2010; Shivaji et al., 2011;Yan et al., 2012; Margesin et al., 2012; Knowlton et al., 2013; Shivaji et al., 2013
Rhodococcus, Brevundimonas	Antarctic rock debris and ice, Himalayan proglacial soil	Peeters et al., 2011; Srinivas et al., 2011; Shivaji et al., 2013
Marisediminicola	Sandy intertidal sediment Antarctica	Li et al., 2010
Pedobacter	Alpine glacier cryoconites, Himalayan glacier soil, and rock-water interface in Antarctica	Margesin et al., 2003; Shivaji et al., 2011; Huang et al., 2013
Lysinibacillus	Antarctic ice and algal mats	Antibus et al., 2012; Shivaji et al., 2013
Rhodopseudomonas	Antarctic lakes	Burke and Burton, 1988 Huang et al., 2013

* present study

Yeasts isolated from Antarctic ice have been found to be capable of reproductive growth at -5°C (Amato et al., 2009) and macromolecular synthesis at temperature as low as -15°C,

indicating that they may represent active members of the snow ecosystems. The prevalence of these species in cryospheric ecosystems could reflect their efficient adaptation to the extreme condition typical of the glacial ecosystems. The consistent recovery of isolates belonging to similar genera in geographically distant icy environments has been noted previously and is likely due to similar adaptation strategies, allowing them to survive and metabolise for extended periods of time in frozen environments (Priscu and Christner, 2004; Christner et al., 2008; Miteva, 2008). A neighbour joining tree of isolates recovered from the Antarctic snow and closely related cultured representatives are shown in Figure 4.2.

Although most of the isolates identified in this study were similar to the species frequently found in the polar habitats, the occurrence of two of them in the polar regions is rare / unreported. *Galbitalea*, first isolated from soil was very recently identified as a new genus (Kim et al., 2014). *Galbitalea* sp. has so far not been reported from any polar environment. Therefore, this is the first report of the genus *Galbitalea* from Antarctica. Similarly, the isolation of *Pigmentiphaga* spp. from polar regions appears to be rare, with only an uncultured clone sequence reported from an epishelf lake in the Canadian High Arctic (Veillette et al., 2011).

4.3.2.1. Spatial distribution of cultured microbes

The culturable microbial diversity in the coastal site comprised 16 different genera (total 33 isolates) with more or less equal representation of all bacterial phylotypes. In contrast, the midland site was dominated by only 1 genus (*Sphingomonas*; total 24 isolates), while *Cryptococcus* spp. dominated the inland site, comprising 60% of the isolates recovered from this site among a total of 25 isolates belonging to 8 different genera. Although the genus *Sphingomonas* was common to all samples along the traverse, the isolates from each site belonged to species that were not present in other sites. The only exception was *Sphingomonas glacialis* which was recovered both from coastal and midland sites. However, only 1 isolate of *S. glacialis* was obtained from coastal region while 12 isolates were recovered from the midland region. The results of this study corresponds well with a previous study that showed that the bacterial concentrations and community compositions were distinctly different from coast to inland with significantly higher community sizes of bacteria in the coastal snowpack (Yan et al., 2012).

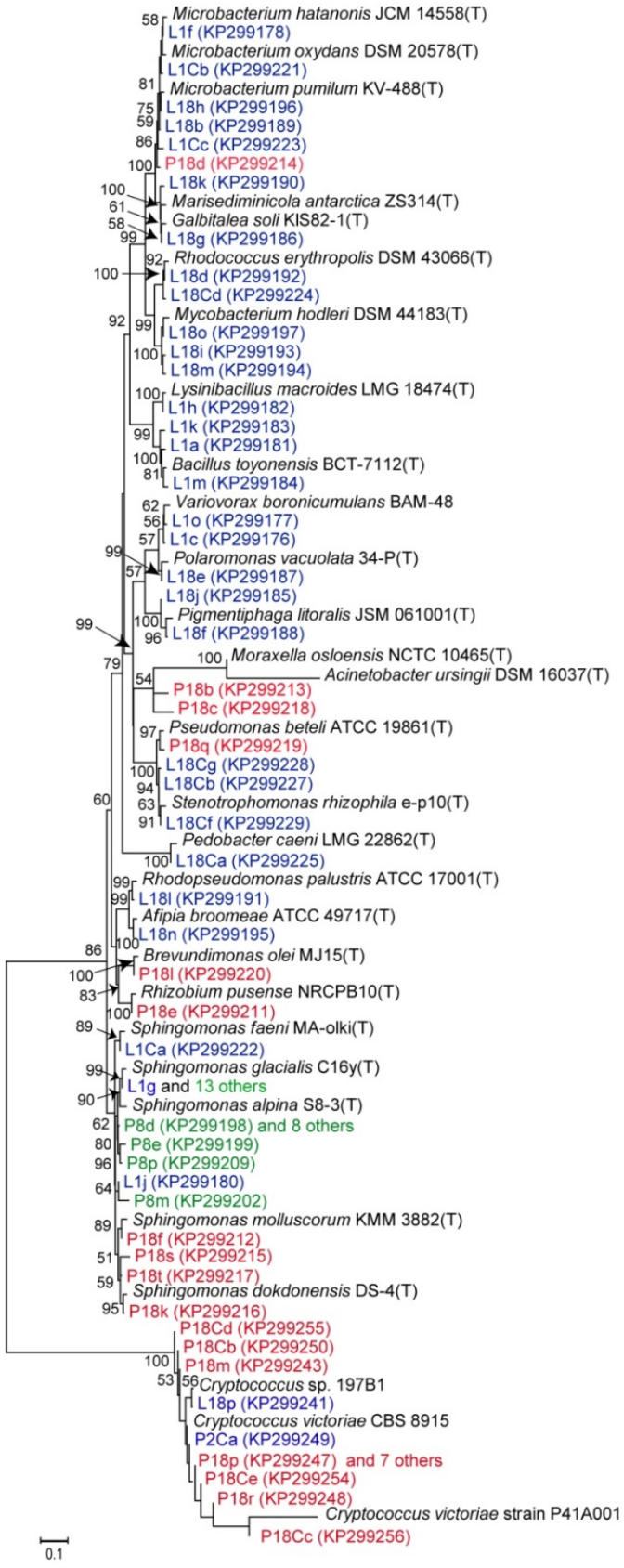
Microbacterium hatanonis JCM 14558(T)
L1f (KP299178)
Microbacterium oxydans DSM 20578(T)
L1Cb (KP299221)
Microbacterium pumilum KV-488(T)
L18h (KP299196)
L18b (KP299189)
L1Cc (KP299223)
P18d (KP299214)
L18k (KP299190)
Marisediminicola antarctica ZS314(T)
Galbitalea soli KIS82-1(T)
L18g (KP299186)
Rhodococcus erythropolis DSM 43066(T)
L18d (KP299192)
L18Cd (KP299224)
Mycobacterium hodleri DSM 44183(T)
L18o (KP299197)
L18i (KP299193)
L18m (KP299194)
Lysinibacillus macroides LMG 18474(T)
L1h (KP299182)
L1k (KP299183)
L1a (KP299181)
Bacillus toyonensis BCT-7112(T)
L1m (KP299184)
Variovorax boronicumulans BAM-48
L1o (KP299177)
L1c (KP299176)
Polaromonas vacuolata 34-P(T)
L18e (KP299187)
L18j (KP299185)
Pigmentiphaga litoralis JSM 061001(T)
L18f (KP299188)
Moraxella osloensis NCTC 10465(T)
Acinetobacter ursingii DSM 16037(T)
P18b (KP299213)
P18c (KP299218)
Pseudomonas beteli ATCC 19861(T)
P18q (KP299219)
L18Cg (KP299228)
L18Cb (KP299227)
Stenotrophomonas rhizophila e-p10(T)
L18Cf (KP299229)
Pedobacter caeni LMG 22862(T)
L18Ca (KP299225)
Rhodopseudomonas palustris ATCC 17001(T)
L18l (KP299191)
Afipia broomeae ATCC 49717(T)
L18n (KP299195)
Brevundimonas olei MJ15(T)
P18l (KP299220)
Rhizobium pusense NRCPB10(T)
P18e (KP299211)
Sphingomonas faeni MA-olki(T)
L1Ca (KP299222)
Sphingomonas glacialis C16y(T)
L1g and 13 others
Sphingomonas alpina S8-3(T)
P8d (KP299198) and 8 others
P8e (KP299199)
P8p (KP299209)
L1j (KP299180)
P8m (KP299202)
Sphingomonas molluscorum KMM 3882(T)
P18f (KP299212)
P18s (KP299215)
P18t (KP299217)
Sphingomonas dokdonensis DS-4(T)
P18k (KP299216)
P18Cd (KP299255)
P18Cb (KP299250)
P18m (KP299243)
Cryptococcus sp. 197B1
L18p (KP299241)
Cryptococcus victoriae CBS 8915
P2Ca (KP299249)
P18p (KP299247) and 7 others
P18Ce (KP299254)
P18r (KP299248)
Cryptococcus victoriae strain P41A001
P18Cc (KP299256)
0.1

Figure 4.2. *Neighbour joining tree showing the closest relatives of 16S rRNA gene sequences from cultured microbes. Isolates recovered from the coastal, midland and inland samples are shown in blue, green and red, respectively.*

4.3.3. Microbial communities based on culture-independent approach

Sequence analysis of clones produced 127 sequences with good quality of which 80 were from bacteria, 34 from Archaea and 13 from eukarya. Clone sequence analyses allowed the identification of 12 different phyla (Actinobacteria, Bacteroidetes, Firmicutes, Ascomycota, Cryptomycota, Proteobacteria, Planctomycetes, Rhizaria, Verrucomicrobiae, Cyanobacteria, Deinococcus-Thermus, Euryarchaeota) and 19 different orders (Sphingomonadales, Rickettsiales, Burkholderiales, Pseudomonadales, Legionellales, Sphingobacteriales, Cytophagales, Actinomycetales, Eurotiales, Cercomonadida, Deinococcales, Halobacteriales, Planctomycetales, Methylophilales, Verrucomicrobiales, Bacillales, Caulobacterales, Rhizobiales and Xanthomonadales).

Although, some predominant microbial phyla detected by both culture dependent and culture-independent approaches were the same (Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes), cloning and sequencing revealed a substantially higher level of microbial diversity by culture-independent method than by the culture based method. For instance, eight phyla identified by culture-independent method (Cyanobacteria, Rhizaria, Deinococcus-Thermus, Planctomycetes, Verrucomicrobiae, Ascomycota, Cryptomycota and Euryarchaeota) were not represented among the cultivable bacteria. This clearly shows that each method targeted different taxonomical groups and the use of both methods together enabled a higher coverage of microbial diversity. Overall, most nucleotide sequences were of Proteobacteria which accounted for 17/25 phylotypes (58/127 clones; 46%). Euryarchaeota accounted for only 1 of 25 phylotypes (Halobacteriaceae), but 27% of the clone sequences (34/127clones). This was followed by Bacteroidetes (3 phylotypes, 7% of clones), Ascomycota (1 phylotype, 7% of clones), Firmicutes (1 phylotype, 5% of clones), Rhizaria (1 phylotype, 2% of clones) and Cryptomycota (1 phylotype, 2% of clones). Verrucomicrobiae, Cyanobacteria, Deinococcus-Thermus, Planctomycetes and Actinobacteria were represented by 1 clone sequence each (Fig. 4.3). In addition, 2 uncultured bacterial phyla were also detected. Though the 16S gene clone libraries are potentially subjected to biases, Proteobacteria have been frequently isolated from ice cores and snow samples from both polar, as well as, non-polar icy regions (Christner et al., 2001; Christner et al., 2003; Xiang et al., 2004; Miteva et al., 2009; Shivaji et al., 2011; Antony

et al., 2012a; Shivaji et al., 2013; Michaud et al., 2014). These data suggest that Proteobacteria are common among these habitats and is consistent with the results of this study. Thus, the clone library method gave an indication of the different bacterial phyla present.

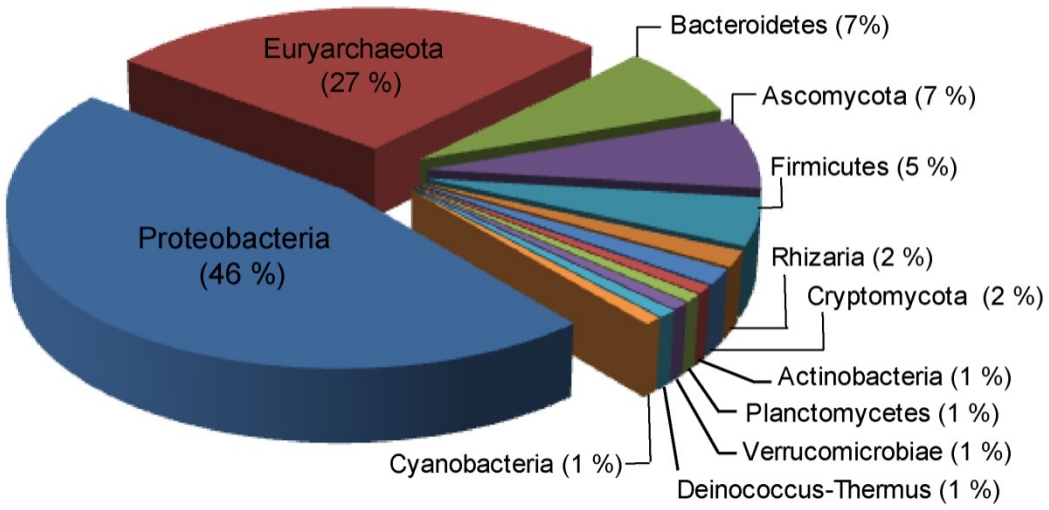

Figure 4.3. *Distribution of different phyla based on clone analysis.*

The identification of clone sequences to at least the generic level (≥95% sequence similarity, Sentausa and Fournier, 2013) was possible for 105 sequences, with 14 sequences identified to only the family level (>86.5% sequence similarity, Yarza et al., 2014). Six clone sequences showed the highest similarity to uncultured microbes described to only the order level in the NCBI database and 2 showed the highest similarity to uncultured bacteria of unknown identity. Clone sequence analyses allowed the identification of 30 genera (Table 4.6 a-c). The clone sequences identified up to the genus level were represented by *Bradyrhizobium*, *Caldimonas*, *Janthinobacterium*, *Legionella*, *Methylophilus*, *Polymorphobacter*, *Methylovorus*, *Mucilaginibacter*, *Polaromonas*, *Rhodanobacter*, *Tumebacillus*, *Aspergillus*, *Penicillium*, *Natronococcus*, *Afipia*, *Altererythrobacter*, *Brevundimonas*, *Deinococcus*, *Paramicrosporidium*, *Dyadobacter*, *Dyella*, *Halalkalicoccus*, *Hydrotalea*, *Mesorhizobium*, *Piscinibacter*, *Rhodococcus*, *Stenotrophomonas*, *Aquicella*, *Methylobacillus* and *Nitrobacter*.

Table 4.6a. *Affiliation of the phylotypes from Antarctic coastal surface snow samples.*

Phylogenetic grouping	Phylum	Nearest neighbor (Closest BLASTn match)	No of clones	% identity
Bacteria	Proteobacteria	Legionellales (NR_025764.1)	1	91.0
		Piscinibacter aquaticus (NR_114061.1)	1	99.0
		Caldimonas sp. (NR_115010.1)	1	95.0
		Janthinobacterium lividum (NR_026365.1)	1	99.8
		Polymorphobacter sp. (NR_125454.1)	1	95.5
		Brevundimonas bullata (NR_113611.1)	1	99.0
	Firmicutes	Bacillales (NR_112564.1)	4	91.0-94.0
	Bacteroidetes	*Mucilaginibacter rigui* (NR_113976.1)	1	98.7
		Sphingobacteriales (NR_042494.1)	1	91.9
		Dyadobacter sp. (NR_074368.1)	1	97.7
	Actinobacteria	*Rhodococcus yunnanensis* (NR_043009.1)	1	99.5
Archaea	Euryarchaeota	*Natronococcus amylolyticus* Ah-36 (NR_028217.1)	20	99.0
		Halalkalicoccus jeotgali B3 (NR_113415.1)	14	99.0
		Halalkalicoccus tibetensis JCM 11890 (NR_113416.1)	1	99.0
Eukarya	Ascomycota	*Penicillium janthinellum* (AB293968.1)	2	99.0
		Aspergillus glaucus (EU263606.1)	1	97.0
		Aspergillus ruber (U00970)	2	99.0
		Ascomycota sp. Ex2 (EU887744.1)	1	96.0

Table 4.6b. *Affiliation of the phylotypes from Antarctic midland surface snow samples.*

Phylogenetic grouping	Phylum	Nearest neighbor (Closest BLASTn match)	No of clones	% identity
Bacteria	Proteobacteria	Uncultured delta proteobacterium 7A_6 (AY251224.1)	1	98.0
		Rhodanobacter sp. (NR_102497.1)	1	97.0
		Rhodanobacter lindaniclasticus (NR_024878.1)	1	98.9
		Rhodanobacter ginsengisoli (NR_044127.1)	1	98.8
		Methylovorus sp. (NR_074780.1)	3	96.6-96.9
		Methylophilus sp. (NR_041257)	1	97.8
		Methylophilus sp. (NR_041257.1)	3	95.4-97.5
		Methylophilus sp. (NR_104519.1)	1	95.4
		Methylophilus leisingeri (NR_041258.1)	2	99.1-100
		Methylophilus leisingeri (NR_041258.1)	1	99.6
		Methylobacillus sp. (NR_104760.1)	1	97.0
		Mesorhizobium sp. (NR_114124.1)	1	96.9
		Legionellales (NR_109416.1)	2	92-93.6
		Legionella sp. (NR_041742.1)	1	95.4
		Dyella ginsengisoli (NR_041370.1)	1	99.1
		Nitrobacter sp. (NR_074313.1)	1	98.0
		Aquicella sp. (NR_025764.1)	1	91.3
		Rickettsiales (NR_044847.3)	1	90.0
	Planctomycetes	Planctomycetales (KF826994.1)	1	96.7
	Bacteroidetes	*Mucilaginibacter* sp. (NR_109510.1)	1	97.8
		Cytophagales (NR_117435.1)	1	93.0
		Hydrotalea sp. (NR_109380.1)	1	96.7
		Sphingobacteriales (JN679200.1)	1	95.9
	Deinococcus-Thermus	*Deinococcus* sp. (NR_117835.1)	1	95.4
	Cyanobacteria	Uncultured Cyanobacteria (HM446050.1)	1	93.7
	Unidentified	Uncultured bacterium (AB630665.1)	1	97.0
		Uncultured bacterium s135 (EU919859.1)	1	90.5
	Firmicutes	*Tumebacillus* sp. (NR_112564.1)	1	97.0
Eukarya	Ascomycota	*Aspergillus penicillioides* 481 (DQ985958.1)	3	99.0
	Cryptomycota	*Paramicrosporidium saccamoebae* KSL3 (JQ796369.1)	2	96.0

