'Organics in ice': Novel organic compounds in ice cores for use in palaeoclimate reconstruction

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Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text

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Abstract for:

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The majority of current ice core studies focus on analysing the inorganic component of atmospheric aerosol, trapped and preserved in the ice as a record of past atmosphere. However, this does not fully represent the make-up of atmospheric aerosol, which can be up to 50% organic. This thesis aims to develop the understanding and quantification of a number of these organic compounds in ice core samples.

A novel and promising area of 'organics in ice' research lies within the groups of primary and secondary compounds released from the terrestrial and marine biospheres; these compounds may help us to form a record of past biosphere emissions, with implications for biological productivity and atmospheric chemistry. A small selection of studies obtaining new records from these types of organic compounds in ice have demonstrated this concept, for example lipid compounds in snow layers dating back 450 years in Greenland, oxidation products of isoprene and monoterpenes in ice up to 350 years old in Alaska, and carboxylic acids and inorganic ions between 1942-1993 from Grenzgletscher (Monte Rosa Massif) in the southern Swiss Alps. Compound concentrations were related back to Northern Hemisphere temperature, atmospheric transport pathways and intensities, and biomass burning signals respectively.

There are many terrestrial and marine biogenic compounds not yet investigated in ice core samples. Thus we are presented with an almost untapped reservoir of new climate information. Therefore, it is timely to produce a method of analysis for a long list of the most promising of these compounds (herein 'target compounds'), namely fatty acids and secondary oxidation aerosol of terpenes (SOA), allowing quantification of these novel analytes in ice core samples to investigate the concept further.

This project begins with an investigation in to the possible contamination sources of the target compounds in ice core samples. It attempts to quantify the threat of contamination throughout the drilling, storage and analyses processes. It finds there is substantial presence of organic compounds in media used during ice core processing, but the risk to target compounds is minor where clean-protocols are followed, and the threat is limited to outer surface ice of a solid ice

core. Recommendations for ice core processing steps in preparation for organics analyses are outlined based on these results.

A method of high performance liquid chromatography-mass spectrometry (HPLC-MS) including rotary evaporation preconcentration of samples is then optimised for detection of target compounds. The final method achieves good average recoveries of 80%, and reproducibility of 9% RSD. The method is reproducible on different instruments, based on an interlab comparison. An extension of this method, direct injection HPLC-MS analysis (where sample preconcentration is eliminated) is tested for the benefits of reducing sample volume and contamination introduced by preconcentration steps. The method is successful for SOA compounds (7% RSD) but not for fatty acids where background contamination is very high.

The method of preconcentration HPLC-MS is then applied to samples from two ice cores; the marine-aerosol dominated Bouvet Island (sub-Antarctic) ice core, and the terrestrial-aerosol dominated Belukha Glacier (Russian Altai Mountains) ice core. Novel organic compounds are detected in both cores. Compound concentration time-series are investigated statistically by comparison to pre-existing inorganic compound records from the same cores and to historical climate data, and by application of back trajectory modelling using the Met Office's Numerical Atmospheric-dispersion Modelling Environment (NAME).

In the case of Bouvet Island, the fatty acid oleic acid is found to have statistically significant correlations with the sea-ice marker methanesulfonic acid, and indeed with sea ice concentration during July to September in a geographical region extending westwards from the island along the maximum sea ice extent margin. The mechanism behind this correlation is suggested to be that of algal blooming during spring months, releasing oleic acid which is transported by strong westerly winds to the island. This highlights the prospects for a suite of marine biogenics in ice cores being used as sea ice markers. In the Belukha core, where sample records are available at sub-annual resolution, a suite of SOA compounds display summer-time peaks in concentrations. This seasonal variability is shown to be related to emission signals of these organic compounds, which is an exciting prospect for future work in improving our understanding of budgets of these aerosols in the atmosphere. The records detected demonstrate great promise for the use of organic compounds as environmental markers.

Acknowledgements

I would like to acknowledge those without whom this thesis would not have been possible. I cannot write enough to thank everyone who has supported me along the way, but in brief;

I would like to thank my supervisors, Eric Wolff, Liz Thomas, and Markus Kalberer for not only giving me the opportunity to undertake this PhD, but for all the opportunities they have given me along the way, some of which have been the best experiences of my life. Also Chiara Giorio, for managing to make me a Chemist and for always being available for help and advice. The support and encouragement I have had from all my supervisors has been invaluable.

I would like to thank my family, my mum and dad for their belief and support no matter what decision I make, and my brother, my constant source of inspiration and reality-checking advice. I do most things in the hope to make them proud.

I have met an uncountable number of inspirational people throughout the last three and a half years, some of which I am now lucky enough to call my friends. They helped me believe I could do this when doubt crept in, and kept me smiling along the way.

This thesis is dedicated in memory of Dr Sophie Miller

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Chapter 1: Introduction

Preface

Chapter 1 is adapted in-part from the published literature review:

Giorio, C; Kehrwald, N; Barbante, C; Kalberer, M; King, A. C. F; Thomas, E; Wolff, E. W; Zennaro, P. 2018. Prospects for reconstructing paleoenvironmental conditions from organic compounds in polar snow and ice. Quaternary Science Reviews, 183, pp.1–22.

Specifically, section 1.2 is taken directly from this paper, having been originally written by the author of this thesis, Amy King, and Table 1 designed by Amy King with contributions from co-authors.

Table 2 in section 1.6 is published in:

King, A.C.F; Giorio, C; Wolff, E; Thomas, E; Karroca, O; Roverso, M; Schwikowski, M; Tapparo, A; Gambaro, A; Kalberer, M. 2019. A new method for the determination of primary and secondary terrestrial and marine biomarkers in ice cores using liquid chromatography high-resolution mass spectrometry. Talanta, 194, pp.233–242.

Written sections on previous work in preconcentration techniques (section 1.5) are adapted from this paper, also originally written by Amy King.

Other sections are originally written for this thesis by Amy King.

Chapter 1: Table of Contents

1. Introduction

Ice cores provide us with information about Earth's past climates over scales ranging from just a few years to much longer records, up to 800,000 years ago. Older cores come from the Antarctic continent and Greenland, but on shorter timescales cores may come from both polar as well as lower latitude ice-covered regions (Jouzel 2013). Such continuous, long-term and/or high-resolution records are of great importance in understanding our climate. Discontinuous records from blue ice areas in Antarctica specifically, where very ancient ice is uplifted to the surface, also provide us with snapshots of Earth's history up to 2 Million years ago. These records show us a time when Earth's orbital cycles were on a different scale of periodicity (Higgins et al. 2015).

Ice core records have revealed some of the most important discoveries in climatological terms. Antarctic ice cores are from extremely remote locations and are therefore excellent records of very well mixed air masses representing long-term global average atmospheric content, generally away from anthropogenic contaminants. For example they show the cyclical link between carbon dioxide and temperature (from oxygen isotopes) over orbital timescales (Figure 1) (Luthi et al. 2008), as well as glacial-interglacial cycles, and abrupt climate changes. Antarctic cores may also be useful over shorter timescales for climate specific to the Antarctic region, for example they show changing accumulation rates over the continent in recent decades, highlighting regional variability such as the anomalous trends for the Antarctic Peninsula (Figure 2) (Thomas et al. 2015). Ice cores also show changes in the extent of sea ice (Abram et al. 2013; Curran 2003) (from methanesulphonic acid, released by phytoplankton during blooms in the spring break up of sea ice), a critical driver in climate feedbacks but as yet not well understood outside the satellite era.

From Northern Hemisphere cores such as Greenland or Alpine cores, less remote and closer to source regions of many gases and particles, there are additional records of both temperature and carbon dioxide, with their increases since the pre-industrial era (MacFarling Meure et al. 2006), records of biomass burning (from ammonium and levoglucosan) demonstrating anthropogenic influences through the modern era, and when comparing Antarctic and Greenland cores evidence of the bi-polar see-saw (the out-of-phase temperature changes between the two hemispheres). In all cores, mineral dust gives us information about dust supply availability (related to glacial-interglacial cycles as well as short term changes), transport variability (related to atmospheric conditions and transport pathways) and source regions based

on mineral composition. These are just examples of the vast array of information contained within ice cores.
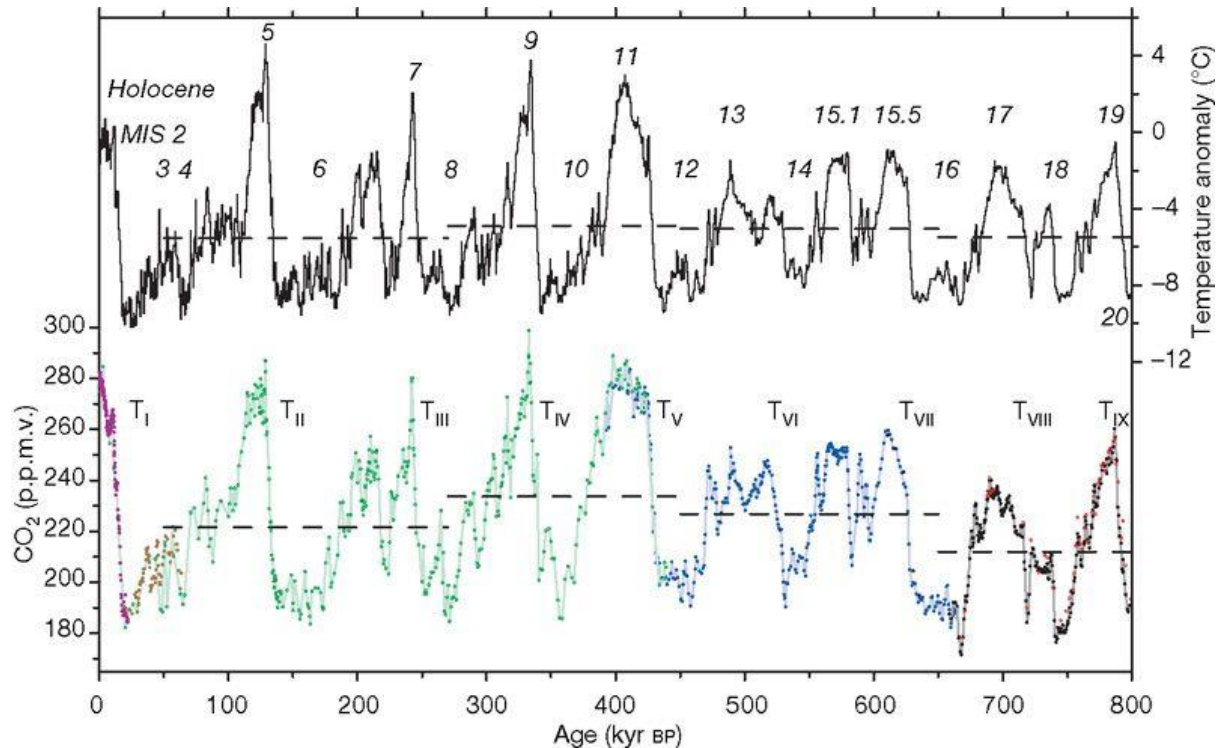
Figure 1: Comparative CO_2 and temperature (from deuterium isotopes) anomaly (with respect to the mean temperature of the last millennium) records for the oldest available continuous ice core records, from Dome C, Antarctica. Different colours for the CO2 data represent sections measured in different labs and in the case of the brown and green colour, from different Antarctic cores. Horizontal lines are time-period averages while glacial terminations are indicated using Roman numerals in subscript (for example T_I). Marine Isotope Stages (MIS) are given in italic Arabic numerals. The figure shows a clear synchronicity between the rise and fall of CO2 and temperature over 100,000 year orbital timescales. (From Luthi et al. 2008).

Figure 2: Annual average snow accumulation (dashed lines) and running decadal means (thick lines) for four ice cores; Gomez (Black), Bryan Coast (red), Ferrigno (blue), all on the Antarctic Peninsula, and WAIS divide WDC05Q (green) which is more central Antarctic continent. Solid horizonta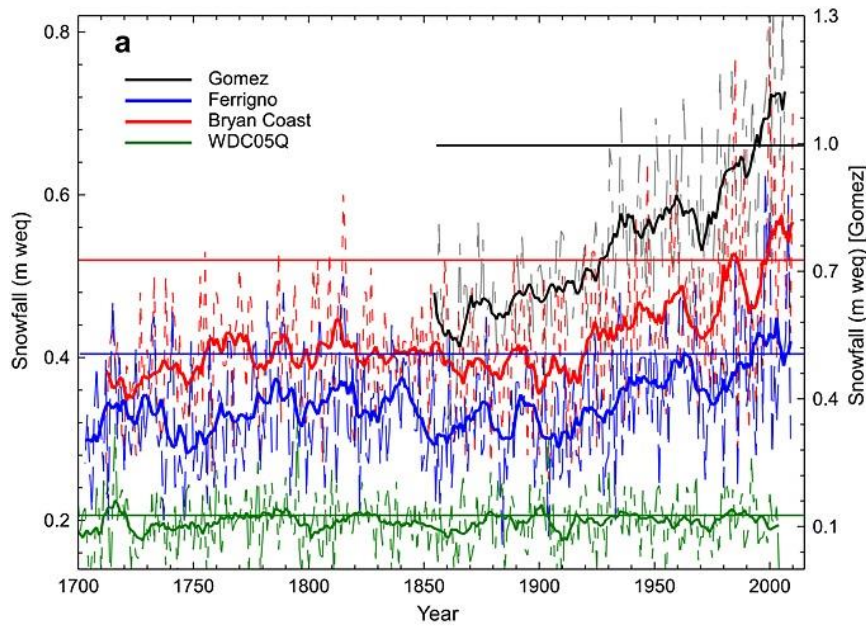l lines indicate the average for each record in the period 1980–2010. The records show the increasing accumulation progressively over the peninsula away from the central continent, in comparison with the overall Antarctic continent record of no change in accumulation. (From Thomas et al. 2015).

Ice core climate records come from three main sources; isotopic content of water molecules forming the ice (oxygen and deuterium), analyses of gases trapped inside air bubbles within the ice (for example carbon dioxide and methane), or from gas and particle impurities trapped within or on snowflakes as they fell through the ancient atmosphere (for example salts and mineral dust). The majority of current ice core studies focus on these dominantly inorganic constituents of atmospheric aerosol, however this does not fully represent the make-up of atmospheric aerosol, which can be up to 50% organic (Jimenez et al. 2009). Studies have shown this organic component is not only present in high altitude and latitude snow and ice-covered regions, both in atmospheric aerosol and buried within snow and ice, but it's measured quantities also have links to past environmental conditions as described further in following sections. Thus we are presented with an almost untapped reservoir of new climate information.