Table 4.6c. *Affiliation of the phylotypes from inland Antarctic surface snow samples.*

Phylogenetic grouping	Phylum	Nearest neighbor (Closest BLASTn match)	No of clones	% identity
Bacteria	Proteobacteria	*Afipia birgiae* (NR_025117.1)	1	98.7
		Altererythrobacter sp. (NR_108901.1)	1	95.0
		Methylophilus sp. (NR_041257.1)	1	95.6
		Pseudomonadales (NR_041702.1)	1	91.0
		Bradyrhizobium diazoefficiens (NR_074322.1)	1	99.4
		Legionella sp. (NR_036805.1)	1	97.3
		Methylophilus flavus (NR_104519.1)	1	99.5
		Methylophilus leisingeri (NR_041258.1)	13	99.0-99.8
		Methylophilus luteus (NR_116847.1)	1	99.4
		Methylophilus sp. (NR_041257.1)	2	95.0-96.8
		Methylophilus glucosoxydans (NR_122095.1)	1	99.7
		Polaromonas aquatica (NR_042404.1)	1	100.0
		Rhizobiales (NR_117911)	1	93.0
		Stenotrophomonas rhizophila (NR_121739.1)	1	99.9
	Bacteroidetes	*Mucilaginibacter rigui* (NR_113976.1)	2	99.1-98.7
	Firmicutes	Uncultured Firmicutes MoB-G8-114 (EF016848.1)	1	99.3
	Verrucomicrobiae	Verrucomicrobiales (NR_026022.1)	1	92.0
Eukarya	Rhizaria	Cercomonadida (FJ790701.1)	2	92.0

4.3.3.1. Bacteria

The dominant phylogenetic group among the bacteria was the phylum Proteobacteria. Out of the 58 Proteobacteria clones, 36 were β-Proteobacteria (Oxalobacteraceae, Aquabacterium, Comamonadaceae, Piscinibacter, Methylophilaceae), 12 were γ-Proteobacteria (Coxiellaceae, Legionellaceae, Pseudomonadaceae, Xanthomonadaceae), 9 were α-Proteobacteria (Caulobacteraceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Phyllobacteriaceae, Caedibacter, Erythrobacteraceae, Sphingomonadaceae) and 1 was related to an uncultured δ-Proteobacteria. The assignment of the most proteobacterial sequences to the class β-Proteobacteria corresponds to other studies of glacier ice (Foght et al., 2004), subglacial habitats (Cheng and Foght, 2007), and mountain snow (Segawa et al., 2005). In comparison to members of α-, β-, and γ-Proteobacteria detected in polar habitats (Christner et al., 2001; Xiang et al., 2004; Christner et al., 2003; Miteva et al., 2009; Antony et al., 2012a; Yan et al., 2012), members of δ-Proteobacteria were less frequently detected (Segawa et al., 2010; Cameron et al., 2012; Michaud et al., 2014). This is consistent with the identification of only 1 δ-Proteobacterium clone in the present samples. The abundance of proteobacterial lineages within the snowpack bacterial community, is

consistent with culture based studies (Shivaji et al., 2013) clone library studies (Xiang et al., 2004; Edwards et al., 2011; Cameron et al., 2012), and high-throughput sequencing (Simon et al., 2009; Zarsky et al., 2013; Edwards et al., 2014) of 16S amplicons from polar and non polar glacial environments. Edwards et al., (2014) suggested that frequent isolation of proteobacterial taxa may reflect their ability to adapt to frequent environmental fluctuations typical of extreme environments. Other bacterial phylotypes identified in this study such as Actinobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Planctomycetes, Verrucomicrobiae and Deinococcus-Thermus were also well represented in several cold habitats (Table 4.7).

Table 4.7. *Microbial phylotypes identified in this study* and other cold habitats.*

Phylotype*	Isolation site	Reference
Firmicutes, Actinobacteria and Bacteroidetes	Greenland ice sheet, Alpine Arctic and Himalayan glaciers as well as various and Antarctic ecosystems such as snow, rock debris cryoconite holes and ice	Wynn-Williams and Edwards, 2000; Segawa et al., 2010; Srinivas et al., 2011; Shivaji et al., 2011; Cameron et al., 2012; Yan et al., 2012; Zarsky et al., 2013; Shivaji et al., 2013; Michaud et al., 2014; Edwards et al., 2014; Musilova et al., 2015
Planctomycetes	Soil from Himalaya and Antarctica, cryoconite holes and snow from Antarctica	de la Torre et al., 2003; Shivaji et al., 2011; Cameron et al., 2012; Michaud et al., 2014; Niederberger et al., 2015
Cyanobacteria	Various Antarctic, Arctic and Alpine environments such as lakes, soils, lake ice covers and glaciers	Priscu et al., 1998; Gordon et al., 2000; Pandey et al., 2004; Singh and Elester, 2007; Zakhia et al., 2008; Quesada and Vincent, 2012; Michaud et al., 2014; Edwards et al., 2014
Verrucomicrobia	Surface snow and accretion ice of subglacial lake Vostoc in Antarctica	Srinivas et al., 2011; Shivaji et al., 2011; Shtarkman et al., 2013; Michaud et al., 2014
Thermus-Deinococcus	Snow and soil from Antarctica	Carpenter et al., 2000; Aislabie et al., 2006; Yergeau et al., 2007

Among these, members of the family Deinococcaceae are the best known for their ability to withstand extremely large amounts of ionising radiation induced damage to their DNA (Battista et al., 1999; Dartnell et al., 2010). Such a property would be advantageous to organisms on the surface of the ice sheet that are exposed to high doses of solar irradiation.

Three of the sequences were the closest to an uncultured bacterium from moss pillars in an Antarctic freshwater lake (AB630665.1), an uncultured bacterium clone from an Arctic fjord (EU919859.1) and an uncultured cyanobacterial sequence from an unidentified environmental sample (HM446050.1). Thus, the microbial phyla and genera recorded in this study were similar to those previously identified within the icy habitats worldwide, suggesting that members of these genera are likely to possess similar adaptive mechanisms and that these members may be actively growing within these environments.

4.3.3.2. Fungi

Fungi made up 9% of the total microbial sequences identified in the snowpack. Sequences related to Aspergillaceae (Ascomycota) constituted around 82% of the identified fungal communities and included members of the genus *Aspergillus* and *Penicillium*. *Paramicrosporidium* (Cryptomycota) - related sequences constituted the remaining 18% of the identified fungal community. Members of Aspergillaceae were recorded in the coastal and midland sites, whereas *Paramicrosporidium*-related sequences were detected only in the midland site. The detection of *Aspergillus* and *Penicillium* in the Antarctic snowpack is consistent with the previous studies that have identified several species of these genera in various cold habitats of Greenland (Knowlton et al., 2013) and Antarctica (Singh et al., 2006). Fungi have also been reported in the deep, ancient Vostok ice core (Poglazova et al., 2001; Abyzov et al., 2004; D'Elia et al., 2009). Viable fungi have been discovered in ice samples as old as 140,000 years (Ma et al., 1999). A study of fungi in Arctic glacial ice and sea ice showed that resident fungal communities are adapted to low temperatures, low water activity, as well as high salt concentrations, thus maintaining a continuous colonization of the ice (Gunde-Cimerman et al., 2003). Particularly, many species of the genus *Penicillium* were found to prefer to grow at lower temperatures (Gunde-Cimerman et al., 2003).

Perhaps, the most striking discovery in this study was the detection of Cryptomycota-related sequences, a clade of mostly undescribed fungus which was recently proposed to represent the earliest diverging branch of fungi (Jones et al., 2011). Despite having fungal characteristics, Cryptomycota is distinct from other fungi so far identified, in not having a chitin-rich cell wall in the major stages of its lifecycle (Jones et al., 2011). Members of Cryptomycota are found in freshwater, soil, sediment, and some marine habitats (Jones et

al., 2011) and it appears from the literature that these have so far not been identified from Antarctic snowpack.

In addition to fungi, 2 clone sequences related to the protist Rhizaria (Cecozoa) were also identified in the study. This may be because primers based on conserved nucleotide sequences in fungi, sometimes also target non fungal populations (White et al., 1990). Rhizaria have been detected in polar and non-polar glacier environments (Cameron et al., 2012; García-Descalzo et al., 2013; Lutz et al., 2015). Although Rhizaria as a whole is one of the most poorly understood supergroups of eukaryotes, in the last decade, it has been established as one of the major lineages of eukaryotes, encompassing a huge diversity of microbes (Burki and Keeling, 2014). At the environmental and ecological levels, members of Rhizaria are increasingly found to be abundant bacterial grazers, and therefore hold key positions in the microbial food webs (Burki and Keeling, 2014).

4.3.3.3. Archaea

Archaea were also detected in the Antarctic snowpack. Majority (59%) of archaeal sequences had high similarities (99%) to alpha-amylase producing haloalkaliphilic archaeon *Natronococcus* sp. (NR_028217.1). The remaining Euryarchaeal sequences were closely related to halophilic archaeon *Halalkalicoccus* sp. (NR_113415.1 and NR_113416.1). Although bacteria and eukarya are prevalent in ice environments (e.g., Abyzov et al., 1998, 1999; Christner et al., 2000; Christner et al., 2003; D'Elia et al., 2009), very few studies have documented the presence of Archaea in these environments. Recently, Archaea have been found in cryoconite holes in Antarctica (Cameron et al., 2012) and Svalbard (Zarsky et al., 2013), Icelandic snow (Lutz et al., 2015), Arctic sea ice (Collins et al., 2010) and in subglacial sediments in a Canadian glacier (Hamilton et al., 2013). The present study revealed a very limited diversity, with Halobacteriaceae (Euryarchaeota) being the only archaeal taxa present, which is consistent with the earlier studies. For example, archaeal phyla detected in glaciers in Iceland (Lutz et al., 2015) and cryoconite holes in Svalbard (Zarsky et al., 2013), were restricted to Thaumarchaeota and Euryarchaeota. Collins et al., (2010) detected only 2 archaeal phyla: Crenarchaeota and Euryarchaeota in the Arctic sea ice. Similarly, Cameron et al., (2012) found a limited number of taxa affiliated with Thaumarchaeaota and Methanobacteriaceae restricted to the Antarctic cryoconite. Low archaeal diversity has also been reported in snow samples collected from the Greenland Ice Sheet (Cameron et al., 2015). In this study, the archaeal

sequences were detected only in snow from the coastal site. There have been no other reports available on Archaea within the Antarctic snowpack. Although, all studies considered together, it appears that the apparent diversity of Archaea on glacier surfaces is low, conclusions about the biodiversity cannot be drawn due to the low number of studies made on microbial communities in these environments.

4.3.3.4. Spatial distribution of clone sequences

Comparison of clone sequences showed that microbes affiliated to 13 genera were present in snow in the coastal site, while 15 and 8 different genera were present in the midland and inland sites, respectively. Among these, the genera *Mucilaginibacter* and *Aspergillus* were common to both coastal and midland sites, while the genera *Methylophilus* and *Legionella* were common to the midland and inland sites. Overall, the composition of microbial communities in snow samples from the 3 sites was distinctly different and no single strain was common to all the three sites. Some phylogenetic groups were predominantly isolated in the snow from the coastal, midland or inland regions. For example, Euryarchaeota (Halobacteriaceae) was the dominant phylotype in the snow samples collected from the coastal site, constituting 64% of the identified clones. In contrast, Proteobacteria were dominant in the snow samples both in the midland and inland regions, forming 62% and 82%, respectively, of the identified clones.

4.3.4. Spatial distribution of total snowpack microbial communities

Combined data from culture dependent and culture independent studies were used to assess the microbial communities present in the snow samples along the transect. While microbial numbers were nearly similar and did not vary from coast to inland, the microbial community composition was different and unique to each location (Fig. 4.4). Overall, higher diversity of culturable microbes was observed in the snow from the coastal region (Shannon-Weiner diversity index (H′) - 2.75) compared to the samples from the midland (H′ – 2.00) and inland regions (H′ – 1.96). Using the combined data from the culture dependent and culture independent studies, 27 distinct genera were recorded in the coastal site, while 16 and 17 different genera were recorded in the mid- and inland sites, respectively. Among the microbial communities identified, 21 genera were unique to the coastal site. In contrast, the mid- and inland sites, had 11 and 10 genera each, which were unique for these sites (Table 4.8).

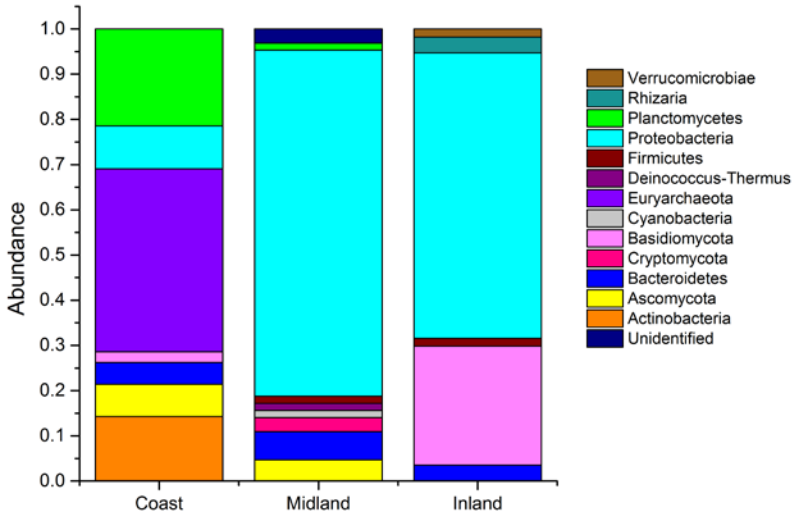

Figure 4.4. *Relative abundance of microbial communities at the phyla level.*

The microbial community compositions along the traverse were different with only 2 genera common to the three sites, viz., *Mucilaginibacter* and *Sphingomonas*. In a study of microbial diversity in the snow cores from Antarctica collected along a 1300 km traverse inland from the coast, it was found that the abundance and diversity of bacteria decreased with increase in latitude, altitude and distance from the coast (Yan et al., 2012). This was attributed to the different bacterial sources and different biogeographies under different regional climate conditions in the upper snow layers. Yan et al., (2012) suggested that near the coast, the oceanic climate may have affected bacterial sources and the microbial communities might have been derived from short-distance marine masses, while the inland region might have been influenced more by continental climate with the bacterial sources being derived from the remote continent via long-range atmospheric transport.

Table 4.8. *Site-specific distribution of microbial genera.*

Coastal		Midland	Inland
Bacillus	*Mycobacterium*	*Aquicella*	*Acinetobacter*
Brevundimonas	*Natronococcus*	*Bradyrhizobium*	*Altererythrobacter*
Caldimonas	*Pedobacter*	*Deinococcus*	*Bradyrhizobium*
Cryptococcus	*Penicillium*	*Dyella*	*Brevundimonas*
Dyadobacter	*Pigmentiphaga*	*Hydrotalea*	*Cryptococcus*
Frigoribacterium	*Piscinibacter*	*Mesorhizobium*	*Microbacterium*
Halalkalicoccus	*Polymorphobacter*	*Methylobacillus*	*Moraxella*
Janthinobacterium	*Rhodococcus*	*Methylovorus*	*Pseudomonas*
Lysinibacillus	*Rhodopseudomonas*	*Paramicrosporidium*	*Rhizobium*
Marisediminicola	*Variovorax*	*Rhodanobacter*	*Verrucomicrobiales*
Microbacterium		*Tumebacillus*	

In addition, the humidity and temperature decline from coast to inland were also believed to have contributed to the observed differences in the microbial abundance and community composition with coastal regions being more favourable for microbial survival than inland. This observation is consistent with the results of this study, where the highest diversity of microbes were recovered from the coastal snow samples. The high diversity of microbial communities in the coastal sample points to the possibility of the ocean being an important source of microbes to this region.

The production of marine aerosols during bubble dispersal at water–air interfaces is an important mechanism for the transportation of microorganisms across large distances (Aller et al., 2005; Smith et al., 2013). Concentrations of $ssNa^+$ which is considered as the most conservative ionic proxy for sea spray in coastal Antarctica, was found to increase with proximity to marine waters, suggesting that the composition of the coastal snowpack was largely influenced by sea spray (see section 2.3). Thus, aerial transport of microorganisms originating from the marine environment could be a major source of microbial assemblages in the Antarctic snowpack. This is in agreement with a previous study where the microbial communities were found to vary with altitude and distance from marine sources within the East Antarctic region (Yan et al., 2012). This is further supported by the fact that snow from the coastal region was dominated by Halobacteriaceae. Members of this family are obligate extreme halophiles, thriving in a great variety of hypersaline environments such as salterns (Lizama et al., 2002), Great Salt Lake, Utah (Wainø et al., 2000), the Dead Sea (Mullakhanbhai et al., 1975), saline lake (Castillo et al., 2006), including the cold, hypersaline lakes in Antarctica (Franzmann et al., 1988; DeMaere et al., 2013). Presence of Halobacteriaceae only in the coastal snow suggests that these Archaea might have been transported and deposited into the snow along with marine aerosols. Indeed, Archaea have previously been detected in the cold marine surface waters of Antarctica and reported to constitute a significant fraction of the prokaryotic biomass (DeLong et al., 1994). Archaea including Halobacteriaceae have also been reported to be associated with Antarctic sea-ice communities (Cowie et al., 2011; Jifei et al., 2014). Thus, airborne microbes originating from marine aerosols could be transported by wind and deposited onto the ice sheets and once deposited, their growth requirements might be supported by the relatively high salt concentrations in the coastal snowpack. As Halobacteriaceae has evolved to thrive in saline environments, the high salt concentrations in the coastal samples might confer a selective advantage to these

organisms, thereby providing a competitive edge and allowing their selected proliferation on the surface of the coastal ice sheet.

Although, microorganisms on the surface of the ice sheet might also be sourced from long-range atmospheric transport through wind and dust (Antony et al., 2012a; Smith et al., 2013), this study suggests that aerial transport of marine air masses might be a significant source of microbial communities to the coastal Antarctic snowpack. These results not only concur with a previous study that demonstrated distinctly different bacterial community compositions from coast to inland with higher microbial diversity in the coastal samples (Yan et al., 2012), but also lends support to the hypothesis (presented in section 2.3) that possible localised differences in bacterial composition and associated metabolic processes might be responsible for the observed variation in organic matter composition along this transect.

4.3.5. Biodiversity and potential implications for biogeochemical processes

Metagenomic and metatranscriptomic analysis of Antarctic microbial communities indicate the presence of bacteria, eukarya and Archaea. The majority of the microbial communities identified in this study are related to species known for their versatile metabolic properties and ecological functions, such as nitrogen cycling, sulfur metabolism, carbon cycling and nutrient recycling (Simon et al., 2009; Williams et al., 2012; Shtarkman et al., 2013). This indicates that some of the identified bacterial groups may possibly be involved in the cycling of these elements on the ice sheet surface. Here, the metabolic potential of some of the microbial groups that were dominant or ubiquitous in the snow samples studied are described. However, interpretations of the functional potential of the identified microbial communities should be viewed with caution as taxonomical data associated with ribosomal gene analyses do not provide the details of the actual role that these organisms play in the Antarctic environment, and the functional potential can differ between species and within the same species (Polz et al., 2013).

Members of the genus *Spingomonas* was prevalent in snow samples from the coastal, midland and inland regions. Their prevalence in all three sites along the 180 km long transect suggests that they may be an important component of the snowpack microbial communities and potentially affect snowpack biochemical processes. A salient characteristic of *Sphingomonas* sp. is its ability to degrade a variety of recalcitrant

aromatic compounds and plant polysaccharides such as celluloses, hemicelluloses, and lignin (Masai et al., 1999; Balkwill et al., 2006; Aylward et al., 2013). This is due to a suite of mono- and dioxygenases that they encode (Aylward et al., 2013), that catalyses the ring cleavage step which is critical to aromatic compound degradation (Harayama et al., 1992). In addition, this species also produces enzymes that aid in the degradation of nitroalkanes and sulfonated compounds. Thus, *Spingomonas* sp. is a specialized heterotroph capable of utilising a wide array of compounds especially components of refractory material as carbon, sulfur, and phosphorus sources (Aylward et al., 2013). This has relevance in the present study because a substantial fraction of the DOM in the snow samples studied comprised vascular plant material, as well as aromatic compounds and compounds containing N, S and P. As suggested by Aylward et al., (2013), *Sphingomonas* sp. may be specially adapted to utilise particular recalcitrant compounds in oligotrophic environments, as a successful ecological strategy, where few alternative nutrient sources would be available and where other microbes may not likely possess the enzymatic machinery necessary to compete for their use. This is supported by the fact that many strains of *Sphingomonas* are oligotrophic and are believed to be important contributors to the total biomass and nutrient recycling in oligotrophic environments (Eguchi et al., 2001; Barton et al., 2004).

Sphingobacteriales were also detected in all the snow samples studied here. Members of the Sphingobacteriales, particularly those belonging to the family Chitophagaceae (detected in this study) exhibit strong positive chemotactic response to inorganic ions such as nitrate and phosphate (Dennis et al., 2013) with some known to reduce nitrate as a terminal electron acceptor (Lim et al., 2009).

Methylophilaceae was recorded in the coastal and midland sites. Members of this family comprise several methylotrophic bacteria capable of utilising single C1 compounds as sources of energy and carbon. Among the several known methylophilic genera, three, viz., *Methylobacillus*, *Methylophilus* and *Methylovorus* (Kalyuzhnaya et al., 2006) were detected in the snow samples in the present study. This has relevance as C1 compounds such as MSA occur in Antarctica (Legrand and Mayewski, 1997), and was detected in all snow samples studied here along the 180 km transect (see section 3.3). MSA formed in the atmosphere by the chemical oxidation of biogenic dimethylsulfide is a major intermediate in the biogeochemical cycling of sulfur and its degradation by bacteria is an important link

in the biogeochemical S cycle (Baker et al., 1991). Previous studies have shown that methylotrophic bacteria isolated from various Antarctic environments are capable of utilising MSA for growth (Moosvi et al., 2005; Antony et al., 2012a). In addition, members of the genus Afipia, which were previously isolated from Antarctic environments and found to be capable of using MSA for growth (Moosvi et al., 2005), were also detected in this study.

Among the eukaryotic sequences identified, those affiliated to *Cryptococcus victoriae* comprised more than half. Yeasts belonging to the genus *Cryptococcus* particularly, *C. victoriae* is ubiquitous in Antarctica (Montes et al., 1999; D'Elia et al., 2009; Buzzini et al., 2012) and are well adapted to growth at low temperatures and minimal nutritional availability (Margesin and Miteva, 2011). A significant proportion of these *Cryptococcus* yeasts exhibit diverse enzymatic activities such as amylase, lipase, esterase, protease, pectinase, cellulose, xylanase and chitinase (Turchetti et al., 2008; Carrasco et al., 2012). *C. victoriae* isolated from Antarctica was found to assimilate as many as 27 carbon sources (Carrasco et al., 2012). The well known ability of *Crytococcus* sp. of producing an extracellular polysaccharide capsule (Vishniac, 2006; Van Bogaert et al., 2009; Pavlova et al., 2011) and its superior ability to assimilate carbon and nitrogen sources (Connel et al., 2008) are among the features that facilitate its apparent dominance in culturable yeast populations in cold ecosystems (Turchetti et al., 2015). This agrees well with the isolation of exopolysaccharide producing *Cryptococcus* sp. in this study (Fig. 4.5).

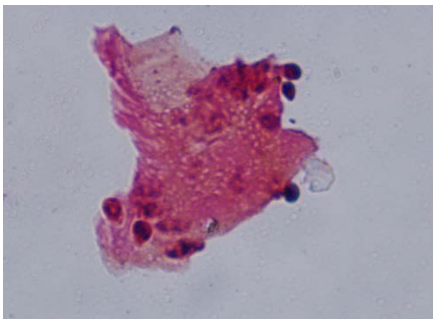

Figure 4.5. *Cryptococcus cells embedded in exopolysaccharide (magnification 1000X using Olympus BX53 microscope).*

The diversity of extracellular enzyme activities in the yeasts, and the diversity of compounds that are degraded, project the importance of the yeast community in nutrient recycling in the Antarctic regions. Other fungal sequences identified in this study include

those affiliated to *Aspergillus* and *Penicillium*. Members of *Aspergillus* and *Penicillium* are capable of degrading a wide array of carbon substrates including plant polysaccharides such as cellulose, hemicellulose, lignin and pectin (de Vries and Visser, 2001; Ali et al., 2012; Dhakar et al., 2014). In addition, *Aspergillus* and *Penicillium* species isolated from Antarctica were found to be capable of degrading several aromatic compounds (Alexieva et al., 2012). Both these genera were also capable of using tannins as sole carbon source and have been implicated in tannin degradation (Cruz-Hernández et al., 2005). Isolation of these genera in this study is very relevant as lignins, tannins and aromatic compounds made up a significant fraction of snowpack DOM pool.

Archaeal sequences in the coastal snowpack were dominanted by the Phylum Euryarchaeota (Halobacteriales). Although members of Halobacteriales play crucial roles in the Earth's global geochemical cycles, such as organic carbon mineralisation, denitrification and sulfidogenesis (Offre et al., 2013), their role in the polar setting is less known. However, as Halobacteriales comprised a significant fraction of the microbial communities identified in the snowpack, it is presumed that they should be having some important ecological role to perform in this environment.

Several other microbial groups with key roles in natural environment such as *Pigmentiphaga litoralis*, *Polaromonas vacuolata*, Planctomycetes and Cyanobacteria were also detected in the snowpack. *P. litoralis*, capable of growth at low temperatures is known to produce phosphatases (Chen et al., 2009) that function to catalyze the hydrolysis of organophosphorus compounds (Singh and Walker, 2006). They are also capable of utilising a number of carbohydrates (Chen et al., 2009). *P. vacuolata* is a heterotrophic gas vacuolate bacteria. This species has been previously isolated from antarctic marine waters and was found to be capable of utilising a number of carbon substrates (Irgens et al., 1996). Planctomycetes is emerging as a group of interest in microbial ecology, because the members perform anaerobic ammonia oxidation (anammox) (Strous et al., 1999), and the metaproteomic analysis indicates that they may be active in coastal Antarctic Peninsular waters (Williams et al., 2012). Cyanobacteria are the most widely distributed photoautotrophs in polar regions (Pandey et al., 2004; Vincent, 2007; Singh and Elester, 2007) and are believed to be important contributors to critical ecosystem processes, particularly in carbon and nitrogen cycling, in addition to supporting the heterotrophic and higher trophic communities (Makhalanyane et al., 2015). Moreover, Cyanobacteria are

known to produce a great variety of secondary metabolites whose ecological functions are still unclear (Welker and von Döhren, 2006). Cyanobacteria are also important as they contribute to the formation of cryoconite granules by densely covering the surface of the granules and trapping mineral particles inside them (Takeuchi et al., 2010). As cryoconite aggregates substantially reduce the albedo of the glacier surface and accelerate melting (Kohshima et al., 1993), these cyanobacteria may potentially affect the albedo of the ablation surface of the glacier. Cyanobacteria production may also be important in sustaining heterotrophic bacteria living within the granules (Takeuchi et al., 2010) and these are believed to be key agents in the functionality of supraglacial cryoconite ecosystems (Edwards et al., 2014). In addition, the microbial groups that are involved in various ecological processes related to N cycling were also detected in the snow sample. For example, sequences related to a N_2-fixing species of *Bradyrhizobium* and *Nitrobacter* which are facultative chemolithoautotrophs and conserves energy from the oxidation of nitrite to nitrate (Lengeler et al., 1998) were also detected.

Thus, microorganisms that play key roles in the natural ecosystem, including the biogeochemical cycling of C, N, S and P, were detected in this study. In addition, several of the identified groups were previously shown to be capable of degradation of biomolecules such as lipids, proteins, carbohydrates, recalcitrant aromatic compounds and vascular plant material such as lignins and tannins. As the snowpack DOM comprises C, N, S and P- bearing aromatic and aliphatic organic compounds of different bimolecular compound classes such as lipids, proteins, carbohydrates, lignins and tannins, the microbial communities present could potentially modify the snow chemistry, through their metabolic activities. Analysis of the role of microbial communities in ecological processes would improve our understanding of the biogeochemical elemental cycles affected by microbial communities in the Antarctic environment. In the next chapter, an attempt has been made to understand the role of these microbes in supraglacial DOM cycling through a combination of *in situ* field experiments and ultrahigh resolution mass spectrometry. Specifically, the transformation of supraglacial dissolved organic matter through microbial communities on the AIS surface has been elucidated at the molecular level, for a better understanding of the interaction between microbes and the wide spectrum of compounds that comprise the supraglacial DOM pool.

CHAPTER 5

Microbially mediated molecular transformations of dissolved organic matter

5.1. Introduction

The biogeochemical diversity of supraglacial DOM is very complex. Proteins, lipids and other organic molecules derived from resident microbial communities dominate supraglacial DOM, which also comprises compounds derived from marine sources, vascular plant material, as well as by-products of fossil fuel combustion and biomass burning (Stibal et al., 2008; Bhatia et al., 2010; Singer et al., 2012; Stibal et al., 2012; Stubbins et al., 2012a; Antony et al., 2014). Further, such glacier-derived DOM is biologically available (Hood et al., 2009) and provides a subsidy of carbon to resident microbial communities as well as downstream ecosystems (Stubbins et al., 2012a). Microbial communities use the supraglacial DOM as a source of carbon and nutrients (Anesio et al., 2010; Stibal et al., 2012; Larose et al., 2013) which in turn, produce organic metabolites with vastly different properties (Kujawinski et al., 2004; Koch et al., 2014). These biochemical transformations greatly modify the properties of DOM and thus exert a strong influence on the nature of DOM on supraglacial surfaces (Jones, 1999; Anesio et al., 2010) and also that exported to downstream aquatic ecosystems. The interaction between DOM and microorganisms is important, as the degree to which DOM on the surface of glaciers and ice sheets is available for microbial decomposition determines its significance for supraglacial biogeochemistry. At one extreme, the highly refractory DOM molecules that are not degraded for extended periods of time could contribute to carbon storage in the ice sheets until melting processes remove the stored carbon from ice and transport it to the downstream ecosystems, where it may impact the ecology of these environments. At the other extreme, the bioavailable DOM would form an important resource for supraglacial heterotrophs (Hood et al., 2009; Singer et al., 2012) and also the inorganic nutrients regenerated during the decomposition of DOM may stimulate autotrophic microbial communities (Kirchman, 2012), thereby impacting the uptake of atmospheric carbon dioxide and sustenance of heterotrophic bacterial communities through production of new carbon. The active microbial communities play an important role in biogeochemical cycling in glacial environments (Anesio et al., 2010; Stibal et al., 2012; Larose et al., 2013). Although the degradation of DOM is known to be an important variable that influences the global carbon cycle, very little is understood about the interactions between microorganisms and the wide spectrum of compounds that comprise DOM. Assessments of biogeochemical transformations of DOM beyond bulk examination and compound specific analyses are lacking and this necessitates a more fundamental

assessment of DOM composition and reactivity, in order to better understand the carbon cycling in ice sheets.

Here, the ESI-FTICR-MS was used to detect molecular changes in Antarctic supraglacial DOM associated with microbial processes. By using the FTICR-MS, this study provides novel molecular insights on the previously undocumented compounds within the supraglacial DOM that are amenable to microbial alteration, resistible to microbial degradation and produced as a result of microbial processing. The new information gained on DOM transformation on the ice sheet surface would help in elucidating the drivers of DOM dynamics and in improving our ability to predict the fate of this material in supraglacial as well as downstream environments.

5.2. Materials and methods

5.2.1. Snow sampling, field incubations and sample processing

Surface snow samples collected from a clean site in the Princess Elizabeth Land region (69°28'S, 76°09'E) in East Antarctica was used for the study. Surface snow was homogenously mixed using a sterile spatula and sub-sampled into 33 acid-cleaned (1 M HCl), pre-combusted (475 °C for 4 hr) sterile quartz tubes with Teflon caps. All required precautions were taken during sampling to reduce the risk of contamination. Snow samples were immediately processed for onsite incubations in the field site itself, as outlined below. One set of tubes, consisting of snow samples with the naturally occurring microbial assemblages, was covered with aluminium foil to prevent light penetration. This set of tubes was incubated in the field under ambient conditions to assess the microbial processing of snowpack DOM. Another set consisting of snow samples poisoned with sodium azide (NaN_3, ca. 6.5 mg L^{-1}) and wrapped in aluminium foil, was incubated as above. This set represented the control. NaN_3 was injected into the snow samples using a sterile Hamilton microliter syringe in several line injections. After the injection, the snow samples were mixed homogenously using a sterile spatula. In addition, field blanks consisting of pre-cleaned sterile quartz tubes exposed to the sampling site conditions for the same length of time as that of the sample were also maintained. Samples, including field blanks and control, were incubated in the field (Fig. 5.1) and retrieved after 5, 10, 15, 35, 45 and 55 days. However, loss of the 5-day incubated samples due to breakage prevented further analysis of these samples from this time point.

Figure 5.1. *Field experimental set up.*

Ambient average daily air temperature during the incubation period varied from -4.4°C to +5.1°C. The snow samples before incubation, and those retrieved from the field at specific time points were processed immediately on site. DOM was extracted from the samples (as well as the field blanks and controls) using 100 mg PPL cartridges (Agilent Bond Elut-PPL), following the method outlined in Dittmar et al. (2008) and as detailed in section 3.2.1. PPL cartridges were stored at -20°C until elution, which occurred just before the FTICR-MS analysis.

5.2.2. FTICR-MS analyses

Samples were analysed using a Bruker Daltonics 12 T Apex Qe FTICR-MS instrument, operated in both negative and positive electrospray ionisation modes, using parameters consistent with those described in section 3.2.1. All peaks found in each mode's field blank were removed from the DOM peak list. Also, peaks in the incubated snow samples that were common with that of the biocide amended control were not considered. The blank-corrected master peak lists for each sample were assigned unique molecular formulas as described in section 3.2.2. For all samples, 68-89% of all peaks were assigned a unique molecular formula. The majority (>83%) of the assigned formulas were within 0.5 ppm mass accuracy and all formulas were within 1.0 ppm mass accuracy. After exact elemental formulas had been assigned, formulas that degrade in the dark incubated snow sample and the biocide amended control were compared. Following incubation, any

formula that degraded in the snow sample and also in the control was attributed to abiotic dark reactions and was not considered. These constituted about 1.4 to 2.5% of the total identified formulas. Similarly, any formula present in the dark incubated snow sample that was also present in the control (but not in the initial sample) was attributed to abiotic dark reactions and was not considered. These constituted up to 30% of the total identified formulas.After assigning the exact elemental formulas, the molecules were categorised by compound class using various chemical metrics as described in section 3.2.2. The DBE-O parameter (obtained by subtracting the number of oxygen atoms from DBE value) was used for a simple approximation for pure C-C unsaturation (Gonsior et al., 2009; 2011). Carbon oxidation state (OS_C) was calculated as $OS_C \approx 2\ O/C - H/C$ (Kroll et al., 2011).

Aliphatic compounds, possibly originating from algal detritus and/or microbial biomass that included the lipid and protein formulas identified in this study, are collectively referred to as 'autochthonous DOM' (O/C <0.6 and H/C >1.7; Schmidt et al., 2009). Identified formulas that were similar in composition to lignin and tannin derived species, indicative of terrestrially sourced DOM, are collectively categorised as 'terrestrial DOM'. The DOM has been classified into 3 major groups based on biological reactivity (some overlap between groups exist) - 1) Bio-labile: compounds that disappeared during dark incubation, 2) Bio-produced: compounds, absent in the initial sample and were generated during the course of the incubation and 3) Bio-resistant: compounds present in the initial sample that resisted degradation until the end of the incubation (See Supplementary Tables S2 and S3 for a list of formulas).

5.3. Results and Discussion

5.3.1. Molecular signatures of snowpack DOM

In the mass spectrum of the initial sample, 2338 formulas were assigned, of which 1404 were aliphatic, 86 were aromatic and 44 were condensed aromatic (with the remaining 804 falling between the aliphatic and aromatic categories). Molecular characterisation revealed that the DOM pool comprised various compound classes, such as lignin (1110), proteins (62), lipids (109), unsaturated hydrocarbons (181), carbohydrates (75) and tannins (29) (Fig. 5.2). Several CRAM (414) and condensed aromatic formulas (44) resembling DBC were also detected.