Only a few organic compounds in snow and ice samples have already been investigated. Mostly this includes anthropogenic contaminants such as persistent organic pollutants (POPs) (e.g. Fuoco et al. 2012) and polycyclic aromatic hydrocarbons (PAHs) (Figure 3), biomass burning markers such as levoglucosan (e.g. Kehrwald et al. 2012), and the sea ice marker methanesulphonic acid (MSA).
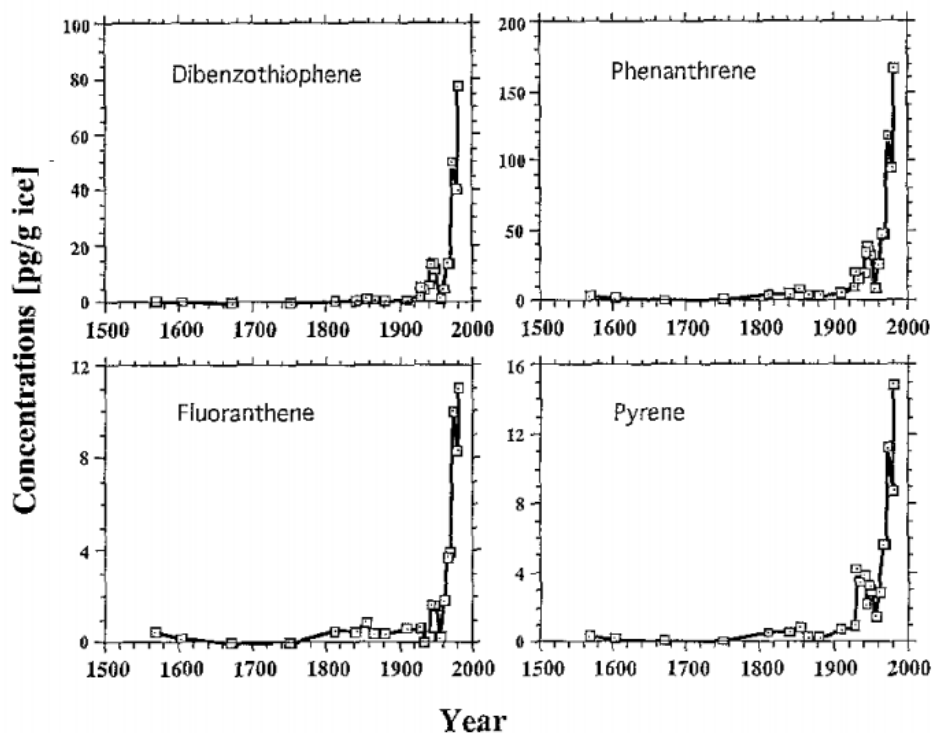
Figure 3: Example ice core records of several PAHs found in a Greenland ice core for the last 400 years. Records show a large increase in concentrations due to anthropogenic production of the compounds since 1930 (Kawamura and Suzuki, 1994).

A novel and promising area of 'organics in ice' research lies within the groups of primary and secondary compounds released from the terrestrial and marine biosphere; these compounds may help us to form a record of past biosphere emissions, with implications for biological productivity and atmospheric chemistry. A small selection of studies obtaining new records from these types of organic compounds in ice have demonstrated this concept; Kawamura et al. (1996) detected lipid compounds in snow layers dating back 450 years at Site J, Greenland, using gas chromatography – mass spectrometry (GC-MS), Pokhrel et al. (2015) detected oxidation products of isoprene and monoterpenes in ice up to 350 years old in Alaska using GC-MS of rotary-evaporation preconcentrated samples, and Muller-Tautges et al. (2016)

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detected carboxylic acids and inorganic ions between 1942-1993 from Grenzgletscher (Monte Rosa Massif) in the southern Swiss Alps using high performance liquid chromatography-mass spectrometry (HPLC-MS) on samples preconcentrated using solid phase extraction (SPE). Further discussion of these analytical techniques can be found in section 1.5. Compound concentrations in these studies were related back to Northern Hemisphere temperature, atmospheric transport pathways and intensities, and biomass burning signals respectively.

There are still many terrestrial and marine biogenic compounds not yet investigated in ice core samples. Therefore, it is timely to produce methods of analyses for a long list of the most promising of these compounds, allowing quantification of these novel analytes in ice core samples to investigate the concept further. This concept not only requires the detection and quantification of these compounds, but investigation of their potential environmental and climate links.

1.1   Challenges of organic analyses of ice

Organic analyses of ice has been enabled only in recent years by improving technological capabilities allowing detection of these compounds at very low levels within samples. Our increasing understanding of organic aerosol, both in terms of emissions and reaction pathways in the atmosphere, has further aided our interpretation of measurements. However, there are still challenges to overcome in the low concentrations of analytes in the ice, and in the high contamination potential of organic compounds in sample collection, processing and analyses.

1.1.1   Low concentrations of analytes

Previous studies collectively show that concentrations of organics in ice should be expected to be extremely low, often at concentrations not more than ppt (equivalent to pg/g or ng/L) and certainly not above ppb (ng/g or ug/L). For terrestrial and marine biogenic compounds specifically, example compound concentrations range between 0.09-0.23 ppb for fatty acids and oxycarboxylic acids in Greenland and Alaska (Kawamura 1993; Pokhrel et al. 2015), and 0.05-692 ppb for secondary oxidation aerosols of isoprenes and monoterpenes in Alaska and Kamchatka (Pokhrel et al. 2015; Fu et al. 2016), though for other types of organics such as

anthropogenic pollutants levels have been as low as 1 ppt in Greenland (Kawamura 1994). In Antarctic ice, even more remote from source locations than those above, the expected concentrations may be substantially lower. Therefore, while new instrumentation certainly presents opportunities to detect analytes at these levels, it has commonly been the case that preconcentration of samples is a necessary requirement for accurate quantification. As described in more detail in section 1.5, several preconcentration methods have successfully recovered organic compounds in ice and snow samples. However these additional processing steps introduce extra challenges in optimising methods when considering multiple types of organic compounds, and in trying to limit introduction of contamination to the samples, to which many organic compounds may be highly susceptible (Giorio et al. 2018).

1.1.2 Contamination potential of organics

1.1.2.1 Drilling processes and ice properties

Physical properties of ice greatly differ with location and depth in the ice sheet; these properties often guide the drilling processes used and thus the possibility of contamination occurring.

At the top of a theoretical vertical column of ice, loosely packed snow falls layer-upon-layer. This snow, although densifying with the increasing weight and compaction of overlying snowfalls, remains an open, porous matrix of snowflakes surrounded by air, which we call firn, for some considerable time (dependent on rate of snow accumulation above). Therefore, air is free to migrate throughout the upper ice column, open to contaminating air flow both in-situ and once sampled. However this upper portion of the core, usually up to 100 m depth, does not require the addition of drilling fluid to lubricate the drill barrel, due to lower ice densities and pressure. Lower down the ice column, where compaction transforms the snow into solid ice, air bubbles become entirely encased and sealed off from surrounding air flow. This portion of ice needs a lubricating drill fluid to stop the core barrel from sticking. While the inner portions of these lower solid-ice sections should be sealed from outer contamination, any cracks or damage to the outer-core surface may allow penetration of contaminants, and the very outer surface is not usually appropriate for contamination-sensitive analyses.

Ice cores are portioned in to several strips, with each going to different analyses. Outer portions of the core are usually used for isotope measurements, since these are not sensitive to

contamination from handling or addition of fluids. Inner portions of ice are reserved for more sensitive analyses, often with additional procedures for limiting contamination. This is described in more detail below.

1.1.2.2 Historical decontamination procedures

The need for limitation of contamination of analytes in ice core samples is not unique to organic compounds, and practices have been established and developed throughout the history of ice core analyses for many of the more commonly analysed inorganic compounds (Boutron & Batifol 1985). They include processes for 'cleaning' the ice before melting and analyses such as scraping and washing to remove the outer ice surface, the use of specialist facilities such as laminar flow benches and clean-rooms, and the preparation of laboratory equipment such as baking of glassware and the use of materials adapted to the potential contaminants (in the case of organic compounds plastic is generally avoided, for example). More detail of existing methods can be found in Chapter 2. While the methods developed for inorganics certainly guide those required for organic compounds, the latter do present some new challenges.

Compared to inorganics, there are very few studies quantifying the contamination sources of organics in ice. Developing methods for laboratory preparation of organics samples is certainly manageable, since materials used and the laboratory environment for sample processing can be easily adapted. However the drilling, storage and cutting of cores may be more difficult to adapt since technology and materials are very well established, and even if possible to adapt difficulties arise when using ice samples from cores drilled before consideration for organics. Major potential sources of organic contaminants may be drill fluids used for drilling deeper core sections, which result in the coating of core-surfaces in hydrocarbon rich fluids, or fluids sourced from coconut oils (rich in fatty acids), that can affect analyses of organic compounds (Legrand et al. 2013). Following the drilling process, the cores are placed inside 'layflat bags' in the field for transportation and storage. Layflats are long, cylindrical plastic bags, and may be coated in resins to protect the plastic from cold field and freezer conditions. Cores are cut in to strips in laboratory environments using band saws which have been cleaned by solvents such as isopropyl alcohol (IPA) solutions. A search of currently available literature found no organic-compound analyses of drill fluids, layflat bags and coatings, or IPA.

There are limited studies looking at the diffusion of contaminants through the outer ice core surface, as a way of quantifying whether contaminant sources are a threat to certain portions of ice used for analyses. Using microspheres to represent microbial contamination (sourced from drill fluids) Miteva et al. (2014) recommend removal of the outer few mm's of ice, while Christner et al. (2005) recommend a much more generous removal of the outer 3cm of ice based on the study of kerosene diffusion. However it is important to know exactly what threat is presented from all materials used in ice processing, specific to organic compounds.

With these issues in mind, Chapter 2 presents a thorough study identifying any contaminants present in a suite of drilling fluids, layflat bags and IPA solution which match this project's target analytes (See section 1.6, Table 2). For these materials it also measures the extent of diffusion of the contaminant through the outer ice surface.

1.2   Terrestrial biogenic markers

The terrestrial biosphere is the largest global emitter of both primary organic aerosols and volatile organic compounds (VOCs). A fraction of the VOCs is oxidised in the atmosphere forming secondary oxidation aerosol (SOA). VOCs and SOA in ice cores may elucidate how the terrestrial biosphere has changed in recent decades due to anthropogenic land use changes, as well as across rapid climate changes or glacial-interglacial transitions. Measurements of compounds associated with primary emissions (such as leaf waxes) reflect the size of the biosphere while the relative proportions of these compounds may provide information on the composition of the relevant part of the biosphere. Clearly, given the distance of Greenland and especially Antarctica from source regions, interpretation in terms of which source is being sampled and how other factors have influenced the concentrations recorded will be critical for these molecules.

Biogenic VOCs, particularly isoprene and terpenes, are prolific in the atmosphere. Emission rates are again expected to scale with the size and composition of the biosphere, modulated by emission factors related to climate. These molecules also play two crucial roles in the atmosphere. They are a major control on the hydroxyl radical (·OH) concentration in the atmosphere; constraining ·OH concentrations over time would be of interest for many reasons, not least in limiting the causes of methane change over time (Levine et al. 2011) . In addition,

biogenic VOCs are on a global level the most important source of SOA, which is increasingly implicated as an important factor in the growth of cloud condensation nuclei (Tröstl et al. 2016). Records of such compounds will be complicated not only by transport effects but also by lifetime limitations caused by chemical degradation, but it would be valuable to have proxies for VOC emissions.

The recent identification of a multitude of biogenic organic markers in snow and ice samples situated both near to and far from source regions raises the exciting potential of new environmental proxies that are present in the ice core record. Such compounds have, for example, been found in Alaska (Pokhrel et al. 2015; Sankelo et al. 2013; Yamamoto et al. 2011), the Chinese Himalayas (Xie et al. 2000), Greenland (Grannas 2004; von Schneidemesser et al. 2008), Franz Josef Land, Russia (Grannas et al. 2006), the Tibetan Plateau (Shen et al. 2015) and the Canadian Arctic (Grannas 2004), as well as in oceanic aerosol samples in the Canadian high Arctic (Fu et al. 2013) and over ocean latitudes ranging from the Arctic to Antarctica (Hu et al. 2013) . Here I discuss the terrestrial organic compounds so far identified in snow or ice samples, or high latitude atmospheric aerosols, which are most promising as biomarkers in ice.

1.2.1 Primary emissions

A number of lipid-based compounds with terrestrial sources have been shown to have long-range transport potential in atmospheric aerosols, and to subsequently be preserved within ice layers. N-alkanes, n-alkenes, n-alkanols, and n-alkanoic acids are sourced from terrestrial leaf epicuticular waxes, soil dust, microbial processes or marine phytoplankton (Pokhrel 2015). Specifically, lipid compounds in epicuticular waxes are plucked from the leaf surface in high winds and entrained in the atmosphere as primary aerosols (Yamamoto et al. 2011), and their concentrations may be expected to demonstrate a record of biogeochemical cycles such as the relative amount of deciduous vegetation and associated carbon storage (Kawamura et al. 1996). Relatively resistant to degradation (Pancost & Boot 2004), these lipid-based compounds persist in the atmosphere at time scales of days to weeks.

The process of emission, long range transport, deposition and entrainment of lipid-based compounds within snow and ice has been demonstrated in previous work, from both shallow snow pit studies and now extending back to ice layers in the order of several hundred years in

age. Identification within shallow snow pits in Japan (Sankelo et al. 2013; Yamamoto et al. 2011) and China (Xie et al. 2000) demonstrates the potential of terrestrial organic compounds to be preserved in snow layers, with the major source of the lipid compounds being large forested regions found proximal to the sample site. Extending to more distant deposition sites, De Angelis et al. (2012) observed terrestrial vegetation and biomass burning emissions dominating the carboxylic acid budget within surface snow layers at Summit, Greenland, concluding that aerosols generated by Northern Hemisphere terrestrial biomass are an important contributor to aerosol deposits in the ice record at this high latitude location. Lipid compounds have further been detected in snow layers dating back to 450 years at Site J in Greenland (Kawamura et al. 1996). Although air masses that pass over Greenland contain a more complex mixture of both marine and terrestrial organic aerosols, they were able to identify compounds of specific terrestrial origin using a number of distinguishing molecular characteristics (see below), which have been used and expanded on in subsequent studies (Bendle et al. 2007; Pokhrel 2015; Yamamoto et al. 2011).