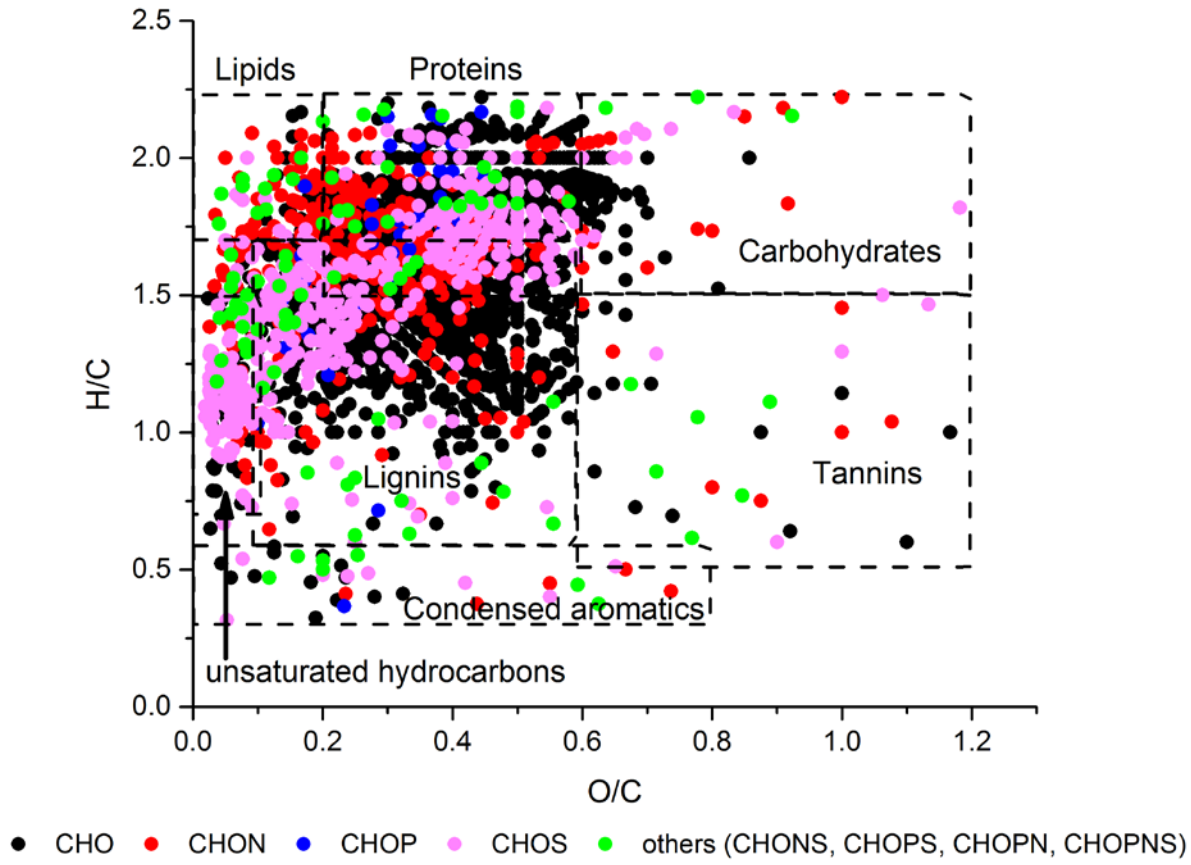

Figure 5.2. *van Krevelen distributions of dissolved organic molecular formulas containing C, H, O, N, S and P, identified in the original snowpack.*

5.3.2. Molecular imprints of microbial transformations of DOM

FTICR mass spectra of surface snow showed large changes in DOM after dark incubation, indicating significant microbial alteration (Fig. 5.3). Further, 89% of the formulas identified in the surface snow DOM disappeared during the initial 10 days of incubation, suggesting that a substantial fraction of the DOM pool was highly labile and turns over very rapidly in supraglacial environments. This agrees with previous reports that the glacier-derived DOM is highly bio-available, with a significant fraction of the organic carbon being readily metabolised by heterotrophic bacteria in short-term laboratory bioassays (Hood et al., 2009; Singer et al., 2012).

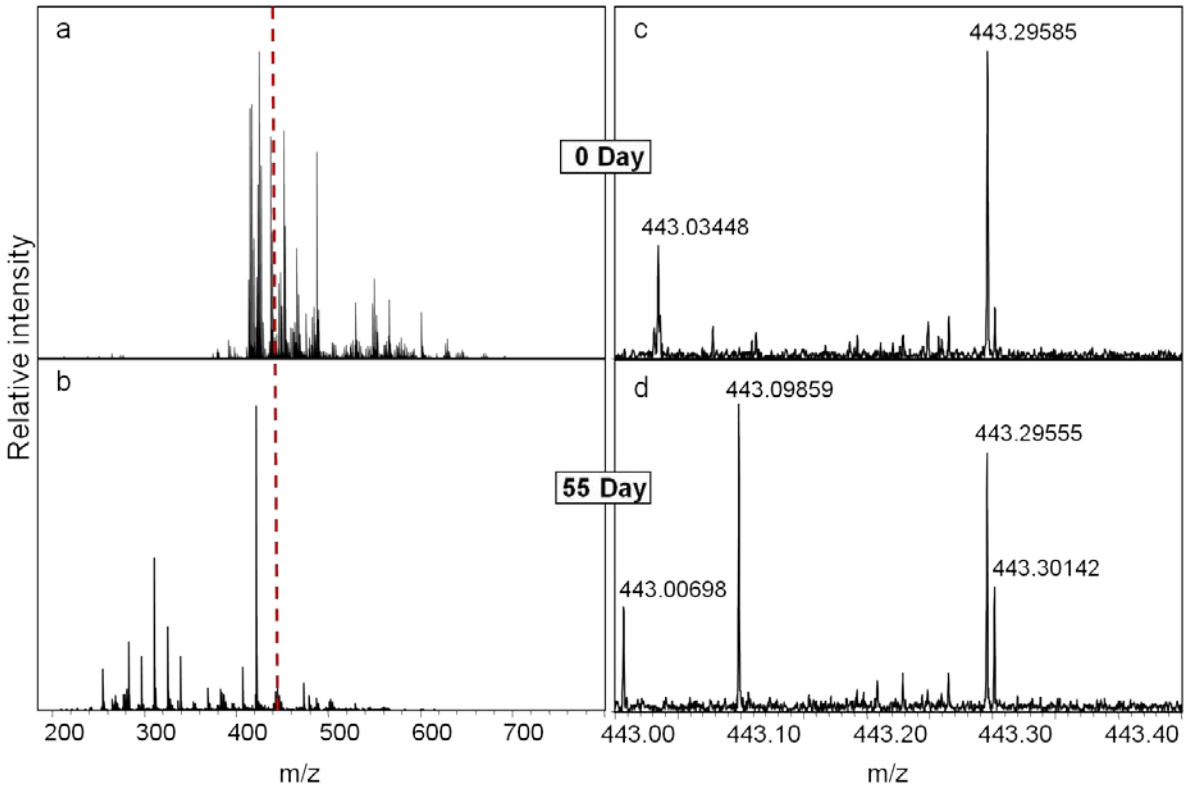

Figure 5.3. *Negative electrospray ionisation FTICR-MS mass spectra of snowpack DOM in the 0- day (a) and 55-day (b) incubation and corresponding expanded region of the mass spectra (range 443.0-443.4), to highlight the complexity at each nominal mass (c, d) and the high resolution obtained by FTICR-MS.*

Aliphatic, aromatic, as well as condensed aromatic compounds present in the initial DOM were found to be highly susceptible to microbial alteration as all the condensed aromatics, 98% of the aromatics and 86% of the aliphatics, originally present in the surface snow DOM disappeared following 10-day dark incubation (Table 5.1). Microbial decomposition of a large fraction of the identified compounds was consistent with the role of heterotrophic bacteria as the major consumers and remineralisers of DOM (Azam, 1998). Interestingly, in addition to loss of compounds, new formulas not observed in the initial DOM were detected. The new components accounted for 53% of the 543 formulas assigned in the 10-day dark incubation.

Table 5.1. *Numbers of all assigned molecular formulas before and after dark incubation.*

Molecular class*	Number of formulas**					
	Period of incubation (days)					
	0	10	15	35	45	55
All identified formulas	2338	543	394	1038	659	494
Terrestrial DOM	1139	236	201	475	310	222
Autochthonous DOM	821	196	113	355	279	147
CRAM	414	74	71	171	83	61
Unsaturated hydrocarbons	181	23	29	72	36	42
Carbohydrates	75	58	17	37	10	26
Aliphatic	1404	364	194	560	456	275
Aromatic	86	24	40	76	22	26
Condensed aromatic	44	22	16	48	15	26

**The number of formulas listed in each column do not add up to the total, because formulas can fall into multiple categories (i.e., the aliphatic and aromatic formulas also include formulas belonging to the terrestrial, autochthonous, CRAM, unsaturated hydrocarbon, and/or carbohydrate classes).*
*** Reported identified formulas after dark incubation includes compounds in the initial sample that resisted degradation as well as new compounds formed during the incubation.*

Comparisons of the mass spectra after subsequent incubation intervals indicated further degradation and production of new compounds (Fig. 5.4). In all cases, unless otherwise mentioned, when lost formulas are referred to, it is with reference to the previous time point. Following the 15-day dark incubation, a loss of 504 formulas was noted with the simultaneous addition of 355 new formulas. Lost formulas include 235 formulas that were present in the initial sample and resisted degradation up to this time point and 269 new formulas (of a total of 290) that were generated in the 10-day time point. A total of 1038 formulas were identified in the 35-day incubation. A loss of 285 formulas was recorded with the simultaneous addition of nearly 3 times as many new formulas (929) (Fig. 5.4). Thus, nearly all the formulas identified on day 35 were those newly added, resulting in higher number of formulas than in the previous two time points. In addition, long term (45- and 55-day) incubations were also carried out. At the end of 55-day incubation, a total of 494 identifiable formulas were observed, which was 79% less than that of the initial sample. The new formulas comprised all bio-molecular compound classes. The majority of the formulas identified in the 55-day incubated sample were lignin-like and/or CRAM (216, 44%) and aliphatic compounds from algal and/or microbial biomass (147, 30%). It may also be possible that due to microbial reworking, the previously non-ionisable

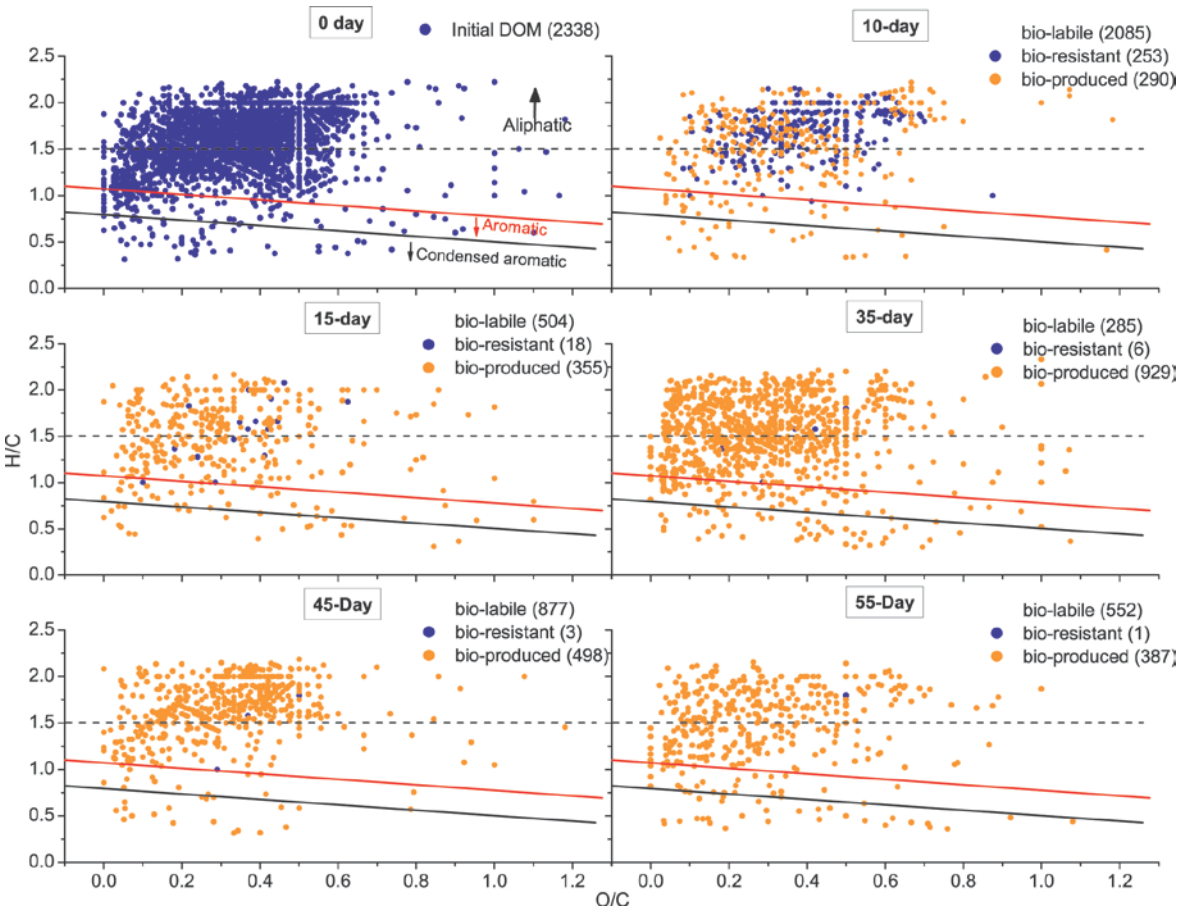

Figure 5.4. *van Krevelen diagram showing all identified DOM formulas in the initial snow sample as well as, bio-labile, bio-produced and bio-resistant DOM formulas identified at the different incubation time points.*

lignin-like compounds were changed to an ionisable form and hence detected. The formation of new CRAM-like formulas is consistent with the finding that the extensive processing of DOM may result in biomolecules that contain an unusually large portion of carboxylic acid functionality (Hertkorn et al., 2006; Lam et al., 2007; Hertkorn et al., 2013). When interpreting these results, it is important to note that an unknown number of molecules are likely to fall beyond the analytical window of ESI-FTICR-MS. This is because they may be directly mineralised to inorganic (Moran and Zepp, 1997; Bushaw-Newton and Moran, 1999; Kieber et al., 1999) or gaseous products (McCallister et al., 2012; Ward et al., 2013; Fasching et al., 2014), or the ionisable molecules are converted to non-ionisable molecules, in which case they would not be detected by FTICR-MS. Thus, not every compound that is degraded, produced, or resisted microbial processing can be identified. Alternatively, microbial processing could alter the previously undetectable non-ionisable molecules present in the initial sample to an ionisable form and, thus would

appear in the mass spectra. Therefore, new products identified may or may not be derived from identifiable bio-labile compounds in the initial sample.

The 0-day sample and 55-day dark treatment shared 172 formulas, representing only 7% of the formulas identified in the initial DOM pool. However, it was seen that all the common formulas (except 1) were removed from the initial sample and regenerated again during the intermittent time points preceding the 55-day time point. Thus, only one formula ($C_{20}H_{36}O_{10}S_1$) among the thousands identified in the initial sample resisted degradation until the end of the 55-day incubation. This bio-resistant compound with chemical characteristics indicative of microbial origin was characterised by a high degree of saturation (low DBE-O), was highly oxygenated and had an O/C and H/C value of 0.5 and 1.8, respectively (Table 5.2).

Table 5.2. *Nature of Bio-labile, Bio-resistant and Bio-produced DOM **

Property	Compound category		
	Bio-resistant	Bio-labile	Bio-produced
C	20.00	23.91	23.67
O	10.00	6.86	6.62
H	36.00	35.65	35.06
P	0.00	0.05	0.07
S	1.00	0.28	0.28
N	0.00	0.39	0.44
O/C	0.50	0.31	0.31
H/C	1.80	1.52	1.52
DBE	3.00	7.36	7.46
DBE/C	0.15	0.30	0.31
DBE-O	-7.00	0.50	0.84
AI_{mod}	0.00	0.19	0.20
OS_c (CHO compounds)	-	-0.86	1.60
MW	467	453	446
Total identified formulas	1	3933	2458

** Parameters listed are the average atomic numbers per formula, atomic O/C and H/C ratios, double bond equivalents (DBE), average DBE/C ratios, DBE minus the number of oxygen atoms (DBE-O), modified aromaticity index (AI_{mod}), and average carbon oxidation state (OS_c), and number-averaged molecular weight (MW).*

Overall, the number of formulas present in the original sample that resisted degradation following 10, 15, 35, 45 and 55- day dark incubation was 253, 18, 6, 3 and 1, respectively (Fig. 5.4). However, the fact is that identical formulas in the initial and microbially processed DOM do not necessarily imply that the molecules have exactly the same structure. Given the considerable degree of unsaturation and heteroatom content of DOM,

it is likely that numerous structural isomers are possible for any single defined molecular mass (i.e., formula). Therefore, similarities in mass spectra do not necessarily indicate conformity of molecular structures. It is also likely that the relative ionisations changed due to changing sample matrix, such that compounds having low abundances and/or ionisation efficiencies were suppressed and thus were below detection.

It is interesting to note that bacteria degraded and produced compounds at both the high and low end (200-1190 m/z) of the apparent molecular weight distribution of DOM. This extensive degradation and production of new compounds are consistent with the role of bacteria not just as consumers, but also as producers of DOM (Guillemette and del Giorgio, 2012). Put together, these provided clear evidence to show that microbial reworking results in extensive molecular alteration of the DOM mediated by parallel processes of degradation and synthesis.

5.3.2.1. Bio-labile compounds

Both aromatic and aliphatic compounds present in the initial DOM appeared to be highly susceptible to microbial alteration. Compounds that are readily degraded by bacteria included bio-labile molecules such as lipids, proteins, carbohydrates, aliphatic compounds derived from algal detritus and/or microbial biomass, as well as compounds traditionally considered to be refractory in nature, such as DBC and lignin- and tannin-like compounds. Proteins and carbohydrates are bio-labile because of the susceptibility of peptide and glycosidic bonds to enzymatic hydrolysis (Nunn et al., 2003; Piontek et al., 2010) and are readily assimilated by bacteria and metabolised at high growth efficiencies (Berggren et al., 2010). This pool of organic matter is rapidly overturned, whereas terrestrially derived compounds such as lignins, with complex macromolecular structures composed of phenylpropanoid units linked by C-C and ether bonds, is assumed to have a chemical stability that can resist extensive microbial degradation (Chen, 2014). This perception that the terrigenous fraction of DOM is largely resistant to microbial metabolism is now changing (Ward et al., 2013; Marín-Spiotta et al., 2014). The findings from this study further expand this changing view and provide detailed molecular evidence of rapid and extensive microbial alteration of terrestrial DOM and show for the first time that these processes could be important in polar snowpack.