Lipid compounds from terrestrial sources may be identified as high molecular weight fatty acids (HFA) (>C24), as opposed to low molecular weight fatty acids (LFA) (<C24) which are indicative of marine and microbial sources (Pokhrel 2015; Yamamoto et al. 2011). Terrestrial particles may further be distinguished from those of anthropogenic sources using the Carbon Preference Index (CPI), determined as the ratio of compounds with odd to even carbon numbers (of atoms in the molecule). CPI values >5 signify an absence of anthropogenic input, whilst values decreasing down to 1 imply an increasing anthropogenic contribution. Furthermore, the average chain length (ACL) within the different compound groups has been utilised as evidence of specific source regions. Within the n-alkane group, for example, greater abundance of longer chain HFAs may indicate warm, tropical source regions, whereas a greater abundance of shorter chain HFAs would suggest more temperate source regions (Bendle et al. 2007). Calculating HFA to total organic carbon (TOC) ratios was further used to identify a tentative link between this ratio and warmer or cooler periods of global temperatures. There were, however, a number of possible explanations of higher ratio values (higher plant emissions, enhanced atmospheric transport, increased area of arid environments, and altering atmospheric transport pathways), and the idea needs refining (Bendle et al. 2007).

To date, these lipid compounds have been studied in a limited manner in snow and ice from Alaska, Greenland and lower latitude glaciers. It is likely to be far more challenging to detect them in Antarctica, which is further from any terrestrial sources.

1.2.2 Secondary oxidation products

Isoprene and terpenes are emitted from all plants including algal sources in ocean regions (Bonsang et al. 1992; Yassaa et al. 2008) and form a considerable contribution to the hydrocarbon budget of the atmosphere (Sharkey et al. 2008). In particular, isoprene and monoterpenes have been measured as substantial terrestrial emissions, with isoprene dominating in flux studies above Amazonian forest canopies (Kesselmeier et al. 2000; Rinne et al. 2002) and on a global level. Sesquiterpenes also contribute to terrestrial emissions, although flux and oxidation pathways of emissions are difficult to study because of their very high reactivity and lower concentrations (Fu et al. 2013). The emission of isoprene and terpenes is conditional on both heat and light, and is therefore proposed as a 'thermo-tolerance mechanism' of plants (Sharkey et al. 2008). The reactive nature of isoprene and terpenes in the atmosphere presents a limitation to the potential for long range transport from the emission site; isoprene, monoterpenes and sesquiterpenes have short chemical lifespans from a few minutes up to a few hours (Kesselmeier et al. 2000), and have not been directly observed in ice. However, the oxidation products of these compounds in both gas and aerosol phase demonstrate a greater potential for longevity in the atmosphere, and subsequent deposition into snow and ice further from the source region.

Isoprene and terpenes emitted by vegetation are subject to several possible degradation processes in the atmosphere, each of which may or may not result in the formation of SOA (Hallquist et al. 2009) with a multitude of secondary products forming from each primary compound. Both degradation pathways and atmospheric concentrations still need further study (Kroll & Seinfeld 2008). However there has been a recent transition in detection of some key SOA components from only laboratory-confined experiments to detection in natural atmospheric samples, as well as in some surface snow and ice core studies, which provides promising results for use of SOA constituents as climate markers (Fu et al. 2016; Pokhrel et al. 2015)

Isoprene has been identified as the largest non-methane VOC emission globally, at ~600 Gt/yr (Guenther et al. 2006). Thus, even a very small percentage of VOC to SOA transformation rates could be significant in the total particulate organic matter budget of the atmosphere (e.g. 6 Gt/yr at only 1% yield) (Carlton et al. 2009). Claeys et al. (2004) first recognised two isoprene

oxidation products, 2-methylthreitol and 2-methylerythritol, in Amazonian air samples, bolstered by the association of two gas-phase products of the oxidation process, methacrylic acid and methacrolein, providing a reaction pathway linking isoprene and the identified SOA composition. It was later shown by Xia and Hopke (2006) that these compounds demonstrate the same seasonal cycle (peak concentrations during summer) as isoprene in forests in the north-eastern United States. The compounds have now been identified in a number of forest-canopy aerosol samples (e.g. Finland (Kourtchev et al. 2013) and Hungary (Ion et al. 2005), and demonstrate the important contribution of isoprene oxidation products to total atmospheric SOA concentrations. However, highly variable lifetimes for these secondary compounds have been suggested (Nozière et al. 2015), which may limit the number of compounds available for consideration.

Methylthreitol (an isoprene SOA marker), pinic acid and pinonic acid (monoterpene SOA markers) were estimated to have short lifetimes of only 0.2 days by Noziere et al. (2015), clearly limiting transportation to high latitude polar regions which may require days. Others, such as MBTCA (3-methyl-1,2,3-butanetricarboxylic acid as a monoterpene SOA marker), nocaryophyllonic acid, caryophyllinic acid and nocaryophyllinic acid (sesquiterpene SOA markers), were estimated to have lifetimes up to 10 days. These values are highly uncertain but in general agree with SOA lifetimes as modelled using global chemistry transport models and general circulation models; with a review of 31 such models by Tsigaridis et al. (2014) finding an average range of SOA atmospheric lifetimes of 2.4-15 days. To add to this uncertainty, Hu et al. (2013) identified both pinic acid and MBTCA in high latitude samples of atmospheric aerosol, in which a substantial contribution to the total concentrations were identified as terrestrially sourced compounds based on atmospheric transport pathway reconstruction for the supplying air masses. More substantially, isoprene and monoterpene oxidation products, including many of those products with proposed short lifetimes of less than 0.2 days, have also been identified in an Alaskan ice core dating back 350 years. For example, (Pokhrel et al. 2015) identified a number of terrestrial and marine sourced SOA compounds within the ice samples, using atmospheric transport pathway reconstruction to determine the compounds' source region(s). Thus, these compounds demonstrate not only the atmospheric transport and deposition of SOA with terrestrial sources, but also the persistence of these compounds to older ice layers. Many of the observed concentration changes were correlated with Northern Hemisphere temperature fluctuations using a combination of compound groups as well as individual compounds for comparisons. This observation suggests also that lifetime of these

compounds in the particle phase may be longer than estimated, perhaps because SOA particles may have low viscosities limiting uptake and diffusion of oxidants (Virtanen et al. 2010). If the study of these compounds is extended to Antarctica, it will be necessary to distinguish compounds with mainly terrestrial sources from those with mainly marine origins. One method is to test for a temporal correlation between the organic markers of interest and uniquely terrestrial molecules, such as the biomass burning marker levoglucosan, or another method is to compare the results against uniquely marine emissions such as MSA, an approach used by Fu et al. (Fu et al. 2013). More details on marine biomarkers can be found in section 1.3.

While there is still much to be investigated regarding SOA compound characteristics, their detection in natural samples in high latitude regions seems to suggest the potential for using these compounds as biomarkers in ice. Furthermore, the ever-expanding number of identified monoterpene oxidation products, e.g. at least 24 monoterpene-derived organic compounds identified above a boreal forest in Hyytiala, Finland (Kourtchev et al. 2013) provides an ever-increasing scope of promising marker compounds, of which any number may have sufficient lifetimes allowing transport and deposition to polar snow.

The groups of compounds discussed here offer excellent potential as biomarkers in ice cores. In the Northern Hemisphere (Greenland), multi-compound studies could, in conjunction with broad determination of their source regions (for example, terrestrial versus marine), allow investigation of past changes in the extent and climate of boreal forests. The same application has yet to be thoroughly tested for the Southern Hemisphere, where the compounds may be expected in even lower concentrations.

1.3   Marine biogenic markers

The marine biosphere also emits primary and secondary organic markers, though total contributions are smaller than those of the terrestrial biosphere (Hallquist et al. 2009). Regionally specific terrestrial versus marine contributions obviously depends on proximity to the source locations, and in coastal Antarctic cores, for example, one can imagine the main organic source being the marine biosphere.

Organic compounds are emitted from the marine biosphere either through biochemical processes, and subsequent oxidation in the atmosphere to SOA, or through physical processes

creating sea spray and bubble bursting in the sea surface microlayer (SML; The top 1000 μm of the ocean, is the boundary between the atmosphere and the ocean and has distinct physiochemical and biological properties that differ from the underlying waters (Wurl et al. 2017)). Studies have shown that the fraction of organic matter in submicrometer marine aerosol is much higher during periods of high biological activity in the sea surface, such as during phytoplankton blooms, which is likely to be strongly associated with the summer months (O'Dowd et al. 2004). Once transported to and deposited in the ice record, these biomarkers may be related to a number of environmental factors such as biogenic productivity, sea ice extent, and wind strength.

1.3.1 Primary emissions

Primary emissions from bubble bursting are mostly known to consist of fatty acids, monocarboxylic acids, dicarboxylic acids and tricarboxylic acids, terpenes and sugars (Schmitt-Kopplin et al. 2012). There may also be marine organisms or fragments of organisms such as phytoplankton, diatoms, bacteria and algae (Leck & Bigg 2005). Organisms may be a source of specific organic compounds, for example oleic acid, an unsaturated fatty acid, is a major constituent in marine diatoms and although these unsaturated fatty acids may be degraded in the atmosphere over short timescales (Mochida et al. 2002) they have been found in ice cores in both Greenland and Alaska (Kawamura et al. 1996; Pokhrel et al. 2015) dating back many hundreds of years at least. This fatty acid is in the group considered low molecular weight fatty acids (LFA), which as previously discussed is indicative of marine sources; other examples of these fatty acids were also detected in the Alaskan core such as lauric acid and myristic acid. In contrast, there are no previous analyses and very little understanding of fatty acids in Antarctic cores and so potential concentrations and association to environmental markers is still very much unknown.

1.3.2 Secondary oxidation products

Similarly to secondary oxidation products of terrestrial sources, isoprene and monoterpene oxidation contributes to SOA in marine sourced aerosol. As discussed for these compounds of terrestrial origin, although highly reactive and expected to have short lifetimes in the

atmosphere, many SOA compounds have been observed in aerosol samples ranging pole to pole over marine locations, and in ice samples with ages up to hundreds of years old. SOA products from marine isoprene and monoterpenes may be expected to be the same as those from terrestrial sources. Unlike fatty acids, which have a set number of known compounds to target, the products of terpene oxidation are still under investigation and many are only known from laboratory generation of the compounds. Therefore, Table 1 provides a collective list of isoprene, monoterpene and sesquiterpene compounds so far detected in robust quantities in both marine and terrestrial (forest canopy) aerosol studies, and in snow and ice samples, to give an overview of those for consideration in this study.

A better understood SOA component of marine-sourced organic aerosol is methasulfonic acid (MSA), which is an oxidation product of dimethylsulphide (DMS). DMS is produced only by marine phytoplankton, and consequentially is seasonally linked to the sea ice zone particularly in the spring/summer break up of sea ice uncovering the open ocean. The greater the sea ice extent during winter, the larger the sea-ice break up area in the following spring-summer promoting greater phytoplankton blooms; therefore there is a statistically significant correlation between sea ice extent and MSA production, as recorded in several coastal Antarctic cores (Abram et al. 2007; Abram et al. 2013; Curran 2003; Wolff et al. 2006). It was one of the first organic compounds to be quantified in ice cores in the polar regions (Legrand et al. 1997). While this thesis aims to deal with novel organic markers, being able to compare any new records to better understood organic records from ice cores may prove valuable in determining environmental correlations, particularly in Antarctic cores where organics are least studied and in lowest concentrations.

Table 1. Isoprene, Monoterpene and Sesquiterpene SOA compounds identified in natural atmospheric aerosol samples from forest locations and in snow samples from remote locations.

Isoprene SOA	Location

2-methyl tetrols:
 	
     2-methylthreitol
 	
     2-methylerythritol
	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2013; Kourtchev et al., 2005), Forested areas China (Wang et al., 2008), Amazonian Forest Brazil (Claeys et al., 2004a), Tibetan Plateau (Shen et al., 2015), Alaskan ice core (Pokhrel et al., 2016), Kamchatka ice core (Fu et al., 2016)

2-methylglyceric acid	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2013; Kourtchev et al., 2005), Forested areas China (Wang et al., 2008), Tibetan Plateau (Shen et al., 2015), Alaskan ice core (Pokhrel et al., 2016), Kamchatka ice core (Fu et al., 2016)
C_5-alkene triols:	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2005), Forested areas China (Wang et al.,

cis-2-methyl-1,3,4,trihydroxy-1-butene; *trans*-2-methyl-1,3,4-trihydroxy-1-butene, 3-methyl-2,3,4-trihydroxy-1-butene	2008), Tibetan Plateau (Shen et al., 2015), Alaskan ice core (Pokhrel et al., 2016)

Monoterpene SOA	Location
3-hydroxyglutaric acid	Canadian High Arctic (Fu et al., 2009), Forested areas China (Wang et al., 2008), Alaskan ice core (Pokhrel et al., 2016), Kamchatka ice core (Fu et al., 2016)
pinic acid, pinonic acid	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2013), Tibetan Plateau (Shen et al., 2015), Alaskan ice core (Pokhrel et al., 2016), Kamchatka ice core (Fu et al., 2016)
pinanoldehyde; pinanediol mononitrate; norpinonic acid; terebic acid; ketolimononic acid; limonic acid; homoterpenylic acid; deterpenylic acid acetate; caric acid; caronic acid; 2-hydroxyterpenylic acid; HHDCA (1-hydroxy-3-(hydroxymethyl)- 2,2 dimethylcyclobutane-carboxylic acid); DHHMDMCP (2,3-dihydroxy-2-(hydroxymethyl)-7,7-dimethylbicycloheptan6-one); HODSA (3-(2-hydroxy-3-oxobutyl)-2,2-dimethylsuccinaldehyde); 2,6,6-trimethylbicycloheptane-2,3,-diol	Boreal Forest Finland (Kourtchev et al., 2013)
norpinic acid	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2013), Forested areas China (Wang et al., 2008)
3-hydroxyglutaric acid, 3-hydroxy-4,4-dimethylglutaric acid	Tibetan Plateau (Shen et al., 2015)
MBTCA (3-methyl-1,2,3-butanetricarboxylic acid)	Canadian High Arctic (Fu et al., 2009), Boreal Forest Finland (Kourtchev et al., 2013), Forested areas China (Wang et al., 2008), Tibetan Plateau (Shen et al., 2015), Kamchatka ice core (Fu et al., 2016)

Sesquiterpene SOA	Location
β-caryophyllinic acid	Canadian High Arctic (Fu et al., 2009), North Carolina, USA (Jaoui et al., 2007), Boreal Forest Finland (van Eijck et al., 2013), Tibetan Plateau (Shen et al., 2015)
DCCA (3,3-dimethyl-2-(3oxobutyl)cyclobutanecarboxylic acid)	Boreal Forest Finland (Kourtchev et al., 2013; van Eijck et al., 2013)
β-nocaryophyllonic acid; 2-(2-carboxyethyl)-3,3-dimethylcyclobutanecarboxylic acid	Boreal Forest Finland (van Eijck et al., 2013)

1.4   Transport, deposition and preservation in ice

As discussed in the previous sections, the route of organic compounds from emission to preservation in ice may be complex. Oxidation of primary emissions may be rapid, and involve a number of different reactions and reactionary products for any particular primary compound. It is also important to consider the mechanisms by which any compound is finally deposited on the snow surface and incorporated in the ice, and what happens to the compound once within the surface snow layers. These processes determine how representative the concentration of the compound in the ice is of the concentration that was in the atmosphere.