5.3.2.1.1. Microbial degradation and transformation of terrestrial DOM

Our understanding of microbial processing of terrestrial DOM is limited, but its importance can be inferred from the microbial mineralisation of lignin-like compounds and related macromolecules over short time scales (Moran and Hodson, 1994; Letscher et al., 2011; Ward et al., 2013). The results of this study revealed that microbial reworking shifted the composition of terrestrial DOM and decreased both the number and magnitude of terrestrial DOM formulas. Microbial degradation resulted in the loss of all lignin- and tannin-like formulas recorded in the initial sample with simultaneous addition of new formulas. Following microbial reworking, the number of identifiable lignin-like formulas dropped by 81%, from 1110 in the initial sample (ie. 0 day sample) to 216 in the 55-day incubation. Similarly, the number of identified tannin-like formulas showed a 79% drop. In addition, a shift in the C, H, O and heteroatom (N, P and S) content of the 55-day microbially reworked terrestrial DOM was observed. Microbially processed lignin-like compounds had lower C, H, O contents, but higher N, P and S contents relative to the initial pool of lignin-like compounds. The average content of C, H, O atoms (average number of each element per molecular formula) dropped by 5-16%, while that of N, S and P increased by 45, 52 and 180%, respectively. In the case of the tannin-like fraction of the DOM pool, the average content of all elements (C, H, O, S and P) except N increased relative to the initial sample. C, H, O and S increased by 31-45%, while P increased by more than 860%. In contrast, the N content of the tannin-like fraction of the DOM decreased by 19%. It is interesting to note that the average molecular weight (MW 443) of lignin-like peaks in the 55-day incubation was lower than that in the 45-day incubation (MW 452). The formation of low molecular weight (LMW) lignin-like compounds is consistent with microbial decomposition of organic matter into smaller molecules (Amon and Benner, 1996) and with the detection of microbes such as *Spingomonas, Cryptococcus*, *Aspergillus* and *Penicillium* in snow (section 4.3.2 and 4.3.3.2) that are capable of degrading vascular plant material. On the other hand, tannin-like formulas had a significantly higher MW (665) in the 55-day, than that of the 45-day incubation (MW 490). Tannins are polyphenolic compounds often with high molecular weight (HMW) and can form chemical complexes with proteins due to the high degree of hydroxylation that offers many possible sites for protein binding (Bhat et al., 1998). Several microbes, including bacteria and fungi, are capable of degrading tannins as well as tannin-protein complexes (Bhat et al., 1998; Cruz-Hernańdez et al., 2005; Pepi et al., 2010; Mutabaruka

et al., 2007). Due to the high affinity of tannins for binding protein and the fact that protein molecules constitute a substantial fraction of the total identified DOM pool, it is hypothesised that such tannin-protein complexes may be formed in Antarctic snow. Further, it is speculated that the observed increase in the number of HMW tannin-like formulas detected in this study may be possibly due to the release of HMW tannins from such complexes following microbial reworking. This is supported by the identification of fungi such as *Penicillium* and *Aspergillus* in the snow from this region that have previously been reported to be capable of degrading tannin as well as tannin-protein complexes (section 4.3.5). Another possibility is that previously undetectable non-ionisable molecules in the initial sample were converted to ionisable molecules following microbial processing and thus, were newly detected in the mass spectra.

Together, these results agree with the observation that microorganisms are capable of overturning the DOM pool by extensive reworking of terrigenous organic matter (Hernes and Benner, 2003; Letscher et al., 2011; Ward et al., 2013). These results are consistent with the isolation of microbes from surface snow from this region that are capable of degrading plant derived material such as cellulose (Antony et al., 2009), and other related macromolecules such as lignin and tannin (section 4.3.5), as well as isolation of microbes from other regions of Antarctica that have cellulolytic and ligninolytic activities (Ferrés et al., 2015). Studies show that terrigenous DOM probably fuels respiration and are either remineralised to CO_2 or the degradation products are recycled through the microbial food web (Ward et al., 2013; Fasching et al., 2014; Berggren et al., 2010). For example, microbial respiration of terrigenous DOM can contribute to CO_2 outgassing to the atmosphere even without photochemical facilitation (Ward et al., 2013; Fasching et al., 2014). Further, LMW terrestrial DOM is readily available and is a quantitatively important component for microbial metabolism (Berggren et al., 2010). The collective results from this study and earlier reports provide strong evidence for the biodegradability of terrestrially derived macromolecules and provide a significant step in understanding their importance as a potential source of bioavailable organic matter in the polar ice sheets.

5.3.2.1.2. Microbially mediated transformation of "refractory"DOM

Stability and transformation of DBC in the environment remain unclear mainly because of their high recalcitrance compared to other natural substances. Although many studies have pointed out possible losses due to biotic or abiotic degradation, most studies have focused

on the photo-lability of DBC (Stubbins et al., 2012b; Ward et al., 2014). Due to its apparent slow decomposition by microbes, very few studies exist on microbially mediated transformations of DBC (Kuzyakov et al., 2009, 2014). As a result, knowledge of microbial DBC degradation processes, especially at the molecular level, is scarce. This study showed that DBC compounds that comprised a range of masses and aromaticity, as well as N, S and P content, were readily degraded by microbes, which is in contrast to findings that BC is mineralised very slowly (Kuzyakov et al., 2009, 2014). All the 44 condensed aromatics in the initial sample were degraded during the first 10 days of incubation. However, a total of 26 DBC formulas were detected in the 55-day incubated sample due to the simultaneous addition of new DBC compounds. The new DBC formulas generated at each incubation interval were removed in the subsequent time points. Barring one, the new formulas generated were unique to the respective time points such that none of the DBC molecules identified at the end of the incubation were identical to those in the initial sample. Microbially processed DBC in the surface snow was characterised by higher DBE values, was more oxygenated, less saturated and contained higher S, but lower P and N contents relative to the initial sample. A 73% increase in O content was observed in microbially altered DBC formulas compared to initial. This agrees with the suggested BC degradation pathways via carboxylation, hydroxylation and/or methoxylation, which increase the O content and generally the water solubility (Willmann and Fakoussa, 1997; Decesari et al., 2002; Hockaday et al., 2007). A small fraction may be incorporated into bacterial biomass or mineralised directly to CO_2 (Kuzyakov et al., 2009). The results of this study indicate that bacteria in supraglacial environments can transform DBC over short time scales (<10 days) and lend support to the emerging studies that document microbial degradation and assimilation of DBC (Hockaday et al., 2007; Kuzyakov et al., 2009, 2014) and also to the growing consensus that DOM transformation (including BC) is driven by the ecosystem properties rather than the inherent molecular complexity or structure of DOM (Marín-Spiotta et al., 2014).

Several CRAM-like compounds that are potentially derived from highly decomposed biomolecules (Hertkorn et al., 2006; Lam et al., 2007) were also observed. Contrary to the common perception that CRAM is bio-refractory, this study recorded microbial degradation of CRAM-like formulas. Ninety two percent of the 414 CRAM-like formulas identified in the initial sample disappeared within 10 days, with simultaneous addition of new formulas during subsequent time points. Only 61 CRAM-like formulas were present

at the end of the 55-day incubation. The microbially reworked CRAM molecules were characterised by higher heteroatom content (129-244% increase from initial). Thus, while rapid microbial reworking of CRAM suggests that these compounds may not be as bio-refractory as previously believed, the formation of new CRAM-like formulas in microbially processed DOM confirms the findings that extensive processing of DOM results in biomolecules that contain an unusually large fraction of carboxylic acids (Hertkorn et al., 2006; Lam et al., 2007; Hertkorn et al., 2013). A portion of the CRAM-like molecules could also possibly be formed through hydroxyl radical initiated oxidation of lignin-like material present in snow (Waggoner et al., 2015).

5.3.2.2. Bio-produced DOM pool

New compounds formed during microbial processing of supraglacial organic matter accounted for majority of the formulas identified in the 55-day time point. Of the 494 formulas identified in the 55-day sample, all but one were new formulas, added during different time points. The observed increase in the oxidative state of the bio-produced DOM (Table 5.2) clearly indicates that diagenetic alterations of DOM were occurring due to microbial oxidation. In general, bio-produced compounds included aliphatic, aromatic and condensed aromatic bio-molecules, represented by all biochemical compound classes. However, unlike the bio-labile pool, the new compounds formed as a result of microbial processing have distinctly different molecular composition (Table 5.2). For example, the new lignin-like formulas formed during the dark incubation were characterised by higher N, S and P content, had slightly higher AI_{mod} values and were more unsaturated compared to the bio-labile lignin-like compounds. On the other hand, newly formed DBC molecules were more oxygenated and had a lower N content than bio-labile DBC. Recently, a new non-pyrogenic mechanism was suggested for the formation of DBC-like compounds in the absence of sunlight through hydroxyl radical initiated oxidation of lignin (Waggoner et al., 2015). Because lignin-like formulas accounted for a significant portion of the identified DOM formulas in the snowpack, it is possible that lignin-like compounds could be responsible for at least a portion of the DBC formed during the incubation through hydroxyl radical initiated oxidation.

5.3.2.3. Changes in the heteroatom composition of DOM

The DOM was more structurally diverse after microbial reworking, as the heteroatom (N,

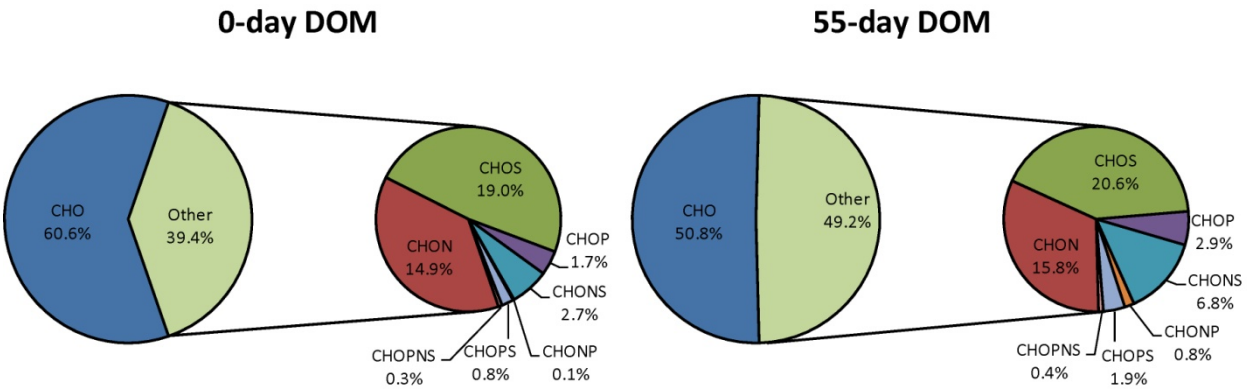

Figure 5.5. *Percentage of each type of molecular formula present on the 0- and 55-day incubation.*

S and P) content of DOM increased following incubation (Fig. 5.5). The contribution of heteroatoms to the total identified DOM formulas increased by 25% upon microbial reworking. Molecular characterisation of DOM in coastal waters revealed that the DOM comprised molecules mainly derived from autochthonous microbial production which tend to have more N, S and P atoms incorporated into the DOM (Sleighter and Hatcher, 2008). This observation is consistent with the results of this study. Increase in formulas containing P either alone or in combination with other heteroatoms was most prominent, wherein the P content of microbially processed DOM was 108% higher than the initial. Also, the contribution of all S-, P- and N-containing formulas to total peak magnitude increased by 100, 28 and 9%, respectively, indicating that they may be present at slightly higher concentrations than the initial. The increase in peak magnitude might also be due to poorly ionised heteroatom compounds, which only showed up following microbial reworking due to a reduction in the competition for charge within the sample. Alternatively, non-ionisable molecules that were undetectable might have been converted to ionisable forms as a result of microbial processing and were detected. The relative peak magnitudes of heteroatom-containing formulas before and after microbial alteration are depicted in Figure 5.6. The compositional variability in heteroatom content of the DOM is not surprising, as the production and cycling of C are inextricably linked to N and P by virtue of the fact that microbially generated organic compounds contain C and N or C, N and P (Karl and Björkman, 2002). Production of dissolved organic phosphorous (DOP) compounds typically begins with biological uptake of inorganic P and incorporation into one of the many intracellular P-containing compounds in the cell (Karl, 2014). DOP can exist in different forms of LMW and HMW compounds and most of these are readily bio-

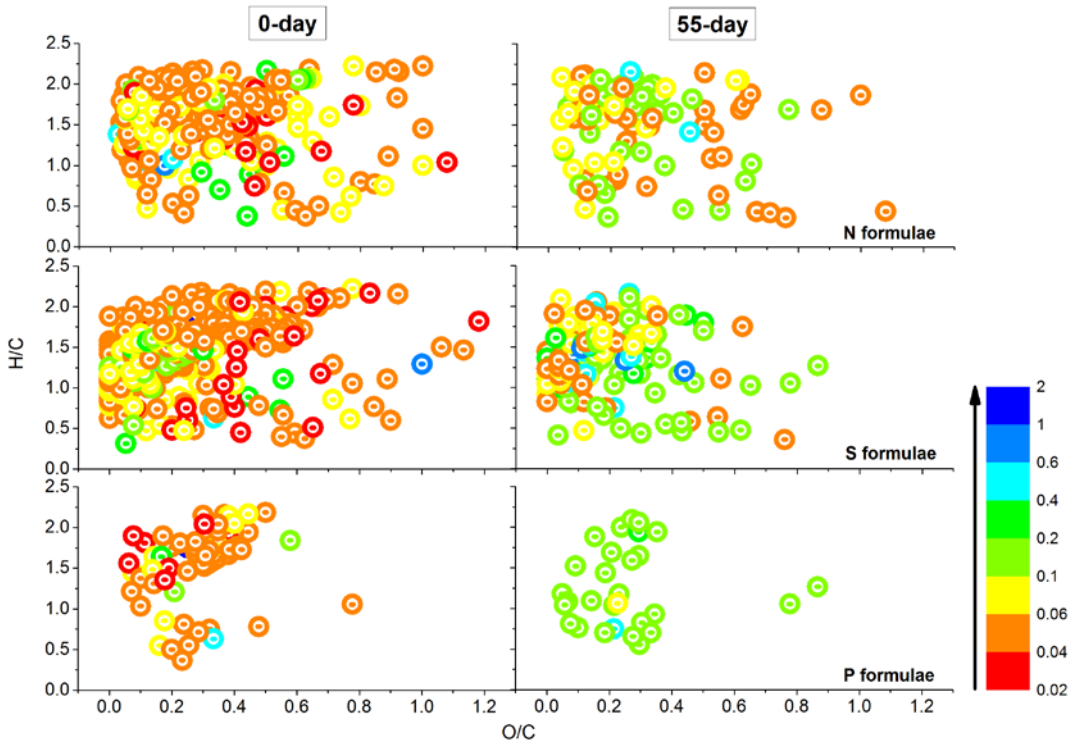

Figure 5.6. *van Krevelen plots of the formulas containing N, S, and P for the 0- and 55-day microbially altered snowpack DOM. Colour represents the relative peak magnitude of associated peaks (percent of the summed total magnitude of all peaks). Points are coloured by the logarithm (base 10) of peak magnitude. Note that formulas with N, S, and P atoms comprised these elements present alone or in combination with each other.*

available albeit at variable rates of assimilation (Karl, 2014). Studies suggest that in marine environments, a fraction of the HMW DOP may be derived from bacteria (Clark et al., 1999). Preferential regeneration of P from HMW DOM has also been reported (Clark et al., 1999). The majority (70%) of the P-containing formulas identified in this study were associated with material derived from microbial biomass which included lipids, proteins and carbohydrates. This is not uncommon as microbial membranes are composed of phospholipid molecules (Nelson and Cox, 2000a). Similarly, during cellular synthesis and metabolism of carbohydrates, intermediates can often be phosphorylated (Nelson and Cox, 2000b). Thus, during cell growth, P is incorporated into a broad spectrum of organic compounds that are lost from the cells to the surrounding environment through exudation and excretion (Karl and Björkman, 2002).

Bacteria are generally thought of as dissolved organic nitrogen (DON) consumers, but they can also be important producers and remineralisers of nitrogenous organic molecules

(Bronk, 2002). Studies of bulk DON in freshwater systems indicate that a large percentage of the DON pool is bioavailable on the time scale of days to weeks (Bronk, 2002). The increase in the number and magnitude of identified nitrogen-containing formulas upon microbial reworking in the present study is in agreement with the studies that show that a substantial fraction of DON in aquatic environments is of bacterial origin (Kaiser and Benner, 2008; McCarthy et al., 1998). The results of this study also concur with previous investigations on DOM alteration that reported increases in DON after microbial incubation (Kujawinski et al., 2004; Koch et al., 2014). Investigations on dissolved organic sulfur (DOS), specifically the production of organic S compounds by microorganisms, is rare especially in comparison with relatively vast amount of research on dissolved organic carbon (DOC) cycling in different environments. Nevertheless, organic S compounds have been recognized as both C and S sources for some microorganisms (Simó et al., 2002; Sievert et al., 2007).

5.3.3. Bioavailability of supraglacial DOM

According to Amon and Benner's (1996) continuum model, the bioavailability of DOM decreases along a continuum of diagenetic state. Heterotrophic bacteria preferentially utilise labile substrates such as carbohydrates and amino acids (Carlson and Ducklow, 1996; Baldock et al., 2004; Kawasaki et al., 2013), resulting in the formation of bio-recalcitrant chemical structures (Baldock et al., 2004; Smittenberg et al., 2006; Kawasaki et al., 2013) and thus contribute significantly to the pool of highly processed, bio-recalcitrant DOM in marine systems (Baldock et al., 2004; Ogawa et al., 2001; Kawasaki et al., 2013). Here, it was seen that the more 'labile' lipids, proteins, carbohydrates, as well as the supposedly 'recalcitrant' compounds such as tannin-like, lignin-like, CRAM, DBC and unsaturated hydrocarbons, were all rapidly degraded. The new products that were concomitantly generated were also rapidly consumed at the following time points. The formation of new DOM by bacteria and subsequent removal on short time scales differed from the earlier view that microbial utilisation and modification is the most important process for the formation of refractory DOM that persists for long time scales (Jiao et al., 2010). It is not known however, if the microbial activity in snow would eventually convert labile DOM to refractory matter on time scales beyond those achieved in the present field experiment. For example Koch et al., (2014) showed that in experimental incubations, Antarctic marine bacteria utilised simple substrates, such as glucose and generated new

DOM that after a period of 2 years had a composition similar to that of refractory DOM. On the other hand, incubations with DOM from fresh biomass resulted in a molecular composition that differed strongly from the refractory pattern even after 2 years of incubation (Koch et al., 2014). Studies of DOM in the deep ocean indicated that the most bio-refractory DOM exists as relatively small molecules of unknown chemical composition (Benner, 2002). Thus, it is also possible that the microbial reworking results in a refractory pool of small molecules that is beyond the analytical window and escape detection.

It is believed that DOM directly derived by bacteria or reworked microbial biomass is characterised by low C/N (Fukuda et al., 1998). Also, the C/N ratio is believed to be an important factor that affects decomposition of organic matter, with low C/N ratios thought to be associated with higher bioavailability and faster rates of decomposition (Hedges et al., 1997; Hunt et al., 2000). However, this study showed that bacteria rapidly degraded compounds with C/N ratios over this wide range contrary to the traditional view that low C/N ratio (<20) implies increased bioavailability.

5.3.4. Implications of microbial processing of DOM on supraglacial surfaces

The collective results from this study present strong evidence of rapid and extensive microbial alteration of DOM in supraglacial settings. This is consistent with the detection of various microbial groups in snow from this region that have been previously shown to have diverse metabolic capabilities such as the utilisation of a wide array of organic compounds including organic C, N, S and P compounds as well as recalcitrant aromatic compounds and plant polysaccharides such as cellulases, hemicellulases, tannin and lignin (section 4.3.5). The results of this study also correspond well with the isolation of bacteria from the Antarctic snow and ice, having a wide diversity of enzymatic activity such as amylolytic, lipolytic, proteolytic, lignolytic, cellulolytic, and oxidase activity etc., (Antony et al., 2009; Antony et al., 2012a; Yan et al., 2012; Shivaji et al., 2013). The findings of this study also corroborate with metagenomic and metaproteomic analyses of the Antarctic microbial communities which indicate that they may be potentially important in the cycling of C, N, and S (Williams et al., 2012; Shtarkman et al., 2013; Michaud et al., 2014). This study also showed that in addition to autochthonous material, terrestrial DOM formed a major source of biodegradable DOM in these environments and might be an important source of carbon and nutrients for supraglacial microbes. Current evidence

suggests that microbial decomposition of terrestrial DOM is one of the major pathways that fuels CO_2 emissions from the aquatic environment to the atmosphere (McCallister et al., 2012; Fasching et al., 2014). Given the ubiquity of terrestrially derived macromolecules on supraglacial surfaces (Grannas et al., 2004; Hood et al., 2009; Bhatia et al., 2010; Singer et al., 2012) and its degradability by microbes, it is quite likely that a fraction of these compounds may contribute to CO_2 outgassing from supraglacial surfaces.

While the importance of black carbon deposited on glacier surfaces on glacier melt rates and mass balance through absorption of solar radiation is well known (McConnell et al., 2007; Gabbi et al., 2015), it is not known how the DBC interacts with resident microbial communities. This study is significant, because it showed that supraglacial microbes is important in DBC cycling and thus has the capacity to greatly influence the DBC residence time on the glacier surface. Arguably, this could in turn influence the persistence of DBC on supraglacial surfaces and its effectiveness in influencing the surface albedo and accelerating glacier melting. Given the possible implications through impacts on surface albedo, the influence of microbial communities inhabiting supraglacial environments on the decomposition of DBC certainly requires consideration. This is the first time that molecular evidence for biodegradability of recalcitrant and terrestrially derived DOM has been demonstrated for Antarctic snowpacks. This study provides a significant step in contributing to the emerging concept that recalcitrance is not molecularly intrinsic, as such, but dependent on physiochemical and biological influences (Marín-Spiotta et al., 2014).

Additionally, shifts in the heteroatom composition of DOM after microbial reworking suggest that these processes may be important in the cycling of not only C but also other elements such as N, S and P. The vast majority of the bio-produced heteroatom containing compounds recorded in this study were bioavailable, suggesting that microbial reworking of supraglacial DOM can provide a new source of N, S and P for resident communities. For example, regeneration of P from DOP plays a significant role in supplying the P necessary for biological production (Karl and Bjorkman, 2001; Dyhrman et al., 2006) and in open-ocean ecosystems, local recycling of P supports approximately 90% of gross primary production (Karl, 2014).

Microbial processing of DOM has implications for not only aspects of carbon and elemental cycling in supraglacial environments, but also for DOM photochemistry.

Microbially processed DOM appears to be more photo-reactive, as evidenced by a higher degree of aromaticity and unsaturation (DBE-O >9) characteristic of photo-labile compounds (Stubbins et al., 2010; Gonsior et al., 2009, 2011). Following microbial transformation, 13% of the identified DOM formulas had characteristics similar to that of photo-labile DOM (DBE-O >9), compared to only 9% of the formulas in the initial DOM. In addition, the contribution of aromatic formulas to the total DOM pool increased by 43% in the 55-day time point. This suggests that microbial reworking may be an important mechanism for the production of photo-labile DOM. Such reworking may aid in faster and more extensive degradation of DOM on the glacier surface during the summer when much of the glacier surface is exposed to longer hours of direct sunlight. A schematic diagram showing microbial processes involved in the transformation of DOM on the ice sheet is given in Figure 5.7.

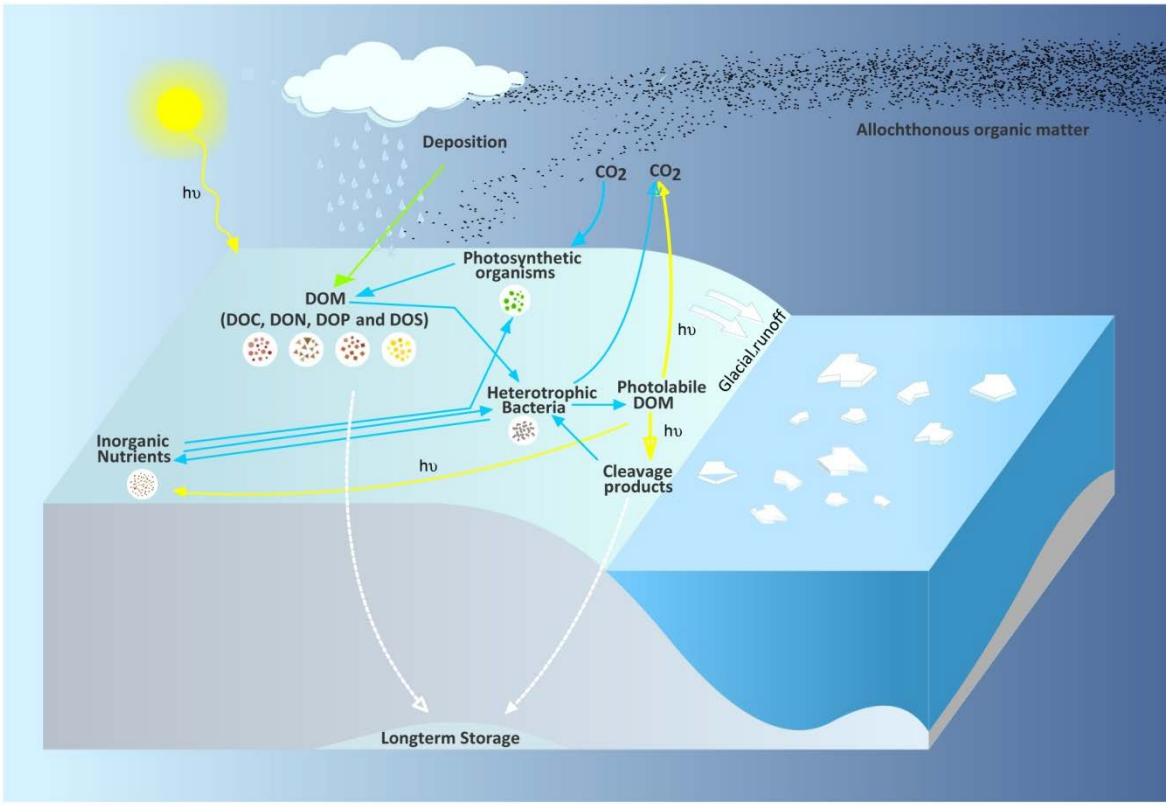

Figure 5.7. *A schematic diagram showing transformation of DOM on the ice sheet. All microbially mediated processes (involving Bacteria, Archaea and Eukarya) are shown in blue, while photochemically influenced processes are shown in yellow.*

Briefly, allochthonous as well as organic material produced by photosynthetic organisms which include DOC, DON, DOS and DOP are mineralised, and transformed by the

resident microbial communities. Heterotrophic bacteria also contribute to the remineralisation of DOM to inorganic forms, which are then available for use by autotrophic communities. Photochemical processing of photo-labile DOM (formed during microbial transformation of DOM) could release LMW organics, N- and P-rich compounds (Bushaw-Newton et al., 1996, Moran and Zepp, 1997; Bushaw-Newton and Moran, 1999) that are biologically available, as well as inorganic ions such as NH_4^+, NO_2^-, thereby stimulating the growth and activity of both heterotrophic and autotrophic organisms (Vähätalo and Järvinen, 2007; Lindell et al., 1995; Wetzel et al., 1995; Bushaw et al., 1996). Finally, DOM that resists degradation contributes to carbon storage in the ice sheets until melting processes remove the stored carbon from ice and transport it to downstream ecosystems where it may impact the ecology of these environments.

Therefore, microbial processes are an important mechanism for cycling of supraglacial DOM and may play a crucial role in determining the composition of bulk DOM in this environment. While this study focused on the East Antarctic ice sheet, the processes investigated here under *in situ* conditions should be generally applicable across different supraglacial environments, given the ubiquity of autochthonous and allochthonous DOM on glacier and ice sheet surfaces. A comprehensive compositional and structural characterisation of DOM from different glacier environments using powerful tools such as FTICR-MS and high field NMR would aid in a better understanding of specific DOM molecules that participate in biogeochemical processes or that are specifically linked to glacier ecosystems or microbial metabolism. This will ultimately lead to novel insights into the supraglacial carbon cycle and help in identifying markers for microbially modified DOM. This is especially relevant as annual release of DOC from the AIS to downstream marine ecosystems is significant (Hood et al., 2015) and is expected to continue increasing in coming decades (Sheperd et al., 2012; Rignot et al., 2013) with unforeseen impacts on marine food webs.

CHAPTER 6
Summary and Conclusions

It is only recently that Antarctica has been recognised as a biome that forms an integral part of the global climate system with important linkages and feedbacks (Goodison et al., 1999). The Antarctic Ice Sheet (AIS) harbours a globally relevant pool of cellular and dissolved organic carbon (Priscu et al., 2008) with potential impacts on global carbon dynamics. The biogeochemical significance of dissolved organic matter (DOM) for supraglacial systems as well as downstream ecosystems that receive supraglacial runoff would largely depend on its availability for resident microbial communities and on the concentration, chemical nature and overall composition of the DOM. Therefore, an increased understanding of the amount and nature of organic matter associated with the AIS is of utmost importance. Despite its importance in biogeochemical cycling of carbon and global carbon dynamics at large, very little is known on the distribution and sources of organic carbon in the vast and climate sensitive landscape of the AIS (Lyons et al., 2007; Federer et al., 2008). In particular, data (quantitative and qualitative) on snowpack DOM and microbial communities as well as the impact of microbial processes on DOM composition are severely limited. Consequently, the carbon dynamics underpinning this ecosystem remains poorly understood. These observations highlight the need for quantifying and also identifying the sources, distribution and reactivity of organic carbon as well as the processes by which DOM is transformed through microbial communities on the AIS surface. The overarching goal of the Ph.D. research therefore is to characterise DOM and microbial communities on the surface of the AIS and to explore the interaction between microbes and the wide spectrum of molecules that comprises the highly complex DOM pool.

In order to meet this goal, measurements of total organic carbon (TOC) using high sensitivity Total Organic Carbon analyser, ionic composition using Ion Chromatography and dust concentrations using Coulter Counter were carried out. An assessment of the abundance and diversity of microbial communities associated with the AIS was also made, using culture-based and culture-independent studies for understanding the microbial communities present. *In situ* experiments were performed through a combination of field studies and ultrahigh resolution mass spectrometry, to gain insights into the interaction between microbes and individual molecules within DOM and to determine how DOM is transformed through microbial communities on the AIS surface. These analyses were carried out systematically along a 180 km traverse perpendicular to the coast in the Princess Elizabeth Land in East Antarctic region.

The salient findings of this study are -

- TOC concentrations in snow samples collected along a 180 km traverse in the Princess Elizabeth land region of East Antarctica ranged from 88 ± 4 to 928 ± 21 μg L^{-1} (mean 259 μg L^{-1}) and exhibited considerable spatial variation with significantly higher values in the coastal site ($p < 0.001$).
- Different sources contributed to the spatial variation of organic carbon in the surface snow. One of these was sea spray whose contribution was more significant in the coastal sites. Sea-salt Na^+ (considered the most conservative ionic proxy for sea spray in coastal Antarctica) showed a spatial trend similar to that of TOC with significantly higher ($p < 0.001$) values at the coastal sites with strong positive ($p < 0.001$) correlation with TOC. This suggests that sea spray may have contributed to the organic carbon load in these samples.
- The information obtained from analysis of bulk parameters was consistent with the results from molecular-level analysis of organic matter. Molecular characterisation showed that DOM was chemically more diverse in samples that were closer to the coast, with over 1600 formulas comprising of C, H, O, N, S and P that were unique to the coastal end member of the sampling transect and was not present in any other sample. In contrast, the inland site had only 240 formulas that were unique, while all other formulas were commonly detected in other surface snow samples. The high diversity of molecular formulas in the coastal samples point to the possibility of the ocean being an important source of DOM to these sites. In addition, CHOS and CHONS compounds progressively decreased from 16 to 8% and 20 to 4%, respectively from coast to inland. The increased heteroatom functionality (N and S contents) observed in the coastal snow samples corresponded well with the earlier studies that have reported higher contributions of N and S containing formulas in marine DOM and enrichment of CHON and CHOS compounds in sea-spray aerosols. Thus, it is apparent that the ocean could be an important source of DOM to the Antarctic snowpack.
- In addition to the marine contribution, *in situ* microorganisms (cellular carbon pool) also accounted for a fraction of the organic carbon. Molecular level characterisation of snowpack DOM showed that aliphatic molecules from microbial and algal biomass comprised about 37-52% of the total formulas assigned for each sample. This indicates that most of the identified supraglacial DOM components originated from the *in situ* microbial activity. This corroborates with the presence of bacteria and diverse

consortia of microalgae in the snow. Total cell numbers ranged from 9.43×10^3 to 9.27×10^4 cells mL^{-1} and accounted for 104-1351 ng carbon L^{-1} (mean 365 ng C L^{-1}). Though this accounted for only a small fraction of the total carbon, when these values were used in computing the microbial cell carbon for the AIS (3.01×10^7 km^3), it equaled to about 1.1×10^{13} g C, an order higher than that of all the world's surface fresh waters.

- In addition, tannin and lignin together accounted for 33-38% of the total identified formulas in each sample, indicating the contribution of terrestrial organic matter in the snow.
- The molecular fingerprint obtained together with back trajectory analysis showed that the dissolved black carbon-like material apparently originating from biomass burning in South America was also present, while a smaller fraction was originating from the soil humics and appears to be photochemically or microbially modified.
- In addition to remote continental sources, signals of oceanic emissions of primary aerosols and secondary organic aerosol precursors were also documented. Accordingly, the snowpack DOM composition appeared to be influenced by the variability in deposited atmospheric species derived from the secondary organic aerosols.
- Based on the existing measurements of DOM in the AIS (Christner et al., 2006; Priscu et al., 2008) and the available data of annual ice loss from the continent, it was estimated that ice loss from Antarctica would release ~32 Gg (0.32×10^{11} g) of carbon annually to the coastal waters of the Southern Ocean, implying that the AIS is a quantitatively important source of organic carbon to marine ecosystems.
- A significant fraction of the supraglacial DOM was potentially highly bioavailable as 22−39% of the total identified formulas had low C/N ratios (≤20). Thus, Antarctic snowpacks are important sources of potentially labile terrestrial and microbial DOM to downstream ecosystems.
- Assessment of the microbial communities associated with the Antarctic snowpack revealed the presence of microbes from 7 major lineages of the domain bacteria: Proteobacteria (includes α-, β-, γ-, and δ-Proteobacteria), Actinobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Verrucomicrobia, Deinococcus-Thermus, 1 major lineage of the domain Archaea (Euryarchaeota) and 4 major lineages of the domain eukaryota: Basidiomycota, Ascomycota, Cryptomycota and Rhizaria.

- Most of the genera recorded in this study are ubiquitous and have been earlier reported from Antarctica, Greenland as well as other non-polar ice regions.
- *Galbitalea* sp. identified in this study is the first report from Antarctica as this genus has so far not been reported from any polar or non-polar glacier environments.
- Clear differences were observed in the microbial communities isolated from different samples from the coast to inland, both in culture-dependent and culture-independent approaches. Higher microbial diversity of 27 genera was observed in the coastal site compared to 16 and 17 genera recorded in the mid- (80 km from coast) and inland (180 km from coast) sites, respectively. The microbial community composition at the coastal, midland and inland sites along the traverse was different except for 2 genera: *Mucilaginibacter* and *Sphingomonas* which were recorded in all three sites. Out of the total 46 genera, 21 genera were recorded only in the coastal site, whereas 11 and 10 genera were recorded only in the midland and inland sites, respectively.
- The high diversity of microbial communities in the coastal site can be attributed to the substantial input of airborne microbes originating from marine aerosols. This is further corroborated by the clear dominance of halophilic microbes such as Halobacteriaceae in the coastal site.
- Majority of the microbial communities identified were related to species known for their role in biogeochemical cycling of C, N, S and P.
- Molecular-level assessment of DOM transformation through microbial communities on the surface of the AIS under *in situ* conditions showed strong evidence of rapid (order of days) and extensive microbial alteration of both autochthonous and allochthonous DOM. Microbes rapidly degraded compounds with C/N ratios over a wide range of 2-78 contrary to the traditional view that low C/N ratio (<20) implies increased bioavailability.
- The supraglacial DOM was rapidly processed by microbial communities through parallel processes of degradation and synthesis of both low and high molecular weight (MW 200-1190) DOM, leading to extensive molecular alteration through changes in chemistry and elemental composition.
- Highly refractory compounds such as dissolved black carbon and carboxylic-rich alicyclic molecules were rapidly and extensively reworked by resident microbial communities. This study provided the first molecular evidence for biodegradability of recalcitrant and terrestrially derived DOM in the Antarctic snow.

- The shifts in the heteroatom composition of DOM suggest that microbial processes may be important in the cycling of not only C but other elements such as N, S and P. The vast majority of the bio-produced heteroatom containing compounds recorded in this study were bioavailable, suggesting that microbial reworking of supraglacial DOM can provide a new source of N, S and P for resident communities. Microbial reworking also resulted in production of photo-reactive DOM molecules with consequent implications for DOM photochemistry. Thus, the biogeochemistry of DOM is highly complex and intimately connected with microbial processes.

Conclusions

This study showed that the AIS that has an immense diversity and quantity of both microbes and DOM. The distinct and diverse microbial assemblages were bacteria, eukaryotes and Archaea; with strong representation from bacterial Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes; archaeal Euryarchaeota; and eukaryotic Basidiomycota and Ascomycota. Microbial community composition was distinctly different from the coast to inland, with higher microbial diversity in the coastal samples, some of which resembled marine assemblages, suggesting that this environment is predominantly seeded by wind-transported marine sources. The diverse DOM on the surface of the AIS was from *in situ* primary production as well as from deposition of organic material derived from marine environment, secondary organic aerosols and long range atmospheric transport of terrestrial and anthropogenic organic matter. Autochthonous as well as terrestrial DOM represented by aliphatic, aromatic and condensed aromatic compounds were all rapidly (order of days) and extensively reworked by resident microbial communities. Shifts in the heteroatom composition suggest that microbial processes may be important in the cycling of not only C, but also other elements such as N, S and P. Therefore, the *in situ* microbial processes mediated by parallel processes of degradation and synthesis are important mechanisms for cycling of supraglacial DOM and may also be playing a crucial role in determining the composition of the bulk DOM in these environments.

This study points out to an underestimation of the effects of microbial processing of DOM on the ice sheet surface as nearly all the identified labile as well as generally considered recalcitrant DOM compounds were found to be microbially transformed. Thus, much of the DOM present on the surface of the AIS could be readily transformed into presumably

more labile and/or otherwise reactive compounds. Given the ubiquity of autochthonous and allochthonous DOM on the ice sheet surface, the processes investigated here under *in situ* conditions could be generally applicable across different supraglacial environments. The novel insights into new and unknown interactions between microbes and specific components of the DOM pool highlight the relevance of microbes for supraglacial DOM cycling – a role that should be seriously considered given the vast expanse of polar ice and climate-driven increase in downstream export of DOM.