A simplified schematic considering the route of an organic compound from source to ice sample is shown in Figure 4. Any compound, in either gas- or aerosol-phase, reaching an ice core site may be deposited on to the surface snow layer by two mechanisms: dry deposition or wet deposition. For aerosol particles, which are those considered in this study, wet deposition dominates where accumulation rate is higher (such as Greenland, coastal Antarctica and sub-Antarctic islands) and dry deposition where accumulation is extremely low, such as the East Antarctic Plateau (Wolff, 2012). For wet deposition, particles are scavenged by the snow flakes and compounds may adsorb on to the surface of the snow flake. The efficiencies of these processes are highly variable depending on a number of factors such as snow properties, compound properties, and temperature (Herbert et al. 2006), and large uncertainties remain (Lei and Wania, 2004).

Organic particles may be highly volatile, adding an additional complication to aerosol deposition; compounds may become re-volatised from the snow surface or photooxidation may occur (Wolff, 2012). This may reduce the concentration of a particular compound in the ice, such that it is not representative of actual atmospheric concentration. However assuming these reactions are constant the time-variability observed in any ice core record would be preserved.

The many complexities of these processes are outside the scope of this overview, and similar to transport may be difficult to determine for this studies list of compounds due to a lack of finite knowledge on their reactionary pathways. However the overview of these processes will be used to guide interpretation throughout Chapters 4 and 5 where ice core records are investigated.
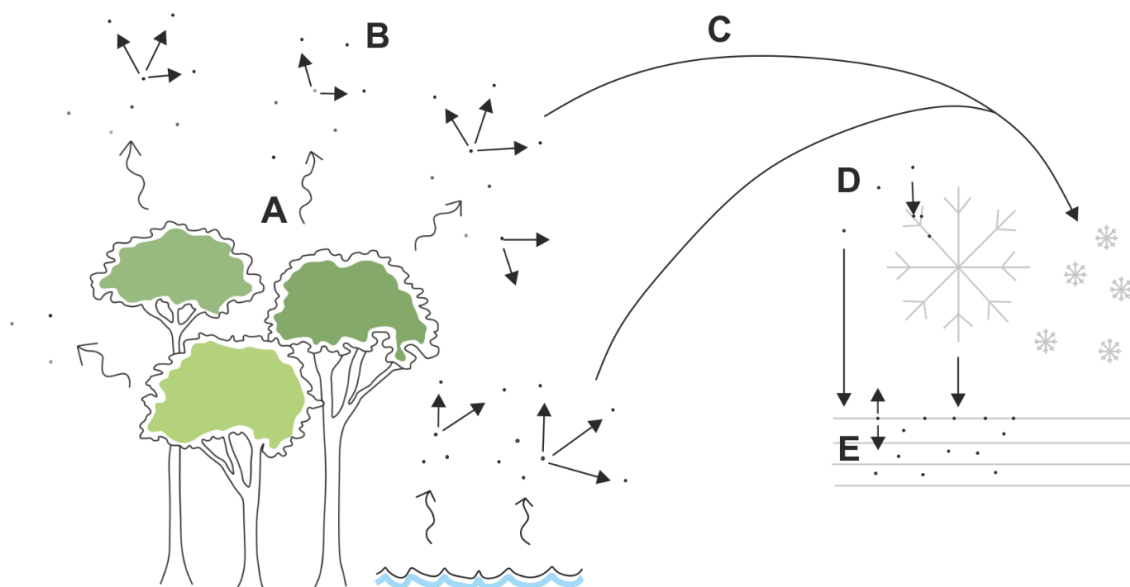
Figure 4: A schematic summary of the route of an organic compound from source to preservation in ice layers. Compounds are emitted (A) and may be oxidised in to secondary products (B), which are transported through the atmosphere (C). Compounds with sufficiently long atmospheric lifetimes will survive to the ice core (or deposition) site. Compounds are adsorbed on to the snow flake surface or fall out directly on to the snow surface (D). They may continue to react with the atmosphere through photo-oxidation, for example, and are finally buried and preserved in the ice (E).

1.5   Analytical methods

Analytical studies of various types of organics in ice generally apply established GC-MS or LC-MS instrumentation. As discussed in section 1.1.1, most studies of organics in ice have in common a compound concentration of parts per billion (ppb) or well below, more commonly parts per trillion (ppt), leading us to the requirement to preconcentrate samples to allow reliable detection. We note here the choice of this thesis to measure and present concentrations, rather than flux of the compounds which is the concentration multiplie by snow accumulation. This is guided by Wolff et al. (2012) and discussed further in Appendix 3. Preconcentration methods applicable to organic compounds in ice, and previously used for the purpose, are Stir Bar Sorptive Extraction (SBSE), Solid Phase Extraction (SPE) and rotary evaporation. Brief descriptions of the methods and previous studies including them are described below. Chapter

3 deals with the development and full optimisation of the HPLC-MS – preconcentration methodology carried forward to analyses of ice samples in this thesis.

1.5.1 Mass spectrometer

All analyses in this study used a HPLC-ESI-HRMS (high performance liquid chromatography – electrospray ionisation – high resolution mass spectrometer) with an Accela system HPLC (Thermo Scientific, Bremen, Germany) coupled to an LTQ Velos Orbitrap (Thermo Scientific, Bremen, Germany). This is except for the use of a UHPLC (ultra-high performance mass spectrometer liquid chromatograph) with an UltiMate3000 coupled to a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap MS at the Department of Chemical Sciences, University of Padua, Italy, for an interlaboratory comparison.

HR-MS is a powerful tool which allows fast detection of many thousands of organic fragments of ions at specific m/z (mass-to-charge) ratios, with clearly separated peaks when coupled to the LC, and even elucidation of the structure of compounds when combined to tandem mass spectrometry (MS-MS) which applies additional energy to each primary or parent ion to break it down in to its constituent fragments, or product ions (Nizkorodov et al. 2011). The LTQ Orbitrap MS used in this study is chosen because of its high mass accuracy of < 2ppm (the difference between the measured m/z of the compound peak and the exact m/z calculated based on the elemental composition of the molecule, the smaller the better (Nizkorodov et al. 2011)). This is an important factor when measuring environmental samples as each may contain many organic ions for which accurate identification relies on very accurate m/z measurements. A drawback is that acquisition time is longer compared to other instruments. Pilot studies of the instrument preceding this project had shown that the sensitivity of the instrument was enough to detect the target organic compounds at expected ice sample concentrations, after preconcentration (i.e. in the range of low ppb).

1.5.2 Stir bar sorptive extraction (SBSE)

Preconcentration via SBSE is based on the adsorption of compounds from a sample to a solid phase of choice (i.e. the stir bar coating, wrapped around a magnetic rod), with the compounds then recovered from the solid phase via re-elution in to a new, smaller volume of liquid of

choice for analyses. Stir bars have been used in only a few studies of organic compounds in ice; Muller-Tautges et al. (2014) used a polydimethylsiloxane (PDMS) coated bar for extraction of α-dicarbonyls (glyoxal and methylglyoxal). Using HPLC-ESI-MS/MS they report recoveries of 78.9±5.6 % for glyoxal and 82.7±7.5 % for methylglyoxal, with limits of detection (LODs) of 0.242 and 0.213 ppb respectively.

Lacorte et al. (2009) used the same type of bar for extraction of persistent organic pollutants (POPs) in Arctic ice. Recoveries using gas-chromatography coupled to mass spectrometry (GC-MS) ranged from 71% to 139%, achieving LODs of 0.1–99 and 102–891 ppt for organohalogenated compounds and polycyclic aromatic hydrocarbons (PAHs) respectively.

1.5.3   Solid phase extraction (SPE)

The SPE technique passes a liquid sample through a sorbent mass (usually held in a syringe), and a series of washing and elution phases then 'clean' the sample of non-target compounds and elute the target compounds from the cartridge. There are a wide range of cartridge types available (greater than possibilities for stir bars), at a range of cartridge masses dependent on sample sizes. This is probably the most complex method of the three considered here with the variability of sorbent masses, but also with the most stages including cartridge preparation with pre- and post-sample washes, equilibration and elution to consider. There are few examples of SPE usage for specific compounds focussed on in this study, but they have been tested for other organics in ice such as POPs.

Muller-Tautges *et al.* (2016) used a strong anion exchange SPE cartridge for concentration of a series of mono-carboxylic and dicarboxylic acids in high-alpine ice and achieved LODs of 0.0002-0.8 ppb with LC-MS analyses. Average sample concentrations ranged from 0.021-15.9 ppb for monocarboxylic acids and 0.022 – 0.52 for dicarboxylic acids. Recoveries are not reported. Wang et al. (2008) used a poly(dimethylsiloxane) fiber mass to quantify organochlorine pesticides (OCPs) and PAHs in a firn core from Dasuopu Glacier, Central Himalayas. Recovery from GC-MS analyses was between 84+/-8.3% and 95+/-4.6% for various OCPs, and 53+/-9% and 120+/35% for PAHs, with LODs between 0.05 and 0.75ppt. Parshintsev et al. (2010) tested two alternative solid phase masses (hydrophilic-lipophilic balance (HLB) sorbent and Oasis MAX, similar to HLB with a modified quaternary amine group) to measure atmospheric aerosol samples. HLB cartridges did not retain compounds

well, but OasisMAX extraction efficiencies were up to 100% with LODs between 9-27ppb LC/MS.

Two further cartridge types are C18 (bonded silica for reversed phase extraction) and HRP (hydrophilic divinylbenzene polymer). Gabrieli et al. (2010) tested concentrations of PAHs in Swiss/Italian Alpine snow/ice using the C18 cartridge with initial recoveries of 71-93%, increasing up to 3.3% with a second elution and 1.1% with a third elution, and LODs ranging 7-460 ppt using LC-MS. Shi et al. (2015) also test the C18 cartridge for analyses of perfluoralkyls (PFAs) in Antarctic ice, but find the HRP cartridge performed better by comparison, with recoveries of 73-117% and limit of quantifications (LOQs, the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met, or ten times standard deviation of the blank) of 0.025-0.5ppt.

1.5.4 Rotary Evaporation

Rotary evaporation involves the evaporation of a liquid sample to complete dryness, in principle leaving the target compound(s) coating the glass evaporator flask. This can then be eluted by a solvent of choice of a lesser volume than the original sample.

Rotary evaporation preconcentration has been previously applied to both the SOA and fatty acid compound groups including compounds which were considered in this study. Pokhrel et al. (2015) quantified fatty acids in rotary evaporated Alaskan ice samples using GC-MS, with average concentrations of individual compounds ranging between 0.09 and 20.3 ppb (LOD 0.001ppb, percentage recovery not reported). Kawamura (1993) achieved LODs of 0.05 ppb for oxocarboxylic acids, and measured dicarbonyls at concentrations of 0.25-1.72 ppb in snow and aerosol samples. In examples of SOA compounds, Pokhrel et al. (2015) and Fu et al. (2016) rotary evaporated Alaskan ice and the Ushkovsky ice cores respectively, with GC-MS analyses detecting isoprene and monoterpene SOA compounds at 6.99±17 to 692±702 ppb in the Alaskan and 0.05−18.4 ppb in the Ushkovsky core (percentage recovery was not reported).

These previous studies present a range of successful methods for many different compounds groups, however none provide comparison of the success of different preconcentration methods for the same compounds. Different preconcentration techniques may be more or less successful for each compound group. Using these previous studies as a starting point for techniques to

apply, this study attempts optimisation of a range of preconcentration methods to find that most successful for a set group of target compounds (Table 2).

1.6 Target compounds

The final target-compound list for this study, presented in Table 2, amalgamates those compounds of both terrestrial and marine biogenic origin considering two main factors; first their promise as biomarkers in ice and second, in practical terms, the availability of laboratory standards to allow quantification of the detected compounds. The first factor, promise as biomarkers, considers long-distance transport and preservation in snow and ice layers without degradation to be essential. Substantial emission quantities and relationships of emissions to an environmental variable are desirable. However, with such novel compounds these emission and transport parameters may not yet be clearly defined. To this list is added the biomass burning marker levoglucosan; this organic biomass burning marker is more commonly quantified and somewhat better understood and is therefore targeted to be used as a reference in terms of methodological success and comparison to other analyses. The compound has been quantified in Alpine, Greenland and even Antarctic ice (for example Kawamura et al. 2012; Kehrwald et al. 2012; Gambaro et al. 2008) using methods of both preconcentration and direct injection for analyses on both gas and liquid chromatography. Unlike other components such as oxalate and soot deposits, levoglucosan is unambiguously generated by pyrolosis of cellulose, and is an organic compound likely to be successfully detected using the method developed for our target compound list.

Table 2: Target compound list for this study, by compound group and in order of increasing number of carbon atoms.