Future scope of the study

Future work envisages more focused experiments, integrating DOM characterisation along with measurements of concentrations and fluxes of organic compounds, inorganic ions and trace gases vis-a-vis the bacterial activity and diversity. This will provide an in depth understanding of the sources and sinks of DOM as well as the interaction between microbes and specific molecules that make up the DOM pool. In addition, a comprehensive compositional and structural characterisation of DOM from different supraglacial environments using powerful tools such as FTICR-MS and high field NMR would aid in better understanding of specific DOM molecules that participate in biogeochemical processes or specifically linked to glacier ecosystems or microbial metabolism. This would ultimately lead to novel insights into the supraglacial carbon cycle and help in identifying markers for microbially modified DOM. Such studies would help in elucidating the important connections and drivers of organic matter dynamics as well as the regional and/or global changes that might be occurring now and in near future.

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Appendix

Appendix

Table S1. Isolation conditions, phenotypic description and phylogenetic relationship of isolates recovered from surface snow.

Sample site	Isolate	Closest Relative	% Identity	Isolation media	Isolation temperature	Description
Coast	L1c	*Variovorax* sp.	95.6	R2A	20°C	pale yellow/opaque/smooth
	L1o	*Variovorax* sp.	98.3	R2A	20°C	yellow/translucent/flat with raised centre
	L1f	*Microbacterium hatanon*is JCM 14558(T)	99.0	10% TSA	20°C	cream/translucent/smooth
	L1g	*Sphingomonas glacialis* C16y(T)	99.5	1% NA	20°C	pale yellow/translucent/smooth
	L1j	*Sphingomonadales*	92.1	1% NA	20°C	yellow/translucent/smooth
	L1a	*Bacillus* sp.	98.4	10% NA	20°C	white/opaque/smooth
	L1h	*Lysinibacillus macroides* LMG 18474(T)	99.3	10% NA	20°C	white/translucent/ smooth
	L1k	*Bacillus* sp.	97.9	25% NA	20°C	white /opaque/filamentous/smooth
	L1m	*Bacillus* sp.	97.0	25% NA	20°C	white /opaque/ filamentous/smooth
	L1Cb	*Microbacterium* sp.	98.0	R2A	4°C	cream/translucent/smooth
	L18j	*Pigmentiphaga* sp.	97.7	R2A	20°C	pale yellow/translucent/smooth
	L18g	*Marisediminicola* sp..	97.9	R3A	20°C	cream/translucent/smooth
	L18e	*Polaromonas* sp.	98.1	ABM	20°C	white/translucent/smooth
	L18f	*Pigmentiphaga* sp.	98.4	1% NA	20°C	white/translucent/smooth
	L18b	*Microbacterium oxyda*ns DSM 20578(T)	99.5	10% NA	20°C	white/translucent/smooth
	L18k	*Galbitalea* sp.	96.7	25% NA	20°C	yellow/translucent/smooth
	L18l	*Rhodopseudomonas* sp.	98.0	25% NA	20°C	white/translucent/smooth
	L18d	*Rhodococcus* sp.	97.3	1% TSA	20°C	white/translucent/smooth
	L18i	*Mycobacterium* sp.	97.9	10% TSA	20°C	cream/translucent/smooth
	L18m	*Mycobacterium* sp.	96.9	25% TSA	20°C	yellow-orange/opaque/smooth
	L18n	*Afipia broomeae* ATCC 49717(T)	98.7	25% TSA	20°C	white/translucent/smooth
	L18h	*Microbacterium pumilum* KV-488(T)	99.5	10% ZMA	20°C	white/translucent/dry
	L18o	*Mycobacterium* sp.	98.4	10% ZMA	20°C	yelllow-orange/translucent/smooth
	L18p	*Cryptococcus* sp.	98.0	10% ZMA	20°C	white/opaque/smooth
	L1Ca	*Sphingomonas* sp.	98.0	ABM	20°C	white/translucent/smooth/

Sample site	Isolate	Closest Relative	% Identity	Isolation media	Isolation temperature	Description
Coast	L1Cc	*Microbacterium* sp.	97.0	10% ZMA	20°C	white/translucent/smooth/
	L18Ce	*Sphingomonas* sp.	97.7	10%TSA	4°C	white/translucent/smooth
	L18Ca	*Pedobacter* sp.	97.8	10% NA	4°C	cream/translucent/smooth/
	L18Cd	*Rhodococcus erythropolis* DSM 43066(T)	99.0	1% TSA	4°C	white/translucent/smooth
	L18Cb	*Stenotrophomonas rhizophila* e-p10(T)	99.0	10% ZMA	4°C	white/translucent/smooth
	L18Cg	*Stenotrophomonas* sp.	97.9	25% ZMA	4°C	white/opaque/smooth
	L18Cf	*Stenotrophomonas* sp.	98.2	25% ZMA	4°C	white/opaque/smooth
	P2Ca	*Cryptococcus victoriae* CBS 8915	100.0	25% TSA	4°C	white/opaque/smooth
Midland	P8d	*Sphingomonas aquatilis* JSS7(T)	99.3	R2A	20°C	yellow/translucent/smooth
	P8e	*Sphingomonas aquatilis* JSS7(T)	98.7	R3A	20°C	yellow/opaque/smooth
	P8c	*Sphingomonas melonis* DAPP-PG 224(T)	99.4	1% NA	20°C	white/translucent/smooth
	P8a	*Sphingomonas aquatilis* JSS7(T)	99.3	10% NA	20°C	yellow/opaque/smooth
	P8m	*Sphingomonas* sp.	97.0	10% NA	20°C	yel/translucent/smooth
	P8i	*Sphingomonas aquatilis* JSS7(T)	98.5	25% NA	20°C	yellow/translucent/smooth
	P8n	*Sphingomonas* sp.	95.9	1% TSA	20°C	cream/opaque/smooth
	P8j	*Sphingomonas* sp.	98.6	10% TSA	20°C	yellow/opaque/smooth
	P8k	*Sphingomonas aquatilis* JSS7(T)	99.7	25% TSA	20°C	yellow/translucent/smooth
	P8b	*Sphingomonas aquatilis* JSS7(T)	99.1	25% TSA	20°C	yellow/opaque/smooth
	P8q	*Sphingomonas aquatilis* JSS7(T)	99.2	10% ZMA	20°C	pale yellow/opaque/smooth
	P8p	*Sphingomonas aquatilis* JSS7(T)	99.3	25% ZMA	20°C	yel/translucent/smooth
	P8g	*Sphingomonas* sp.	96.7	ABM	20°C	yellow/opaque/smooth
	P8Cc	*Sphingomonas glacialis* C16y(T)	98.8	R2A	4°C	yellow/translucent/smooth
	P8Cg	*Sphingomonas* sp.	97.7	R3A	4°C	yellow/opaque/smooth
	P8Cf	Sphingomonas sp.	98.5	ABM	4°C	yellow/opaque/smooth
	P8Ck	Sphingomonas sp.	98.3	1% NA	4°C	pale yellow/translucent/smooth
	P8Ch	*Sphingomonas* sp.	98.6	10% TSA	4°C	yellow/opaque/smooth
	P8Cj	*Sphingomonas* sp.	98.1	10% NA	4°C	yellow/opaque/smooth
	P8Ca	*Sphingomonas* sp.	97.2	25% NA	4°C	pale yellow/translucent/smooth
	P8Cb	*Sphingomonas* sp.	98.5	25% NA	4°C	white/translucent/smooth

Sample site	Isolate	Closest Relative	% Identity	Isolation media	Isolation temperature	Description
Midland	P8Cl	*Sphingomonas glacialis* C16y(T)	98.9	1% TSA	4°C	pale yellow/translucent/smooth
	P8Ci	*Sphingomonas glacialis* C16y(T)	99.6	10% TSA	4°C	yellow/opaque/smooth
	P8Ce	*Sphingomonas glacialis* C16y(T)	99.0	25% TSA	4°C	cream/translucent/smooth/
Inland	P18o	*Cryptococcus victoriae* strain P41A001	98.0	ABM	20°C	white/opaque/smooth
	P18m	*Cryptococcus* sp.	96.0	ABM	20°C	white/opaque/smooth/mucoid
	P18h	*Cryptococcus victoriae* strain P41A001	99.0	ABM	20°C	white/opaque/smooth
	P18e	*Rhizobium* sp.	98.5	R2A	20°C	white/opaque/smooth/mucoid
	P18f	*Sphingomonas* sp.	98.1	R2A	20°C	yellow/opaque/smooth
	P18b	*Moraxella osloensis* NCTC 10465(T)	98.8	R3A	20°C	white/opaque/smooth/mucoid
	P18n	*Cryptococcus* sp.	98.0	1% NA	20°C	white/opaque/smooth
	P18d	*Microbacterium xylanilyticum* S3-E(T)	99.0	25% NA	20°C	cream/translucent/smooth/mucoid
	P18a	*Cryptococcus victoriae* strain P41A001	99.0	1% TSA	20°C	white/opaque/smooth
	P18s	*Sphingomonas* sp.	98.5	1% TSA	20°C	pale yellow/trans/smooth
	P18k	*Sphingomonas* sp.	98.0	1% TSA	20°C	cream/translucent/smooth
	P18p	*Cryptococcus victoriae* strain P41A001	99.0	1% TSA	20°C	white/opaque/smooth
	P18t	*Sphingomonas* sp.	98.6	1% TSA	20°C	yellow/trans/smooth
	P18c	*Acinetobacter* sp.	98.3	10% TSA	20°C	white/translucent/smooth
	P18q	*Pseudomonas* sp.	97.0	25% TSA	20°C	white/opaque/smooth
	P18r	*Cryptococcus* sp.	97.0	25% TSA	20°C	white/opaque/smooth
	P18l	*Brevundimonas olei* MJ15(T)	98.9	10% ZMA	20°C	white/translucent/smooth
	P18Cb	*Cryptococcus* sp.	98.0	R2A	4°C	white/opaque/smooth
	P18Cj	*Cryptococcus victoriae* strain P41A001	99.0	ABM	4°C	white/opaque/smooth
	P18Ca	*Cryptococcus victoriae* strain P41A001	99.0	1% NA	4°C	cream/translucent/smooth
	P18Cg	*Cryptococcus victoriae* strain P41A001	99.0	10% NA	4°C	white/opaque/smooth
	P18Ce	*Cryptococcus victoriae* strain P41A001	99.0	25% NA	4°C	white/opaque/smooth
	P18Cd	*Tremellales*	94.0	1% TSA	4°C	white/translucent/smooth
	P18Cc	*Cryptococcus victoriae* strain P41A001	99.0	10% TSA	4°C	white/opaque/smooth
	P18Ci	*Cryptococcus victoriae* strain P41A001	99.0	25% ZMA	4°C	white/opaque/smooth

Table S2. List of 100 representative molecular formulas (of a total of 2458) identified in the snowpack that were bio-produced in the dark incubations during the different time points.

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
203.10666	0.02	CHO	0.15	1.08	7	5	0.50	C13H14N0O2S0P0
203.12778	0.07	CHO	0.40	1.80	2	-2	0.00	C10H18N0O4S0P0
203.14304	0.19	CHO	0.07	1.29	6	5	0.41	C14H18N0O1S0P0
205.08592	0.15	CHO	0.25	1.00	7	4	0.52	C12H12N0O3S0P0
205.12231	0.07	CHO	0.15	1.23	6	4	0.42	C13H16N0O2S0P0
207.13796	0.03	CHO	0.15	1.38	5	3	0.33	C13H18N0O2S0P0
207.13796	0.03	CHO	0.15	1.38	5	3	0.33	C13H18N0O2S0P0
207.17434	0.04	CHO	0.07	1.57	4	3	0.26	C14H22N0O1S0P0
211.09648	0.07	CHO	0.36	1.27	5	1	0.33	C11H14N0O4S0P0
211.13287	0.00	CHO	0.25	1.50	4	1	0.24	C12H18N0O3S0P0
211.20564	0.00	CHO	0.07	1.86	2	1	0.11	C14H26N0O1S0P0
213.07334	0.29	CHO	0.63	1.75	2	-3	0.00	C8H14N0O5S0P0
213.07575	0.00	CHO	0.50	1.20	5	0	0.33	C10H12N0O5S0P0
213.11213	0.07	CHO	0.36	1.45	4	0	0.22	C11H16N0O4S0P0
213.18491	0.03	CHO	0.15	1.85	2	0	0.08	C13H24N0O2S0P0
215.08899	0.05	CHO	0.63	2.00	1	-4	0.00	C8H16N0O5S0P0
215.10666	0.03	CHO	0.14	1.00	8	6	0.54	C14H14N0O2S0P0
215.16417	0.00	CHO	0.25	1.83	2	-1	0.05	C12H22N0O3S0P0
217.03428	0.22	CHO	0.88	1.00	5	-2	0.33	C8H8N0O7S0P0
217.06826	0.02	CHO	0.86	2.00	1	-5	0.00	C7H14N0O6S0P0
217.06826	0.16	CHO	0.86	2.00	1	-5	0.00	C7H14N0O6S0P0
217.10705	0.09	CHO	0.50	1.60	3	-2	0.07	C10H16N0O5S0P0
217.12231	0.07	CHO	0.14	1.14	7	5	0.46	C14H16N0O2S0P0
217.12231	0.07	CHO	0.14	1.14	7	5	0.46	C14H16N0O2S0P0
217.14343	0.16	CHO	0.36	1.82	2	-2	0.00	C11H20N0O4S0P0
217.14343	0.11	CHO	0.36	1.82	2	-2	0.00	C11H20N0O4S0P0
219.10157	0.18	CHO	0.23	1.08	7	4	0.48	C13H14N0O3S0P0
219.12270	0.18	CHO	0.50	1.80	2	-3	0.00	C10H18N0O5S0P0
219.13796	0.16	CHO	0.14	1.29	6	4	0.38	C14H18N0O2S0P0
219.15908	0.11	CHO	0.36	2.00	1	-3	0.00	C11H22N0O4S0P0
202.06110	0.23	CHON	0.20	0.70	9	7	1.08	C10H7N3O2S0P0
209.16484	0.00	CHON	0.08	1.67	4	3	0.26	C12H20N2O1S0P0
220.13320	0.16	CHON	0.15	1.31	6	4	0.41	C13H17N1O2S0P0
226.14377	0.05	CHON	0.25	1.58	4	1	0.21	C12H19N1O3S0P0
230.17507	0.05	CHON	0.25	1.92	2	-1	0.00	C12H23N1O3S0P0
232.12918	0.47	CHON	0.44	1.89	3	-1	0.00	C9H17N3O4S0P0
238.12264	0.04	CHON	0.06	0.94	10	9	0.62	C16H15N1O1S0P0
241.19105	0.32	CHON	0.15	1.85	3	1	0.10	C13H24N2O2S0P0
242.13868	0.14	CHON	0.33	1.58	4	0	0.17	C12H19N1O4S0P0
242.24784	0.08	CHON	0.07	2.07	1	0	0.00	C15H31N1O1S0P0
241.02775	0.36	CHONS	0.44	0.89	7.5	4	0.75	C9H8N2O4S1P0
246.05942	-0.86	CHONS	0.15	1.00	8	6	0.55	C13H13N1O2S1P0
246.12707	0.99	CHONS	0.20	1.90	3.5	2	0.00	C10H19N3O2S1P0

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
250.05324	0.36	CHONS	0.25	0.92	8.5	6	0.59	C12H11N1O3S1P0
250.12938	0.37	CHONS	0.09	2.09	2	1	0.00	C11H23N1O1S2P0
251.06962	0.03	CHONS	0.63	1.75	3.5	-2	0.00	C8H14N2O5S1P0
255.04100	0.53	CHONS	0.50	1.50	4.5	1	0.00	C8H12N2O4S1P0
259.03832	0.32	CHONS	0.56	1.11	6.5	2	0.43	C9H10N2O5S1P0
259.03832	0.28	CHONS	0.56	1.11	6.5	2	0.43	C9H10N2O5S1P0
264.14503	0.91	CHONS	0.08	2.08	2	1	0.00	C12H25N1O1S2P0
325.17855	-0.73	CHOP	0.43	2.21	0	-6	0.00	C14H31N0O6S0P1
329.15233	0.57	CHOP	0.31	1.69	4	-1	0.08	C16H27N0O5S0P1
333.22002	0.18	CHOP	0.24	2.06	1	-3	0.00	C17H35N0O4S0P1
347.19928	0.51	CHOP	0.29	1.94	2	-3	0.00	C17H33N0O5S0P1
347.19928	0.45	CHOP	0.29	1.94	2	-3	0.00	C17H33N0O5S0P1
349.21493	0.16	CHOP	0.29	2.06	1	-4	0.00	C17H35N0O5S0P1
351.23058	0.45	CHOP	0.29	2.18	0	-5	0.00	C17H37N0O5S0P1
353.13120	0.78	CHOP	0.14	1.10	11	8	0.49	C21H23N0O3S0P1
353.13120	0.58	CHOP	0.14	1.10	11	8	0.49	C21H23N0O3S0P1
355.20437	0.43	CHOP	0.21	1.74	4	0	0.09	C19H33N0O4S0P1
312.17340	0.15	CHOPN	0.19	1.75	4.00	1	0.12	C16H28N1O3S0P1
312.17340	0.31	CHOPN	0.19	1.75	4	1	0.12	C16H28N1O3S0P1
332.23600	0.47	CHOPN	0.18	2.12	1	-2	0.00	C17H36N1O3S0P1
346.21527	0.24	CHOPN	0.24	2.00	2	-2	0.00	C17H34N1O4S0P1
348.23092	0.52	CHOPN	0.24	2.12	1	-3	0.00	C17H36N1O4S0P1
362.21018	0.49	CHOPN	0.29	2.00	2	-3	0.00	C17H34N1O5S0P1
364.22583	0.46	CHOPN	0.29	2.12	1	-4	0.00	C17H36N1O5S0P1
366.16871	0.49	CHOPN	0.47	2.00	2	-5	0.00	C15H30N1O7S0P1
378.20510	0.62	CHOPN	0.35	2.00	2	-4	0.00	C17H34N1O6S0P1
380.22075	0.48	CHOPN	0.35	2.12	1	-5	0.00	C17H36N1O6S0P1
326.06214	-0.12	CHOPNS	0.29	1.29	7	3	0.33	C14H18N1O4S1P1
358.12474	0.51	CHOPNS	0.25	1.63	5	1	0.09	C16H26N1O4S1P1
478.08835	-0.76	CHOPNS	0.20	0.88	16	11	0.59	C25H22N1O5S1P1
530.11965	-0.10	CHOPNS	0.17	0.90	18	13	0.57	C29H26N1O5S1P1
557.99603	-0.03	CHOPNS	1.06	1.13	9	-8	0.00	C16H18N1O17S1P1
558.03241	0.24	CHOPNS	0.94	1.29	8	-8	0.00	C17H22N1O16S1P1
563.94320	-0.81	CHOPNS	0.50	0.33	22.00	10	0.93	C24H8N1O12S1P1
644.33915	0.47	CHOPNS	0.25	1.75	6	-2	0.00	C32H56N1O8S1P1
664.04140	-0.11	CHOPNS	0.12	0.39	35	30	0.86	C41H16N1O5S1P1
668.28163	0.75	CHOPNS	0.19	1.33	14	7	0.29	C36H48N1O7S1P1
325.10327	0.94	CHOPS	0.19	1.44	6	3	0.24	C16H23N0O3S1P1
325.10327	0.72	CHOPS	0.19	1.44	6	3	0.24	C16H23N0O3S1P1
353.13457	0.67	CHOPS	0.17	1.50	6	3	0.21	C18H27N0O3S1P1
359.14514	0.56	CHOPS	0.24	1.71	4.00	0	0.04	C17H29N0O4S1P1
367.17135	-0.26	CHOPS	0.31	2.06	1	-4	0.00	C16H33N0O5S1P1
413.15570	0.64	CHOPS	0.25	1.55	6	1	0.13	C20H31N0O5S1P1
417.17175	-0.79	CHOPS	0.50	2.19	0	-8	0.00	C16H35N0O8S1P1
419.05124	-0.98	CHOPS	0.17	0.74	16	12	0.66	C23H17N0O4S1P1
439.01994	0.69	CHOPS	0.16	0.52	20	16	0.79	C25H13N0O4S1P1
439.11971	-0.89	CHOPS	0.53	1.71	4	-5	0.00	C17H29N0O9S1P1

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
217.16206	0.47	CHOS	0.08	2.00	1.5	1	0.00	C12H24N0O1S1P0
229.16206	0.01	CHOS	0.08	1.85	2.5	2	0.04	C13H24N0O1S1P0
241.16206	0.03	CHOS	0.07	1.71	3.5	3	0.12	C14H24N0O1S1P0
245.09946	0.28	CHOS	0.07	1.07	8.5	8	0.48	C15H16N0O1S1P0
245.19336	0.21	CHOS	0.07	2.00	1.5	1	0.00	C14H28N0O1S1P0
273.03782	0.17	CHOS	0.08	1.15	8	7	0.43	C13H14N0O1S2P0
273.03782	0.28	CHOS	0.08	1.08	8	7	0.43	C13H14N0O1S2P0
277.21958	0.81	CHOS	0.13	2.13	0.5	-2	0.00	C15H32N0O2S1P0
279.12717	-0.03	CHOS	0.42	2.00	1	-4	0.00	C12H24N0O5S1P0
281.04892	-0.06	CHOS	0.38	1.08	7	2	0.37	C13H14N0O5S1P0

Table S3. List of 100 representative molecular formulas (of a total of 3933 formulas) identified in the snowpack that were bio-labile.

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
201.11213	0.07	CHO	0.40	1.60	3	-1	0.13	C10H16N0O4S0P0
201.12739	0.09	CHO	0.07	1.14	7	6	0.48	C14H16N0O1S0P0
203.10666	0.02	CHO	0.15	1.08	7	5	0.50	C13H14N0O2S0P0
203.12778	0.07	CHO	0.40	1.80	2	-2	0.00	C10H18N0O4S0P0
203.14304	0.19	CHO	0.07	1.29	6	5	0.41	C14H18N0O1S0P0
205.08592	0.15	CHO	0.25	1.00	7	4	0.52	C12H12N0O3S0P0
205.12231	0.02	CHO	0.15	1.23	6	4	0.42	C13H16N0O2S0P0
205.12231	0.03	CHO	0.15	1.23	6	4	0.42	C13H16N0O2S0P0
207.13796	0.07	CHO	0.15	1.38	5	3	0.33	C13H18N0O2S0P0
207.13796	0.03	CHO	0.15	1.38	5	3	0.33	C13H18N0O2S0P0
207.17434	0.04	CHO	0.07	1.57	4	3	0.26	C14H22N0O1S0P0
211.10933	0.22	CHO	0.08	1.23	6	5	0.44	C13H16N0O1S0P0
211.13046	0.06	CHO	0.30	2.00	1	-2	0.00	C10H20N0O3S0P0
211.13287	0.00	CHO	0.25	1.50	4	1	0.24	C12H18N0O3S0P0
211.20564	0.00	CHO	0.07	1.86	2	1	0.11	C14H26N0O1S0P0
213.07334	0.47	CHO	0.63	1.75	2	-3	0.00	C8H14N0O5S0P0
213.07575	0.04	CHO	0.50	1.20	5	0	0.33	C10H12N0O5S0P0
213.07575	0.00	CHO	0.50	1.20	5	0	0.33	C10H12N0O5S0P0
213.11213	0.16	CHO	0.36	1.45	4	0	0.22	C11H16N0O4S0P0
213.11213	0.02	CHO	0.36	1.45	4	0	0.22	C11H16N0O4S0P0
213.12739	0.05	CHO	0.07	1.07	8	7	0.52	C15H16N0O1S0P0
213.14611	0.04	CHO	0.30	2.20	0	-3	0.00	C10H22N0O3S0P0
213.14852	0.05	CHO	0.25	1.67	3	0	0.14	C12H20N0O3S0P0
213.18491	0.03	CHO	0.15	1.85	2	0	0.08	C13H24N0O2S0P0
215.08899	0.05	CHO	0.63	2.00	1	-4	0.00	C8H16N0O5S0P0
215.10425	0.03	CHO	0.17	1.33	5	3	0.36	C12H16N0O2S0P0
215.10666	0.02	CHO	0.14	1.00	8	6	0.54	C14H14N0O2S0P0
215.10666	0.03	CHO	0.14	1.00	8	6	0.54	C14H14N0O2S0P0
215.12538	0.03	CHO	0.44	2.22	0	-4	0.00	C9H20N0O4S0P0
215.12778	0.16	CHO	0.36	1.64	3	-1	0.11	C11H18N0O4S0P0
202.06110	0.23	CHON	0.20	0.70	9	7.00	1.08	C10H7N3O2S0P0
209.16484	0.00	CHON	0.08	1.67	4	3	0.26	C12H20N2O1S0P0
209.16484	0.00	CHON	0.08	1.67	4	3	0.26	C12H20N2O1S0P0
220.13320	0.16	CHON	0.15	1.31	6	4	0.41	C13H17N1O2S0P0
222.14645	0.12	CHON	0.18	1.91	2	0	0.06	C11H21N1O2S0P0
222.18524	0.09	CHON	0.07	1.64	4	3	0.24	C14H23N1O1S0P0
226.14377	0.04	CHON	0.25	1.58	4	1	0.21	C12H19N1O3S0P0
230.17507	0.05	CHON	0.25	1.92	2	-1	0.00	C12H23N1O3S0P0
230.17507	0.05	CHON	0.25	1.92	2	-1	0.00	C12H23N1O3S0P0
232.12918	0.47	CHON	0.44	1.89	3	-1.00	0.00	C9H17N3O4S0P0
239.12126	0.54	CHONS	0.08	1.50	5.5	4.5	0.29	C12H18N2O1S1P0
241.02775	0.32	CHONS	0.44	0.89	7.5	3.5	0.75	C9H8N2O4S1P0

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
241.02775	0.36	CHONS	0.44	0.89	7.5	3.5	0.75	C9H8N2O4S1P0
246.05942	-0.86	CHONS	0.15	1.00	8	6.00	0.55	C13H13N1O2S1P0
246.12707	0.99	CHONS	0.20	1.90	3.5	1.5	0.00	C10H19N3O2S1P0
250.05324	0.36	CHONS	0.25	0.92	8.5	5.5	0.59	C12H11N1O3S1P0
250.12938	0.37	CHONS	0.09	2.09	2	1.00	0.00	C11H23N1O1S2P0
252.98896	0.40	CHONS	0.71	0.86	6.5	1.5	1.00	C7H6N2O5S1P0
255.04100	0.53	CHONS	0.50	1.50	4.5	0.5	0.00	C8H12N2O4S1P0
259.03832	0.35	CHONS	0.56	1.11	6.5	1.5	0.43	C9H10N2O5S1P0
325.17855	-0.73	CHOP	0.43	2.21	0	-6	0.00	C14H31N0O6S0P1
329.15233	0.57	CHOP	0.31	1.69	4	-1	0.08	C16H27N0O5S0P1
333.22002	0.18	CHOP	0.24	2.06	1	-3	0.00	C17H35N0O4S0P1
347.19928	0.51	CHOP	0.29	1.94	2	-3	0.00	C17H33N0O5S0P1
351.23058	0.45	CHOP	0.29	2.18	0	-5	0.00	C17H37N0O5S0P1
353.13120	0.78	CHOP	0.14	1.10	11	8	0.49	C21H23N0O3S0P1
355.20437	0.43	CHOP	0.21	1.74	4	0.00	0.09	C19H33N0O4S0P1
361.06352	-0.88	CHOP	0.19	0.71	15	11	0.69	C21H15N0O4S0P1
361.14216	0.49	CHOP	0.44	1.69	4	-3.00	0.00	C16H27N0O7S0P1
361.17855	0.37	CHOP	0.35	1.82	3	-3	0.00	C17H31N0O6S0P1
312.17340	0.15	CHOPN	0.19	1.75	4.00	1.00	0.12	C16H28N1O3S0P1
312.17340	0.31	CHOPN	0.19	1.75	4	1.00	0.12	C16H28N1O3S0P1
332.23600	0.47	CHOPN	0.18	2.12	1	-2	0.00	C17H36N1O3S0P1
346.21527	0.24	CHOPN	0.24	2.00	2	-2	0.00	C17H34N1O4S0P1
348.23092	0.52	CHOPN	0.24	2.12	1	-3	0.00	C17H36N1O4S0P1
362.21018	0.49	CHOPN	0.29	2.00	2	-3	0.00	C17H34N1O5S0P1
364.22583	0.46	CHOPN	0.29	2.12	1	-4	0.00	C17H36N1O5S0P1
366.16871	0.49	CHOPN	0.47	2.00	2	-5	0.00	C15H30N1O7S0P1
378.20510	0.67	CHOPN	0.35	2.00	2	-4	0.00	C17H34N1O6S0P1
380.22075	0.48	CHOPN	0.35	2.12	1	-5	0.00	C17H36N1O6S0P1
326.06214	-0.12	CHOPNS	0.29	1.29	7	3.00	0.33	C14H18N1O4S1P1
358.12474	0.51	CHOPNS	0.25	1.63	5	1	0.09	C16H26N1O4S1P1
478.08835	-0.76	CHOPNS	0.20	0.88	16	11	0.59	C25H22N1O5S1P1
500.26050	0.75	CHOPNS	0.20	1.76	5	0	0.03	C25H44N1O5S1P1
530.11965	-0.10	CHOPNS	0.17	0.90	18	13	0.57	C29H26N1O5S1P1
546.02654	0.02	CHOPNS	0.48	0.78	16	5	0.59	C23H18N1O11S1P1
557.99603	-0.03	CHOPNS	1.06	1.13	9	-8	0.00	C16H18N1O17S1P1
558.03241	0.24	CHOPNS	0.94	1.29	8	-8.00	0.00	C17H22N1O16S1P1
563.94320	-0.81	CHOPNS	0.50	0.33	22.00	10.00	0.93	C24H8N1O12S1P1
644.33915	0.07	CHOPNS	0.25	1.75	6	-2	0.00	C32H56N1O8S1P1
325.10327	0.94	CHOPS	0.19	1.44	6	3	0.24	C16H23N0O3S1P1
353.13457	0.89	CHOPS	0.17	1.50	6	3	0.21	C18H27N0O3S1P1
359.14514	0.56	CHOPS	0.24	1.71	4.00	0.00	0.04	C17H29N0O4S1P1
367.17135	-0.26	CHOPS	0.31	2.06	1	-4.00	0.00	C16H33N0O5S1P1
409.14553	-0.33	CHOPS	0.41	1.82	3	-4	0.00	C17H31N0O7S1P1
411.04615	-0.64	CHOPS	0.24	0.81	14	9	0.61	C21H17N0O5S1P1
413.15570	0.86	CHOPS	0.25	1.55	6	1	0.13	C20H31N0O5S1P1
417.17175	-0.04	CHOPS	0.50	2.19	0	-8	0.00	C16H35N0O8S1P1

ExactMass	Mass error (ppm)	Elemental constituents	O/C	H/C	DBE	DBE-O	AI_{mod}	Formula
417.17175	-0.79	CHOPS	0.50	2.19	0	-8.00	0.00	C16H35N0O8S1P1
419.05124	-0.98	CHOPS	0.17	0.74	16	12	0.66	C23H17N0O4S1P1
217.16206	0.08	CHOS	0.08	2.00	1.5	0.5	0.00	C12H24N0O1S1P0
217.16206	0.47	CHOS	0.08	2.00	1.5	0.5	0.00	C12H24N0O1S1P0
229.16206	0.18	CHOS	0.08	1.85	2.5	1.5	0.04	C13H24N0O1S1P0
229.16206	0.01	CHOS	0.08	1.85	2.5	1.5	0.04	C13H24N0O1S1P0
241.16206	0.03	CHOS	0.07	1.71	3.5	2.5	0.12	C14H24N0O1S1P0
243.17771	0.09	CHOS	0.07	1.86	2.5	1.5	0.04	C14H26N0O1S1P0
245.19336	0.21	CHOS	0.07	2.00	1.5	0.5	0.00	C14H28N0O1S1P0
257.19336	0.01	CHOS	0.07	1.87	2.5	1.5	0.04	C15H28N0O1S1P0
273.03782	0.10	CHOS	0.08	1.08	8	7	0.43	C13H14N0O1S2P0
273.03782	0.17	CHOS	0.08	1.15	8	7	0.43	C13H14N0O1S2P0

Media

- **Nutrient broth** **gL^{-1}**

Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Final pH	7.3±0.1

- **Tryptone Soy broth** **gL^{-1}**

Pancreatic digest of casein	17
Papaic digest of Soyabean meal	3
Sodium chloride	5
Dibasic potassium phosphate	2.5
Dextrose	2.5
Final pH	7.3±0.2

- **Zobell Marine broth** **gL^{-1}**

Peptic digest of animal tissue	5
Yeast extract	1
Ferric citrate	0.1
Sodium chloride	19.45
Magnesium chloride	8.8
Sodium sulfate	3.24
Calcium chloride	1.8
Potassium chloride	0.55
Sodium bicarbonate	0.16
Potassium bromide	0.08
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Disodium phosphate	0.008
Final pH	7.6±0.2

- **Antarctic bacterial medium** **gL^{-1}**

Peptone	0.5
Yeast extract	0.2
Final pH	6.9±0.2

- **R3A broth** **gL^{-1}**

Casein acid hydrolysate	1
Yeast extract	1
Biopeptone	1
Dextrose	1
Starch soluble	1
Dipotassium phosphate	0.6
Magnesium sulfate	0.048
Sodium pyruvate	0.6
Final pH	7.2±0.2

- **R2A broth** **gL^{-1}**

Casein Enzymic hydrolysate	0.25
Peptic digest of animal tissue	0.25
Casein Acid hydrolysate	0.5
Yeast extract	0.5
Glucose	0.5
Starch soluble	0.5
Dipotassium phosphate	0.03
Magnesium sulphate, heptahydrate	0.5
Sodium pyruvate	0.03
Final pH	7.2±0.2

- **Luria Bertani broth** **gL^{-1}**

Tryptone	10
Yeast Extract	5
Sodium chloride	10
Final pH	7.5±0

List of publications from thesis (Citations - 18)

1. Runa Antony, K. Mahalinganathan, Meloth Thamban and Shanta Nair. Organic Carbon in Antarctic Snow: Spatial Trends and Possible Sources. *Environ. Sci. Technol.,* 2011, 45 (23), pp 9944–9950, DOI: 10.1021/es203512t **(IF: 5.25)**
2. Runa Antony, Amanda M. Grannas, Amanda S. Willoughby, Rachel L. Sleighter, Meloth Thamban, and Patrick G. Hatcher (2014) Origin and Sources of Dissolved Organic Matter in Snow on the East Antarctic Ice Sheet, *Environ. Sci. Technol.* 48 (11), pp 6151–6159, DOI: 10.1021/es405246a **(IF: 5.25)**
3. Runa Antony, Amanda S. Willoughby, Amanda M. Grannas, Victoria Catanzano, Rachel L. Sleighter, Meloth Thamban, Patrick G. Hatcher and Shanta Nair. Molecular insights on dissolved organic matter transformation through microbial communities on the Antarctic ice sheet surface. *Environmental microbiology* (*under review*)

List of other publications

1. **Runa Antony,** K.P. Krishnan, C.M. Laluraj, Meloth Thamban, P.K. Dhakephalkar, Anupama S. Engineer, S. Shivaji (2012) Diversity and physiology of culturable bacteria associated with a coastal Antarctic ice core. *Microbiological Research*, 167, 372–380. (**IF: 2.30**)
2. **Runa Antony**, K. Mahalinganathan, K.P. Krishnan, and M. Thamban (2011), Microbial preference for different size classes of organic carbon: A study from Antarctic snow, *Environ. Monit. Assess*. DOI 10.1007/s10661-011-2391-1. (**IF:1.44**)
3. **Runa Antony**, M. Thamban, K.P. Krishnan, and K. Mahalinganathan (2010), Is cloud seeding in coastal Antarctica linked to bromine and nitrate variability in snow? *Environ. Res. Lett.,* 5, 014009. (**IF: 3.63**)
4. **Runa Antony**, P.P. Sujith, S.O. Fernandes, P. Verma, V.D. Khedekar, P.A. Loka Bharathi (2010), Cobalt immobilization by manganese oxidizing bacteria from the Indian Ridge system, *Curr. Microbiol.,* 62(3), 840-849. (**IF: 1.81**)
5. **Runa Antony**, K.P. Krishnan, S. Thomas, W.P. Abraham, and M. Thamban (2009), Phenotypic and molecular identification of *Cellulosimicrobium cellulans* isolated from Antarctic snow, *Antonie van Leeuwenhoek*, 96(4), 627-634. (**IF: 2.09**)

Papers presented at International Conferences/Symposiums (from thesis):

1. Runa Antony, Grannas A.M., Priest A.S., Sleighter R.L., Meloth T., Hatcher P. 'Microbial and long-range terrestrial contributions of organic matter to Antarctica'. *AGU fall meet*, San Francisco, USA, 3-7 December 2012 (Poster).
2. Runa Antony. 'Biogeochemical processes on Antarctic snowpacks'. *15th Meeting of the Asian Forum for Polar Sciences*, Kuala Lumpur, Malaysia, 7-8 October 2014 (Oral).
3. Runa Antony, Willoughby A.S., Grannas A.M., Catanzano V., Sleighter R.L., Thamban M., Hatcher P.G. and Nair S. 'Molecular insights on dissolved organic matter transformation through microbial communities on the Antarctic ice sheet surface'. *International Symposium on Antarctic Earth Sciences*, Goa, India, 12-17 July 2015 (**Best poster award**).
4. Grannas A.M.., Fede A., Boschi V., Catanzano V., Antony R., Willoughby A., Hatcher P. 'Natural organic matter in cryosphere-atmosphere interactions: Chemistry and Characterisation' *Atmospheric Chemistry Gordon Conference*, *New Hampshire*, USA, 2-7 August, 2015 (Poster).