Source	Compound name	Neutral Formula	CAS Number	Molecular Weight
Isoprene-derived SOA	Meso-erythritol*	$C_4H_{10}O_4$	149-32-6	122.12
Isoprene-derived SOA	Methyl tetrols	$C_5H_{12}O_4$	42933-13-1	136.15
Monoterpene-derived SOA	Pimelic acid*	$C_7H_{12}O_4$	111-16-0	160.17
Monoterpene-derived SOA	1,2,4-butanetricarboxylic acid (BTCA)*	$C_7H_{10}O_6$	1703-58-8	234.16

Source	Compound name	Neutral Formula	CAS Number	Molecular Weight
Cntd.				
Monoterpene-derived SOA	3-methyl-1,2,3-butanetricarboxylic acid (MBTCA)	$C_8H_{12}O_6$	77370-41-3	204.18
Monoterpene-derived SOA	Terebic acid	$C_7H_{10}O_4$	79-91-4	158.15
Monoterpene-derived SOA	Pinolic acid	$C_{10}H_{18}O_3$	473-71-2	186.25
Monoterpene-derived SOA	*Cis*-pinonic acid	$C_{10}H_{16}O_3$	61826-55-9	184.23
Monoterpene-derived SOA	Keto-pinic acid	$C_{10}H_{14}O_3$	40724-67-2	182.22
Sesquiterpene-derived SOA	β-caryophyllinic acid	$C_{14}H_{22}O_4$	N/A	253.15
Sesquiterpene-derived SOA	β-caryophyllonic acid	$C_{15}H_{24}O_3$	N/A	251.17
Sesquiterpene-derived SOA	β-nocaryophyllonic acid	$C_{14}H_{22}O_4$	N/A	253.15
Biomass burning	Levoglucosan	$C_6H_{10}O_5$	483-07-7	162.14
Biogenic SOA	D-malic acid	$C_4H_6O_5$	636-61-1	134.09
Primary biogenic	Salicylic acid	$C_7H_6O_3$	69-72-7	138.12
Low molecular weight fatty acids (LFA) (<C24); marine / microbial sources	Lauric acid	$C_{12}H_{24}O_2$	143-07-7	200.32
	Myristic acid	$C_{14}H_{28}O_2$	544-63-8	228.38
	Heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	270.46
	Oleic acid	$C_{18}H_{34}O_2$	112-80-1	282.47
	Nonadecanoic acid	$C_{19}H_{38}O_2$	646-30-0	298.51
	Arachidonic acid	$C_{20}H_{32}O_2$	506-32-1	304.74
	Behenic acid	$C_{22}H_{44}O_2$	204-010-8	340.59
	Tricosanoic acid	$C_{23}H_{46}O_2$	2433-96-7	354.62
High molecular weight fatty acids (HFA) (>C24); terrestrial biomass	Heptacosanoic acid	$C_{27}H_{54}O_2$	7138-40-1	410.73
	Octacosanoic acid	$C_{28}H_{56}O_2$	506-48-9	424.75
	Melissic acid	$C_{30}H_{60}O_2$	506-50-3	466.82

*surrogate standards (analytes chemically similar to those being extracted where actual standard not available.

1.7 Ice core locations

Target compounds for this study are emitted from the terrestrial and/or marine biosphere. This study measures ice cores from two opposing types of location, one being a location expected to be dominated entirely by terrestrially-sourced air masses far from marine sources, and the other a very remote marine location dominated entirely by marine-sourced air masses. This allows a direct comparison between the two, to help understand the expected dominant

compound types from each source, without overly complicating interpretation for sites with mixed air mass input. Locations of cores were also targeted based on proximity to the proposed source; closer to the source would give the greatest chance for transport to and preservation in the ice core site, minimising compound degradation.

1.7.1 Belukha glacier

Belukha glacier is found on Belukha Mountain, part of the Siberian Altai Mountains in Southern Russia. The site is completely landlocked, far from marine sources, with nearby forested regions (Figure 5). Therefore, the Belukha glacier is considered a terrestrial-input ice core site for this study.

The Belukha glacier ice core was drilled in 2001, (49°48'26"N, 86°34'43"E, 4062 m a.s.l.) to a depth of 140m. Previous studies of the core have analysed glacier characteristics (Olivier et al. 2003), major ion chemistry (including the biogenic markers oxalate, formate and ammonium), oxygen isotopes (Olivier et al. 2003; Olivier et al. 2006) and biological constituents such as pollen and (non-marine) diatoms (Papina et al. 2013), giving a suite of pre-existing chemical analyses to compare to new organic records. These are discussed in more detail in Chapter 5.
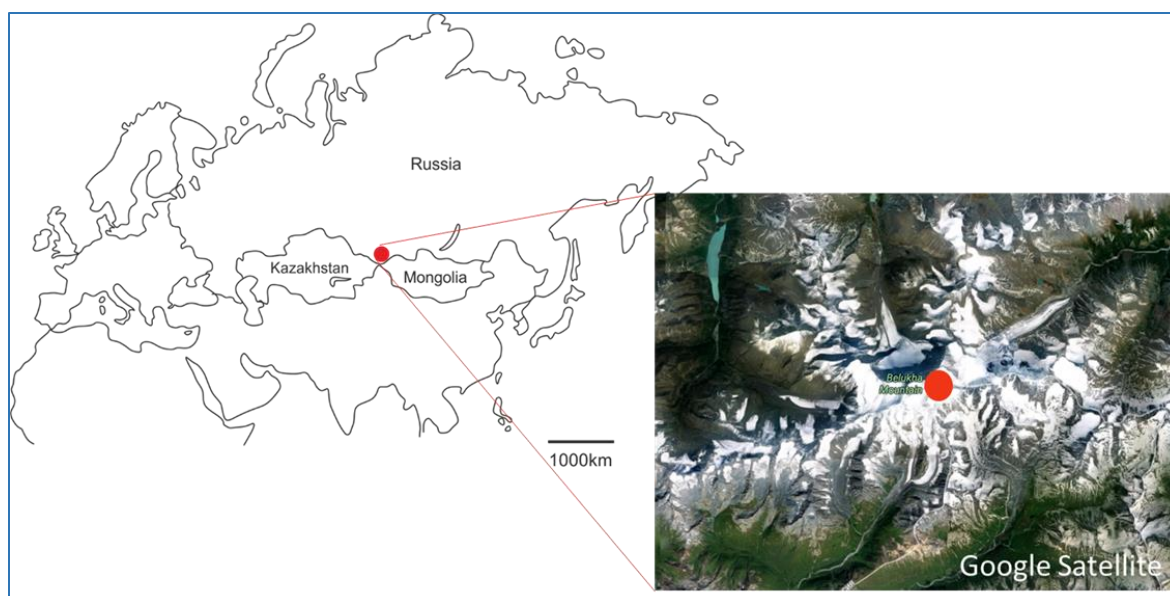

Figure 5: Location map for the Belukha Mountain ice core.

1.7.2 Sub-Antarctic: Bouvet Island

Bouvet Island, or Bouvetøya, is an ice-capped, volcanic sub-Antarctic island (Figure 6). The ice core is the first core ever collected here, as part of the Antarctic Circumnavigation Expedition (ACE) in 2015/2016. The core, drilled to 14m on Bouvet Island and dated back to 2001, is therefore a shallow core representing very modern climate with climate reanalyses data available for comparison.

This core, part of a suite of sub-Antarctic Island cores, contributes important information to a region virtually devoid of palaeoclimate archives, and critical for studying westerly winds, sea ice extent, and atmospheric aerosol content in the area. Bouvet Island sits at the very limit of maximum (winter) sea ice extent, centrally in the westerly wind belt. The highly remote location (Bouvet Island is in fact known as the most remote island in the world!) surrounded by ocean means that this site is considered to be a marine aerosol dominated ice core. More details about the core is found in Chapter 4.

Figure 6: Location map of the sub-Antarctic island Bouvet.

1.8   Back trajectory modelling

A critical component of understanding records retrieved from ice cores is being able to pin-point the location(s) from which any particles detected in the ice were originally sourced. Back trajectory analyses allow us to re-trace atmospheric aerosol pathways, from ice core locations to the particle source. For this, the met office's Numerical Atmospheric-dispersion Modelling Environment (NAME) (Jones et al. 2007) was used.

Simply, NAME is a lagrangian particle model, releasing 'model' particles from a location (i.e. the site from which the ice core was drilled) over a vertical column of air, and tracking these particles through the modelled atmosphere based on a three-dimensional wind field. Historic meteorology comes from a combination of the Met Office Unified Model and ECMWF Numerical Weather Prediction (NWP) meteorology data, with a random element of flow included to represent turbulence. This is available for 2006-2016 on the current model version.

NAME is run over a user-defined grid area, relating to the longitude-latitude area and surface height relative to ground level desired for the study. It runs over user-chosen timescales appropriate to the atmospheric lifetimes of the compounds being investigated. The model may be run over days to months, outputting daily (24hr) back trajectory files. Raw output files, containing particle concentration in the latitude-longitude defined grids, are processed using RStudio software, where particle concentrations can be averaged over months or seasons and plotted as concentrations on a two-dimensional global map.

In order to determine the length of back trajectory analyses to apply requires an understanding of the atmospheric lifetime of the organic compounds analysed in this study. If the compound is detected in the ice core sample, it can only be reasonably modelled that it comes from a distance manageable within this lifetime. For example, it would not be realistic to run back trajectory analyses for several days, and say the particle came from a forest site thousands of kilometres away, if the compound would have become oxidised within an hour of emission. Calculating lifetimes of specific organic aerosol in the atmosphere however can be extremely challenging, as they require rate constant estimations that in turn can be highly complex since there are in turn thousands of other organic compounds involved in the chemical reactions (Donahue et al. 2013). Calculating these for each of the compounds on the target list of this study is well outside the scope of the project, however previous estimations of the lifetimes for these compound groups are used as a guide for a realistic estimation. As discussed in section

1.2.2, lifetimes of SOA compounds can be very short, in the order of a few hours, to a few days. Noziere et al. (2015) (following Donahue et al. 2013) suggest isoprene and monoterpene markers (including pinonic acid, identical to this study) have lifetimes of less than 1 day, while sesquiterpene markers, identical to those in this study, survive between 1-10 days though the maximum value is the less common. Median lifetime in the broader review of SOA compounds by Tsigaridis et al. (2014) is 6 days. Fatty acids are similarly challenging, with estimates of atmospheric lifetimes not only differing between compounds but also within compounds. Oleic acid for example has been given lifetime estimations between minutes and days (Rogge et al. 1991; Shi et al. 2002) but in field observations it was shown to degrade much more slowly than expected based on its high reactivity towards ozone tending lifetimes towards the higher end of this range.

Based on these values, trying to encompass the longer-age estimations while not losing out on information for the shorter age compounds, all back trajectories were run for 5 days.

Further details of the model and running parameters can be found in Chapters 4 and 5, where the model is applied to each of the ice core locations.

1.9   Summary aims and objectives

In summary of this introductory Chapter, the major aims and objectives of this project are presented here to guide the reader through the following Chapters. The aim, to investigate the potential for analysing a suite of organic compounds in ice cores, exploring their application to palaeoclimate reconstruction, will be addressed with the following objectives:

1. To identify a long-list of organic compounds which show potential for preservation and subsequent detection in ice core samples, which may be related in concentrations to an environmental parameter at the time of initial compound emission.
2. To test the potential sample contamination of this target compound list, concluding on whether detected concentrations in the samples are a valid environmental signal.
3. To develop a method of high resolution liquid chromatography mass spectrometry (HPLC-MS), including sample pre-concentration, the optimal of which would be that which performs best on-average for all compounds on the target list.

4. To apply the developed method to samples of two distinct ice cores, one of a dominantly terrestrial aerosol input (Belukha Glacier, Russian Altai Mountains) and one of a dominantly marine aerosol input (Bouvet Island, Sub-Antarctic).

5. To investigate the potential environmental signals shown by any detected time-series records of compounds in the samples, by various methods of comparison to better-known ice core compounds, statistical analyses, and back-trajectory modelling.

6. To conclude on the potential application of these detected target compounds as ice core biomarkers.

2. References

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Chapter 2: Mitigating contamination

Overview

The aim of this chapter is to investigate contamination sources for a pre-defined list of potential organic biomarkers in ice cores. The possible contamination sources tested are drilling fluids, plastic core storage bags (layflats), and the laboratory cleaning solution isopropyl alcohol (IPA). This chapter includes a discussion of pre-existing decontamination protocols for organic compound analyses. It then tests for the diffusion potential of contaminants from the tested media through a solid synthetic ice core outer surface, over timescales of several months. The conclusions define the contamination risk of these compounds from current ice core drilling and sample processing, and outline the most contaminant-free procedures possible for analyses of organic compounds in ice. These procedures will be carried forward for use in sample analyses throughout future chapters.

Chapter 2: Table of Contents

1. Introduction

Detection of organic compounds in ice cores presents new analytical challenges compared to their inorganic counterparts; sources of more ubiquitous organics such as fatty acids are prolific in 'inorganic' laboratories which are filled with plastics, for example. Organics are also to be expected at extremely low concentrations in ice samples, and therefore even for more specific marker compounds like isoprene secondary oxidation aerosols, compounds remain very sensitive to even the smallest contamination. Contamination potential and decontamination protocols must now be carefully considered.

Although the accurate determination of concentrations of organic compounds in ice cores is very complex, with each different compound class providing different challenges in analyses and interpretation, there are some overarching considerations. First, individual analytes are likely to be present at very low concentrations in ice samples, generally below ppb or even ppt levels. Second, the process from drilling, to transport, storage and laboratory analyses poses opportunities for the introduction of contamination, compounded further by the first point. Therefore, the whole ice coring process must be structured using contamination free, or at least contamination limiting, protocols.

Although pre-existing protocols for inorganic compounds may be used to inform the development of similar methods for new organic compound analyses, the latter do pose a new challenge; many of the materials used in the drilling process and in the sampling and analyses stages are unsuitable for organic analyses. For example, drilling fluids used when drilling deeper core sections are frequently oil-based esters. Although these may not directly contact the shallower cores which do not require addition of a drilling fluid, the atmosphere surrounding a drilling environment may be rich in contaminants either from fluids, fuels, machinery and/or people. Furthermore, the standard procedure in many 'clean' laboratories optimised for inorganic analyses is to keep samples and equipment sealed inside plastic bags – again containing organic contaminants. Therefore, we must reassess existing procedures. To adapt and develop protocols for new compound analyses, it is important to first understand whether target molecules are present in the contaminating media for the target compound list of this study (Table 1).

The following sections discuss in more detail the decontamination protocols already in use for inorganic analyses, and describe some of the possible contaminating media and processes for ice cores.

Table 1: Target compound list for this project, by compound group and in order of increasing number of carbon atoms.

Source	Compound name	Neutral Formula
Isoprene-derived SOA	Meso-erythritol	$C_4H_{10}O_4$
Isoprene-derived SOA	Methyl tetrols	$C_5H_{12}O_4$
Monoterpene-derived SOA	Pimelic acid	$C_7H_{12}O_4$
Monoterpene-derived SOA	1,2,4-butanetricarboxylic acid (BTCA)	$C_7H_{10}O_6$
Monoterpene-derived SOA	3-methyl-1,2,3-butanetricarboxylic acid (MBTCA)	$C_8H_{12}O_6$
Monoterpene-derived SOA	Terebic acid	$C_7H_{10}O_4$
Monoterpene-derived SOA	Pinolic acid	$C_{10}H_{18}O_3$
Monoterpene-derived SOA	*Cis*-pinonic acid	$C_{10}H_{16}O_3$
Monoterpene-derived SOA	Keto-pinic acid	$C_{10}H_{14}O_3$
Sesquiterpene-derived SOA	β-caryophyllinic acid	$C_{14}H_{22}O_4$
Sesquiterpene-derived SOA	β-caryophyllonic acid	$C_{15}H_{24}O_3$
Sesquiterpene-derived SOA	β-nocaryophyllonic acid	$C_{14}H_{22}O_4$
Biomass burning	Levoglucosan	$C_6H_{10}O_5$
Biogenic SOA	D-malic acid	$C_4H_6O_5$
Primary biogenic	Salicylic acid	$C_7H_6O_3$
Low molecular weight fatty acids (LFA) (<C24); marine / microbial sources	Lauric acid	$C_{12}H_{24}O_2$
	Myristic acid	$C_{14}H_{28}O_2$
	Heptadecanoic acid	$C_{17}H_{34}O_2$
	Oleic acid	$C_{18}H_{34}O_2$
	Nonadecanoic acid	$C_{19}H_{38}O_2$
	Arachidonic acid	$C_{20}H_{32}O_2$
	Behenic acid	$C_{22}H_{44}O_2$
	Tricosanoic acid	$C_{23}H_{46}O_2$
High molecular weight fatty acids (HFA) (>C24); terrestrial biomass	Heptacosanoic acid	$C_{27}H_{54}O_2$
	Octacosanoic acid	$C_{28}H_{56}O_2$
	Melissic acid	$C_{30}H_{60}O_2$

1.1   Existing decontamination protocols

Decontamination protocols have been discussed (Boutron & Batifol 1985) and developed over succeeding years (Candelone et al., 1994), with multiple methods employed between different investigators for each type of, or even individual, compound to be analysed.

While detailing each decontamination procedure is beyond the remit of this discussion, they would be expected to follow particular principles. In general, an outer layer of ice is removed from the core or core-section, to the extent deemed appropriate to leave remaining ice totally free of the contaminants added throughout the coring and sampling process and diffusing through the outer ice surface. This may be by shaving, cutting, or washing away the outer ice layers, or a combination of these methods. This process often takes a number of repeated steps to limit carry-over of contaminants from outer to inner layers, which may follow very high gradients of change (Christner et al., 2005). The ice is usually considered to be contaminant free when the concentration profile of a compound from outer to inner core reaches a plateau (Candelone et al., 1994), which also indicates the total thickness of ice to be removed from the outer core to reach this 'clean' ice.

Accompanying the removal of outer ice layers will be appropriate methods for cleaning of lab utensils and sample vials (with choice of appropriate materials, e.g. ceramic, stainless steel, glass, also an important consideration), all in a laboratory environment with a contamination-free (or at the least, -limiting) atmosphere. This may be, for example, a specially adapted clean-room, laminar flow bench, or fume hood, or within a cold-room with thorough surface-cleaning procedures.

To pin-point the targeted contamination-free state of the ice sample, the process may also include any number of procedural blanks and tested samples from the outer to inner ice area.

Although this study focuses on solid ice, there is also the possibility that analyses would be of either fractured ice lengths or from the shallow firn layers (the upper 10's of metres of ice which, due to less overlying snow and hence pressure, have not become totally sealed off to the diffusion of gases through the layers). In these cases, contamination potential is much higher, with fractures allowing drill fluids to penetrate deep within the core, and firn susceptible to contamination by particles and vapour transport in the atmosphere at all times it is exposed. There is also the potential for compound migration within the core when exposed to

temperature changes. All of the preceding factors must be taken in to account when planning both decontamination and analyses.

1.2   Contamination sources – Drilling fluids and storage (layflat) bags

The use of drilling fluid is required for deeper ice core drilling (>100m depth) if high quality core retrieval is to be achieved (Sheldon et al., 2014a). The fluid both lubricates the drill-ice surface contact and helps to control pressures to which the ice core surface is exposed, reducing fracturing. The fluid is also used to keep the bore hole open in the winters of multi-year drill campaigns.

While the use of these fluids is generally not considered problematic in the analyses of inorganic compounds in the ice, the coating of core surfaces in hydrocarbon rich fluids, or fluids sourced from coconut oils (rich in fatty acids), may affect analyses of organic compounds. This was demonstrated by Legrand et al. (2013) in their study of organic carbon (OC) concentrations in a series of Greenland and Antarctic ice cores: OC values in the only Antarctic core acquired using a drilling fluid far exceeded values in Greenland ice, despite Greenland's much greater proximity to continental sources of OC.

Many aspects of environmental and physical-ice properties are considered when choosing an appropriate drilling method for a site, and drilling campaigns have utilised several different fluids based on the properties required. Previous to the Montreal Protocol, which initiated the phasing out of hydrochlorofluorocarbons (HCFC's), commonly used drilling fluids were n-butyl acetate (or butyl ethanoate), (e.g. GISP2 B and D cores, Greenland (Aydin et al., 2007)) and the kerosene based Exxsol^{TM} D-series (e.g. NorthGRIP, Greenland and EPICA Dome C, Antarctica (Sheldon et al., 2014a)). In particular, HCFC-141b, a hydrochlorofluorocarbon used as a densifying agent was added to the D-series fluids, and a suitable replacement after a ban in production was not found, while n-butyl acetate became unpopular due to adverse health effects (Talalay, 2016). However the D-series may still be used without a densifying agent.

With the exclusion of these early generation fluids has come the development of alternatives, sourced from esters including plant based esters. Examples include ESTISOL 140 (aliphatic synthetic ester), ESTISOL 165 (aliphatic carboxylic acid ester) (Sheldon et al., 2014b), ESTISOL 240 (distilled ester of coconut oil extract) and COASOL (di-isobutyl esters). A blend

of the latter two was first utilised on the Greenland NEEM ice core in 2009 (Miteva et al., 2014), while ESTISOL 140 has been recently applied to drilling in Antarctica at Aurora Basin North (Sheldon et al., 2014b). Silicon based oils have also been suggested to use for ice core drilling (Talalay & Gundestrup 2002) but due to cost have not been widely used so far. Further testing of contamination from these types of fluid would of course be needed if eventually used.

Evidence suggests that at least partial ice surface penetration occurs when drilling fluids are applied to the outer core. For example, Miteva et al. (2014) used fluorescent microspheres as a proxy for microbial cell (contained within drill fluids) penetration in to the ice core, coating the outer surface of the core for up to 2.5 years. They concluded contamination was mostly confined to surface layers, though diffusion may be further for the smallest size fractions of cells and/or in fractured ice. Christner et al. (2005) applied more extensive analyses of the kerosene content of successive inward layers of a coated ice core using systematic decontamination methods of melting, washing and scraping. They agree with the surface-limited effects of the contamination, though to be completely free of contamination suggest removal of at least 3cm of core surface. Again, the presence of ice fractures and cracks which penetrate the ice core may enhance contamination effects.

Throughout this PhD project, samples tested will be from shallow cores not requiring the use of drilling fluids. However the testing of contamination potential from drilling fluids is applicable to the future planning of analyses of deeper, older core samples and whether this is feasible with this contamination in mind.

Following the drilling process, the cores are placed inside 'layflat bags' in the field for transportation and storage. Layflats are long, cylindrical plastic bags. Two types of layflat bags exist; one of the bags (referred to hereafter as 'EVA layflat') is coated on the inner surface with a substance called 'EVA'. Specifically, this is Exxon LD362BR, a low-density polyethylene resin compounded with 4.5% Vinyl Acetate (information from supplier). The other bag type is not coated in EVA (herein 'non-EVA layflat'). The EVA coating protects the bag from the low temperature conditions of storage, and is the type of bag usually taken in to the field for core-storage, while the non-coated bags are generally for laboratory use. A search of currently available literature found no organic compound analyses of layflats or the EVA coating.

Conclusions from the studies highlighted suggest that a more thorough understanding of organic compound ice core contamination is required, both via compound specific analyses of

the drilling fluids applied, the substances contacting the core during storage, and in the diffusion rates of these compounds through the outer ice core surface.

Contamination sources also include all materials used in the laboratory processing stages of the samples, and the environment in which the samples are prepared and analysed. However, unlike the use of drilling fluids and core storage bags, most of these aspects can be easily adapted and therefore pose limited risk. It is usual for samples being used for organic analyses to be stored in pre-cleaned glass vials with polytetrafluoroethylene (PTFE) lined plastic lids, and/or in aluminium foil. Glassware can be baked at high temperatures over several hours to remove organics, and both glass and foil may be washed with organic solvents. Utensils which are ceramic or stainless steel, rather than plastic, can be cleaned similarly.

The solvents themselves, either for use in cleaning or as eluents in mass spectrometry, are available at various grades of purity, and the highest purity grades (generally UHPLC grade) should be sufficient for trace level organic analyses. However as shown by King et al. (2019) following the work of Chapter 3, the ozonation of solvents may further reduce background contamination of certain fatty acid compounds, even in these 'super-clean' solvents.

Sample preparation and analyses can also be carried out in a clean-room, however many clean laboratories are designed to be low in inorganics and contain plastics, while the air filtering system may not be designed to exclude organic compounds. More challenging still, a clean laboratory environment when samples must also be kept below freezing requires a clean cold-room. Most cold-rooms where cores are cut are not also clean-rooms, and even less likely clean with respect to organics. Therefore, the contaminants present should be quantified before analyses. At the British Antarctic Survey, Cambridge, UK, for example, all surfaces including the saw blades are cleaned before ice cutting with isopropyl alcohol (IPA) solution. Therefore, this solution is also tested here as a contaminant source alongside the drilling fluids and storage bags.

2 Methods

All contamination-source and ice-diffusion sample analyses was performed using the Orbitrap^{TM} high resolution mass spectrometer coupled with an LTQ Velos linear ion trap in

the Department of Chemistry, University of Cambridge, using a variety of sample infusion methods dependent on media being tested. These are detailed further in the following sections.

2.1 Drill fluid and isopropyl alcohol (IPA)

Drilling fluids tested in this study were Exxsol^{TM} D60, ESTISOL 140, ESTISOL 240, and COASOL. Two separate batches of ESTISOL 140, batch one and batch two herein, were used to look for compound variability within fluid types.

All drill fluids and IPA were prepared in 1:10 dilutions with a solvent mixture of 49% Acetonitrile/49% Chloroform (both >99.9%, Optima™ HPLC/MS, Fisher Chemical), containing 2% water with 0.1% formic acid (Optima™ LC/MS, Fisher Chemical). Pilot studies of other solvent/fluid ratios showed lower dilutions were below clear detection of the mass spectrometer.

Prior to sample infusion, a 'lock mass' function was applied to the Orbitrap^{TM}, whereby an observed common contamination peak of known mass and formula is used to shift the entire mass spectrum by the difference in m/z that is recorded, compared to that which is known. Thus, the entire mass spectrum is adjusted along the m/z scale, reducing m/z measuring error. In negative ionisation mode, the mass peak 311.16897, corresponding to the formula $C_{17}H_{27}O_3S$, was applied. In positive ionisation mode the lock mass peak 305.157070, corresponding to $C_{12}H_{26}O_7Na$, was applied.

Samples were injected for analyses using 'Nanomate® ESI direct infusion', whereby the sample is directly infused in to the LTQ Orbitrap^{TM} from an automated sample tray for immediate ionisation (as opposed to applying preceding methods of chromatographic separation such as liquid chromatography, gas chromatography, for example). Multiple repeats of both sample and blank (solvent mixture only) infusions were performed over spectrum ranges from 50-600m/z and 100-1000m/z in both positive and negative ionisation mode of the LTQ Orbitrap^{TM}. Distinct spectrum ranges allows for optimal detection along the entire m/z scale, while overlapping is accounted for when combined together following a Mathematica processing script method outlined later.

2.2 Layflat analyses

Both non-EVA and EVA layflat bags were analysed using a 'LESA universal adaptor plate' as a direct surface analysis tool; rather than direct injection of a liquid sample in to the Orbitrap™, a droplet of solvent (mixture of 49% Acetonitrile/49% Chloroform (both >99.9%, Optima™ HPLC/MS, Fisher Chemical), containing 2% water with 0.1% formic acid (Optima™ LC/MS, Fisher Chemical) is pipetted on to a section of the layflat bag, attached inside-face upwards on to the LESA plate within the Nanomate® (Figure 1). The droplet remains on the surface for 5 seconds, during which time soluble compounds from the bag are extracted in to the droplet, which is then drawn back in to the pipette tip for direct injection. Thus, each recorded spectrum corresponds to a single solvent droplet, for a point on the layflat surface. Three separate strips of non-EVA and EVA layflat bag were analysed with five randomly placed droplets on each to account for heterogeneity in layflat surface properties. Solvents were the same as that used in the drill fluid analyses, as was the lock mass function. Individual spots on the same layflat strip were combined to be a single representative sample using a Mathematica processing method later outlined.
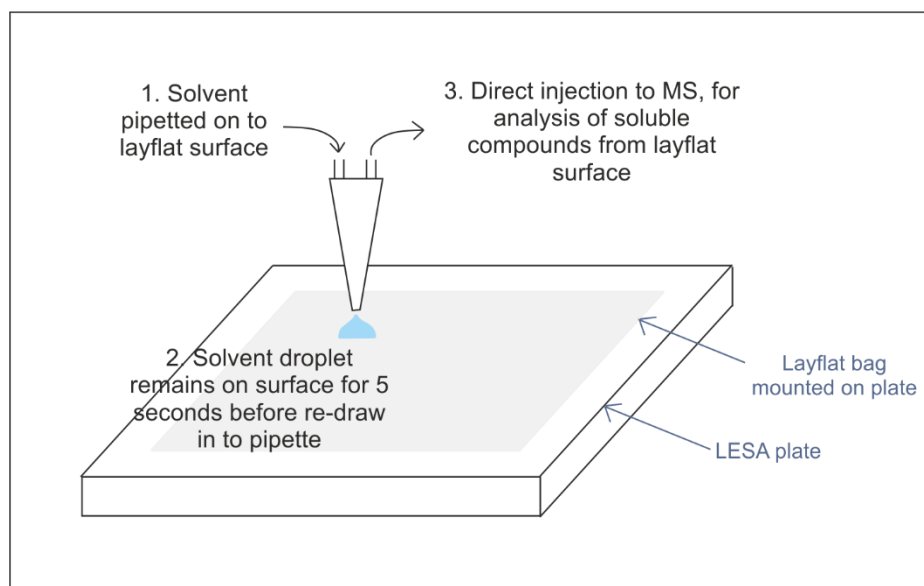

Figure 1: Schematic illustration of the LESA universal adaptor plate, housed inside a Nanomate® which controls the pipette and solvent injection to the attached mass spectrometer. Media, in this case a layflat bag, are mounted on the plate to allow surface compound analyses.

2.3 Compound diffusion through ice core surfaces

Representative proxy ice cores were prepared by freezing Milli-Q ultrapure water inside non-EVA layflat bags. Cores were frozen at -25°C for at least one week prior to further processing.

The outermost surface of each core was coated in a thin layer of ESTISOL240 drilling fluid, by placing inside a non-EVA layflat bag coated on the inner surface. The cores were rolled inside the bag to ensure complete surface coverage, and then placed inside a cold-room (kept between 20-25°C) for long-term storage. ESTISOL 240 was chosen as it is sourced from distilled ester of coconut oil extract, rather than the synthetic esters used for the other fluids, and would be predicted to pose most risk in contamination especially for fatty acids. Coated cores were left for 3 months before analyses, to represent the time taken for transport of cores from the field and storage before processing.

The core was cut in to 1-inch thick cross-sections using a band saw, the blade of which had been cleaned using IPA solution. Worktop surfaces had been covered in aluminium foil. The ice was cut from a non-fractured length of core. The 2.5cm slice was cut in half across the diameter line, and a 3D cubic rectangular section cut from the inner to outer core (Figure 2). Cut surfaces of the ice section were scraped with a clean metal scalpel to remove possible contamination from the band saw blade, working surfaces, or drilling fluid from the outer core contacting the inner core layers after cutting.

The ice section was cut in to individual 2 mm layers (the minimum resolution at which enough liquid sample was produced to infuse in to the Orbitrap^{TM}) working from the outer core to inner core using a Microtome. This instrument uses a metal blade to cut layers of ice from the ice section held between blocks that can be adjusted on a micrometer scale. Each 2 mm section was scraped from the microtome blade in to a separate amber glass sample vial with PTFE-lined caps and stored in a freezer until analyses.

Sample analyses were carried out by firstly using high performance liquid chromatography (HPLC) with a post-column injection of ammonium hydroxide in methanol, before infusion in to the mass spectrometer. Chromatographic separation and instrumental analyses had been optimised for detection of analytes in Table 1, the details of which can be found in Chapter 3, describing the full LC-MS optimisation for sample analyses. This method was applied as it allows a more specific determination of the presence of this studies target compounds (Table

1) than the direct injection – Mathematica processing method. Processing used Xcalibur software. Secondly, samples were analysed using direct injection with processing using the Mathematica software, as previously applied to the drilling fluids and detailed below; this allows investigation of a wide range of compounds present in the samples (not just target analytes) for a broader assessment of the contamination potential via diffusion of the drilling fluid into the inner part of the ice core.

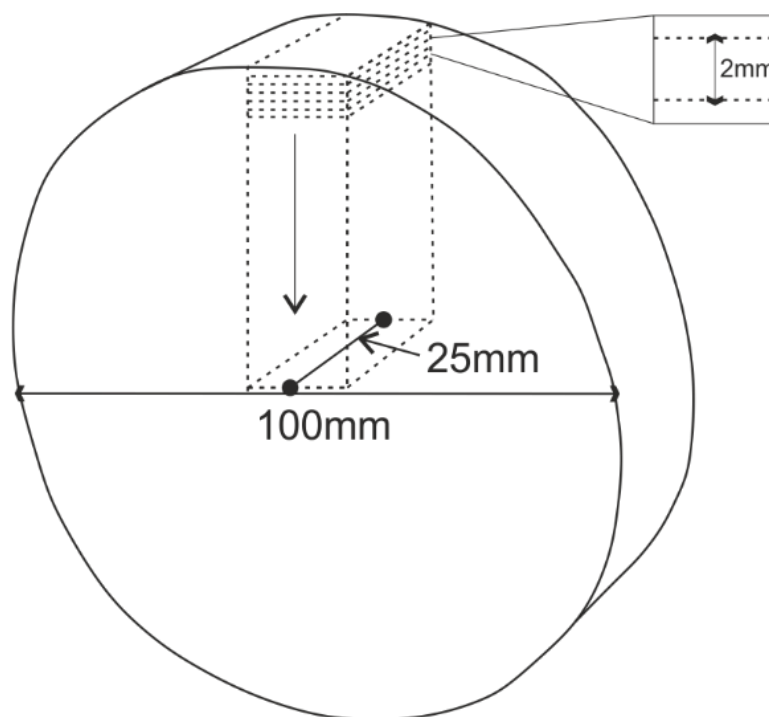**Figure 2:** Schematic diagram of cross-sectional core slice, with dashed lines showing subsequent mm-scale slicing on a microtome. Each 2 mm shaving is kept as an individual sample.

2.4 Data processing and MS-MS analyses

Mass spectrum files acquired by direct injection analysis of the mass spectrometer simply provide a series of 'peaks' recorded as relative intensity at the detected compounds' mass to

charge (m/z) ratio. Data requires subsequent further processing to define each peak as a formula-assigned compound, which was done using in-house developed codes run through the software Mathematica 10.0 (Wolfram Research Inc., UK) (Zielinski et al. 2018).

As well as assigning a formula to the identified peaks within pre-selected boundaries of formula possibilities (in this case allowing $1 \leq {}^{12}C \leq 100$, $0 \leq {}^{13}C \leq 1$, $1 \leq {}^{1}H \leq 200$, $0 \leq {}^{16}O \leq 50$, $0 \leq {}^{14}N \leq 5$, $0 \leq {}^{32}S \leq 2$, $0 \leq {}^{34}S \leq 1$, with an additional $0 \leq {}^{23}Na \leq 10$ for positive ionisation mode files only), the Mathematica code applies a series of 'rules' to filter unrealistic formula assignments such as carbon and sulphur isotope ratios and the nitrogen rule. It then further treats the data by correcting for mass drift, noise levels and shoulder ions, and filters out multiple assignments to single peaks or multiples created by repeated runs of the same samples. The processing steps have been previously successfully applied to environmental samples such as the detection of organic compounds in aerosol following ultrahigh resolution mass spectrometry using the Orbitrap^{TM} (Kourtchev et al. 2013).

The outputted compound formula list was searched for matching compound formulas to the pre-defined organic biomarkers lists (Table 1). A number of compound formulas were flagged as matches. While the Mathematica code is a good way to assign formulas to very large datasets in a time-efficient manor, multiple possibilities of compound formulas for any particular compound mass (a risk which increases for increasing compound mass) may result in incorrect assignments. Therefore it was decided to more precisely determine the composition of the flagged formula assignments using MS-MS analyses.

For MS-MS analyses a defined m/z value (defined by the user based on the compound targeted as identified above) is fragmented into constituent fragment ions in the ion trap part of the Velos LTQ Orbitrap. Therefore, it can be determined more accurately whether the code-assigned formula is the same as the actual target ions, or whether the formula has been incorrectly assigned. However it should be noted there is still an error associated with this process, as the same formula may be found in structural isomers. For example, m/z 172.178, identified in the EVA layflat bag in negative polarity, was assigned to the most likely formula of C8H12O4, which could correspond to norpinic acid. The MS-MS fragment ions displayed peaks at m/z values of 154, 128, and 111 showing that the compound lost fragment ions of M-18 (i.e. 172 minus 154), M-44 and M-61. These peaks correspond to the loss of H_2O (M-18) and CO_2 (M-44), respectively, however a molecular mass of 61 has no possible fragments without the inclusion of a nitrogen (N) atom. As C8H12O4 does not contain N, it is therefore

ruled an invalid formula assignment, and is removed from the contaminant list as it would not interfere with the identification and quantification of norpinic acid. This process was completed for all 58 matching compounds found across the contaminant samples.

The applied direct injection method does not allow for quantification of these confirmed contamination peaks. We can therefore only conclude on their presence. Applying HP-LC analysis would allow this, as it allows quantification from calibration standards. However, while this is possible for the more dilute ice core diffusion samples, injecting purer drilling fluid samples in to a HP-LC system and column risks exceptional contamination of the system and therefore was not applied at this time.

3   Results and discussion

3.1   Drill fluids, IPA and layflat bags

Output files, following Mathematica processing, gave a long-list of assigned compound formulas for both positive and negative ionisation mode analyses run for each of the tested contamination media. These files show every individual compound formula assigned within pre-defined boundaries. Total number of assigned formulas ranged from 319, in batch two of the ESTISOL 140 drilling fluid, to as high as 23,995 in the non-EVA layflat bag (Table 2). There were greater numbers of organic compounds assigned in the layflat bags compared to drill fluids. However, it can be said that all contaminant sources display a substantial number of organic molecules.

Additional MS-MS fragmentation analyses showed there to be four compounds potentially matching those on the target list; two were sourced from the EVA layflat (tentatively assigned as β-caryophyllinic acid and MBTCA), one from the non-EVA layflat (tentatively assigned as levoglucosan) and one from batch one of ESTISOL 140 (tentatively assigned as β-caryophyllinic acid), and all detected in negative ionisation mode. MS-MS analyses eliminated all fatty acid compounds from the initial list of matching compounds, and retained these few SOA compounds; this could be due to the greater error in the Mathematica processing for large compounds (in this case, fatty acids) due to more possible formula combinations producing the same compound mass. Smaller compound assignments by comparison are more likely to be confirmed as correct by MS-MS.

Table 2: Table showing the total number of formulas assigned by the Mathematica processing code, in both positive and negative ionisation mode, for each contaminant source analysed. Also shown are the number of these formulas which matched compounds on the potential biomarkers list, and finally the number of these compounds which were confirmed as matches following detailed MS-MS fragmentation analysis.

	ESTISOL 140 Batch 1	ESTISOL 140 Batch 2	ESTISOL 240	COASOL	Exxsol™ D60	IPA	EVA Layflat	Non-EVA Layflat
Positive Ionisation Mode								
Total number assigned compound formulas	370	319	665	417	1297	462	23,382	23,995
Total formulas matching biomarkers list	3	5	2	0	4	2	5	4
Confirmed compound matches by MS-MS	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Negative Ionisation Mode								
Total number assigned compound formulas	792	770	746	1293	792	1255	26,431	23,366
Total formulas matching biomarkers list	3	1	1	1	3	10	9	5
Confirmed compound matches by MS-MS	**1**	**0**	**0**	**0**	**0**	**0**	**2**	**1**

Elemental ratios of the long-list of assigned formulas can be used to group compounds of similar properties. This idea applies the Van Krevelen method (van Krevelen 1950) which plots the atomic H/C ratio against the atomic O/C ratio of the assigned formulas. The method was originally used for assigning origins of oil in the petroleum industry based on the presence of major compound groups such as lipids. Compounds of particular groups such as these tend to cluster in certain areas of the plot because of common elemental ratios.

An example Van Krevelen plot is shown in Figure 3 for batch one of ESTISOL140 measured in negative ionisation mode. Alongside, H/C and O/C ratios of the compounds on the pre-defined biomarkers list are plotted similarly on a Van Krevelen plot, as well as some specific examples and their molecular formulas plotted on the main Van Krevelen plot to help with comparison. There are clear distinctions between compound types, for example fatty acids cluster together at low O/C ratios and high H/C ratios; this is in corroboration with the defined areas of the Van Krevelen plots of Minor et al. (2015) and Minor et al. (2014), defining the same area as indicators of lipid content in petroleum fluid. Sesquiterpenes, which are the largest compounds of all the SOA types here tested, plot closest to the fatty acids which are of similarly larger size, while the smaller compounds on the list, the isoprene and monoterpene SOA compounds, have higher O/C ratios compared to fatty acids.

The Van Krevelen plot is useful to give an impression of which contaminant sources pose greatest risk to particular compound groups. Further Van Krevelen plots in Figures 4, 5, 6 and 7 show each of the analysed drilling fluids, layflats, IPA and also a comparison for the two different batches of ESTISOL 140. Figure 3 additionally highlights the compounds found to match the compound list after MS-MS analyses, as in Table 2. It is notable that all potential contaminating sources show a high concentration of compounds plotting in the region where fatty acids are grouped in the Van Krevelen plot (as in Figure 3). This is not surprising; fatty acids are common compounds in the general environment. The lowest concentration of fatty acids appears to be found in the COASOL drill fluid (Figure 5), though conversely this seems to be the only fluid which displays a high concentration of compounds in the region of the plot containing isoprene SOA compounds. It may therefore be lower risk to analyse particular compounds types in different cores depending on the fluid that has been applied to core if already drilled, or to choose the fluid dependent on future analyses planned.

Comparing the two batches of ESTISOL 140, shown in Figures 3 and 5, shows considerable variability on a compound-to-compound basis in fluid composition. This difference may be

caused by either a difference in bulk composition at the manufacturing stage, or may be due to differences caused after this such as contaminants from containers the fluid has been stored in. Either way, it indicates that one study is not enough to specifically characterise contaminants in a particular type of drill fluid, and to do this most accurately a sample of the specific fluid used for a drill campaign would need testing.

Considering the number of noteable risks for contamination potential discussed above, it is somewhat reassuring that only (maybe less than) four of the target compounds were highlighted as present across all media tested. Three of the four were from layflat bags, and in new drill campaigns this would be easily moderated against if the core was wrapped in foil rather than (or even inside of) layflat bags. For older cores already in storage in layflats, and for cores for which drilling fluid is required, the option becomes removal of the contaminated outer ice. This requires knowledge of the diffusion potential of the present contaminant over time, and subsequently how much ice to remove to leave totally contaminant free ice. This is examined in the following section.
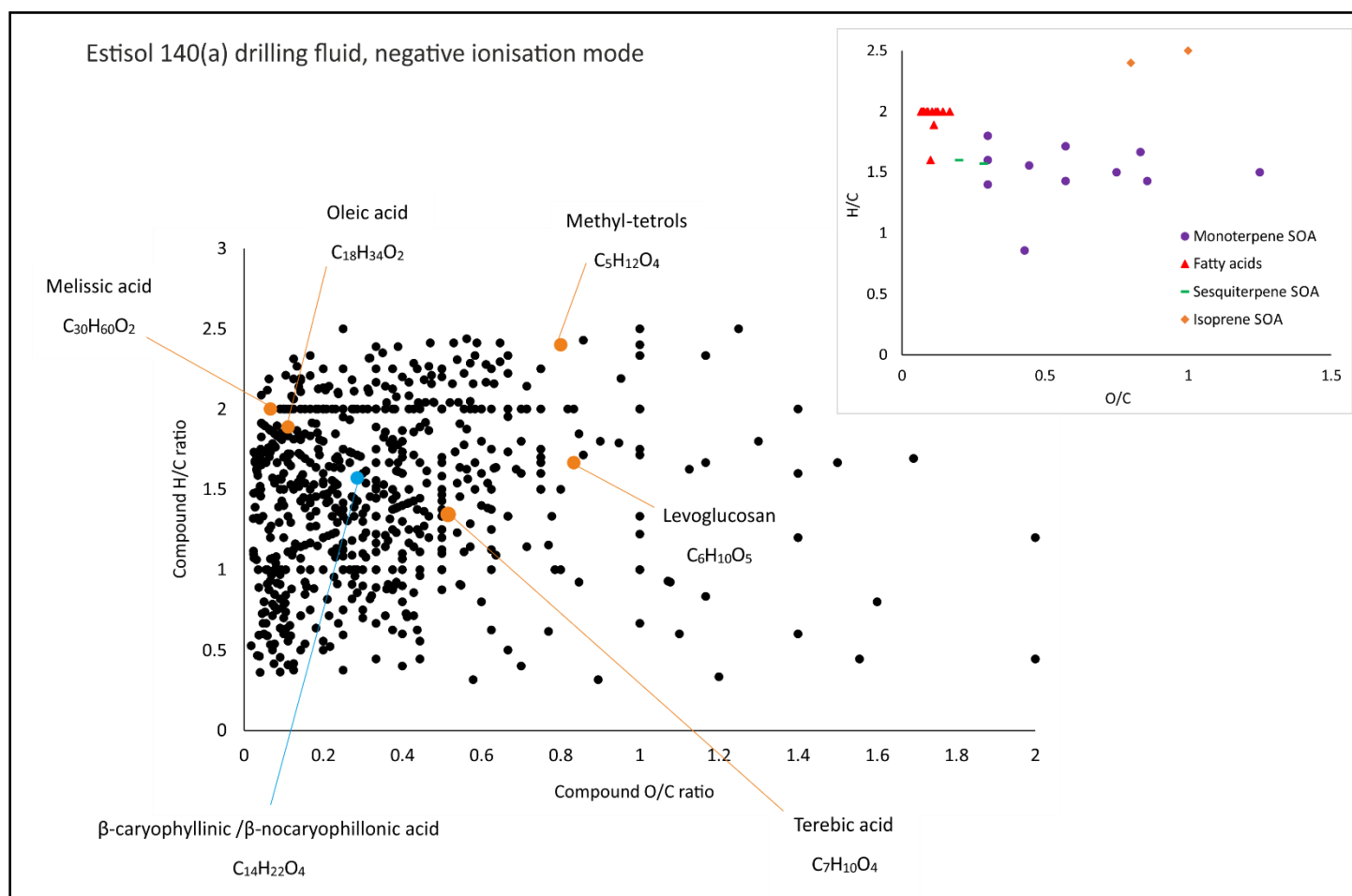
Figure 3: Van Krevelen plot for batch one of ESTISOL 140 measured in negative ionisation mode. Highlighted are some specific examples of this studies compounds and their molecular formulas to help with comparison: orange circles indicate compounds not found in the fluid, while blue circles indicate compounds found to be present in the fluid and therefore a possible contaminant source. Alongside, H/C and O/C ratios of the compounds on the pre-defined biomarkers list are plotted similarly on a Van Krevelen plot to show expected groupings.
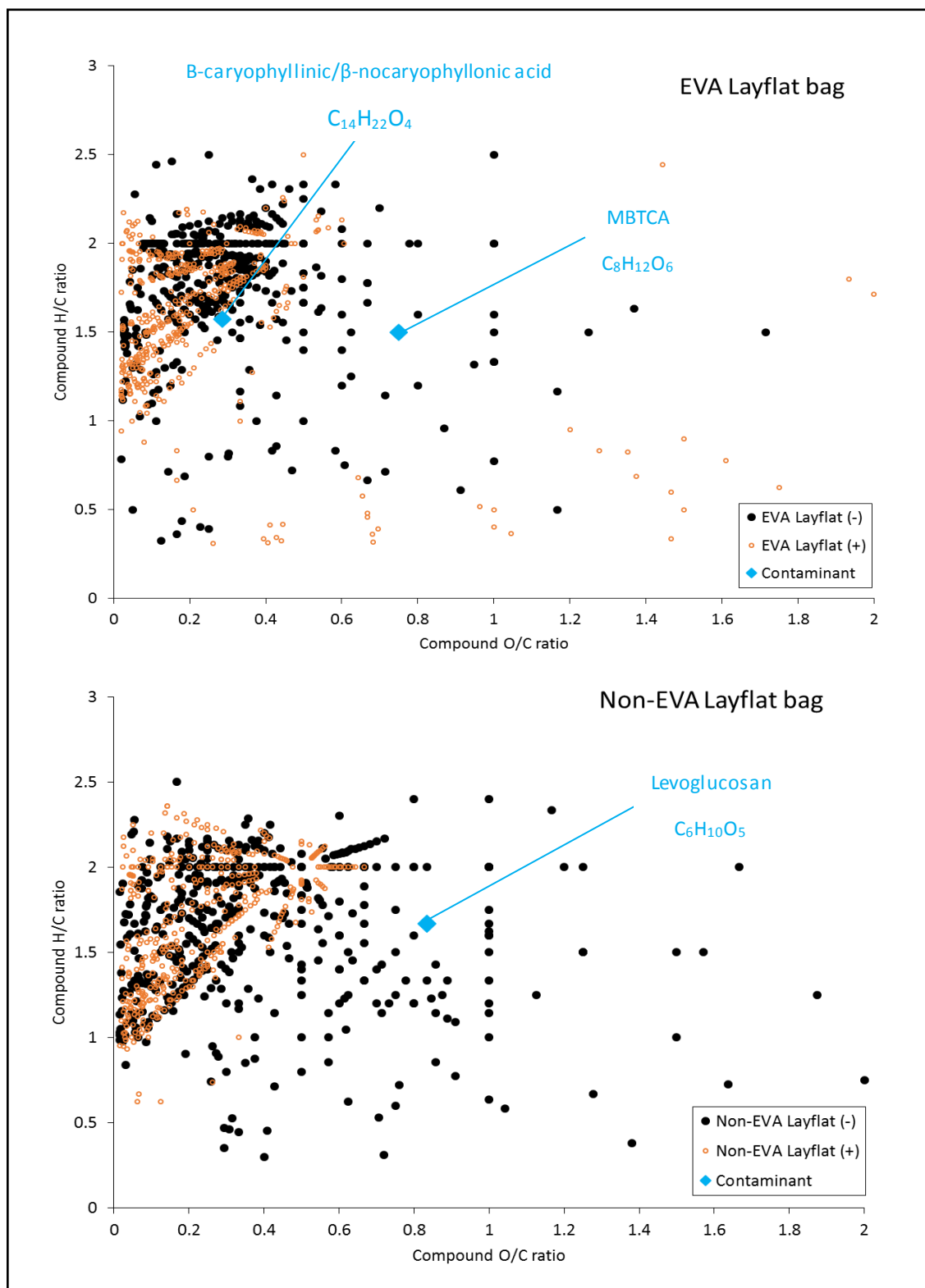
Figure 4: Van Krevelen plots for EVA and non-EVA layflat bags. Due to the very high number of compound formulas assigned during processing (<20,000 in both ionisation modes), only the 450 highest relative intensity points are plotted to allow clear visualisation. Blue dots indicate compounds (matching the target list) found to be present in the fluid.
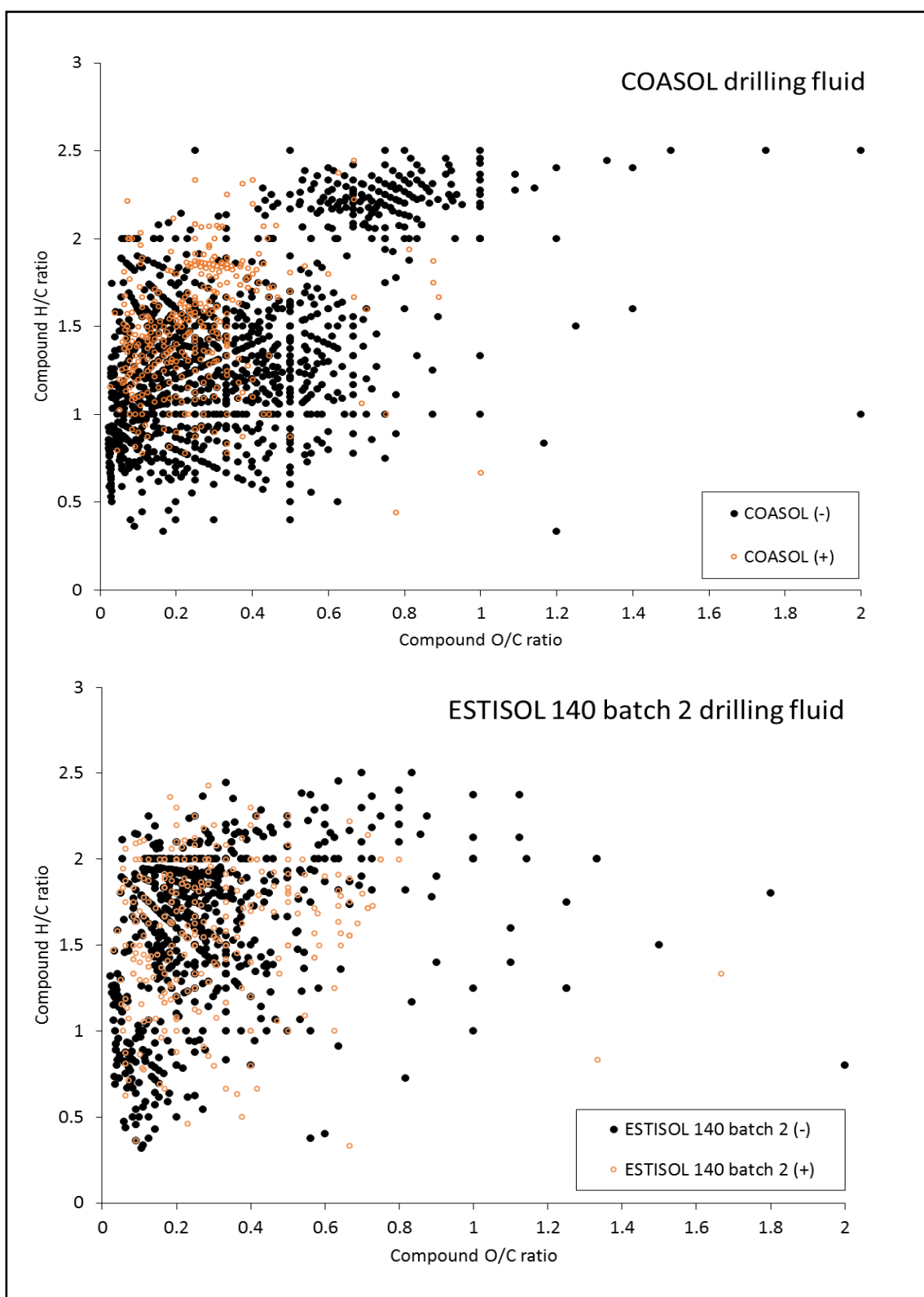
Figure 5: Van Krevelen plots for COASOL and the second batch of ESTISOL 140 (as compared to batch one in Figure 2).
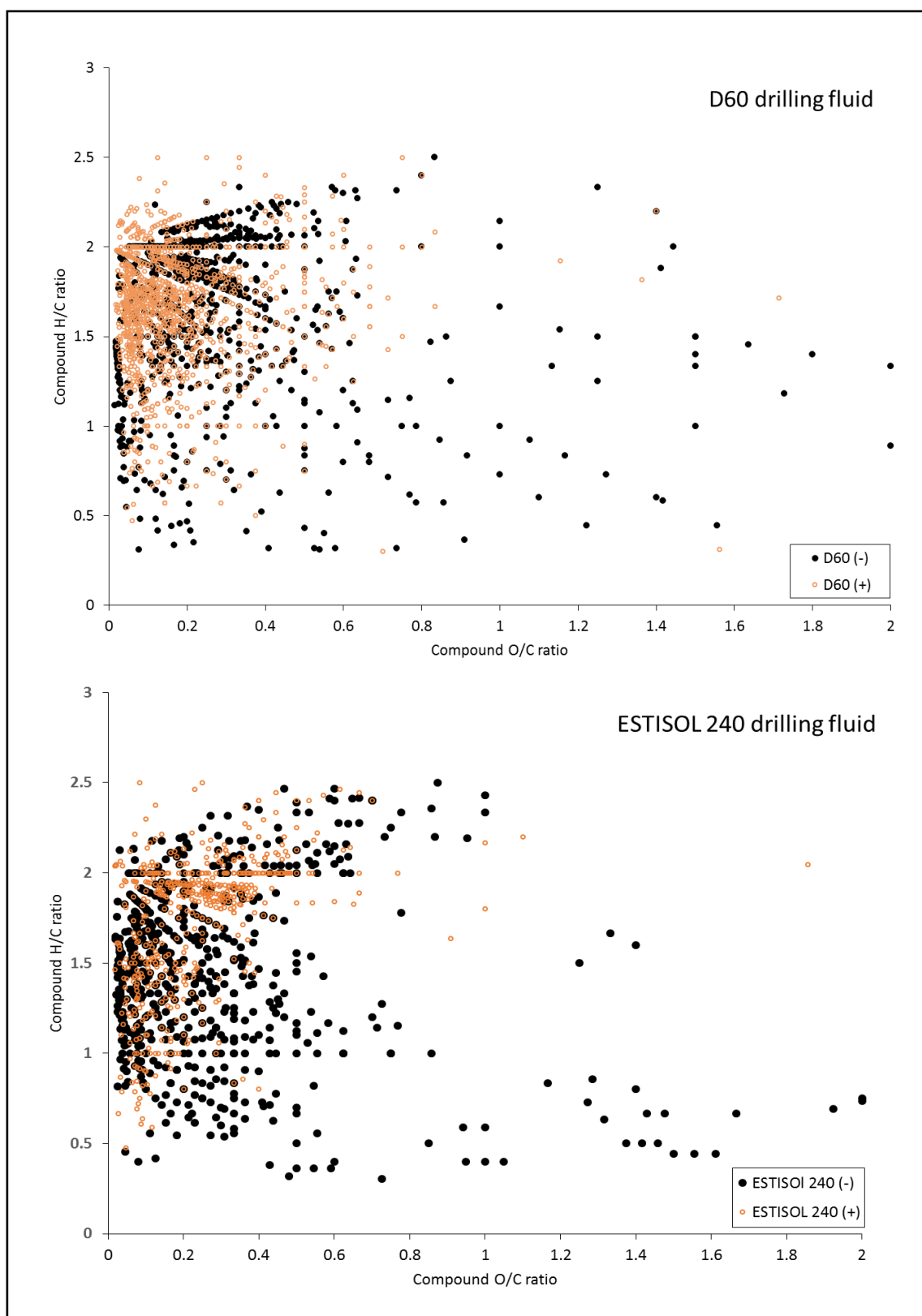
Figure 6: Van Krevelen plots for D60 and ESTISOL 240 drilling fluids.
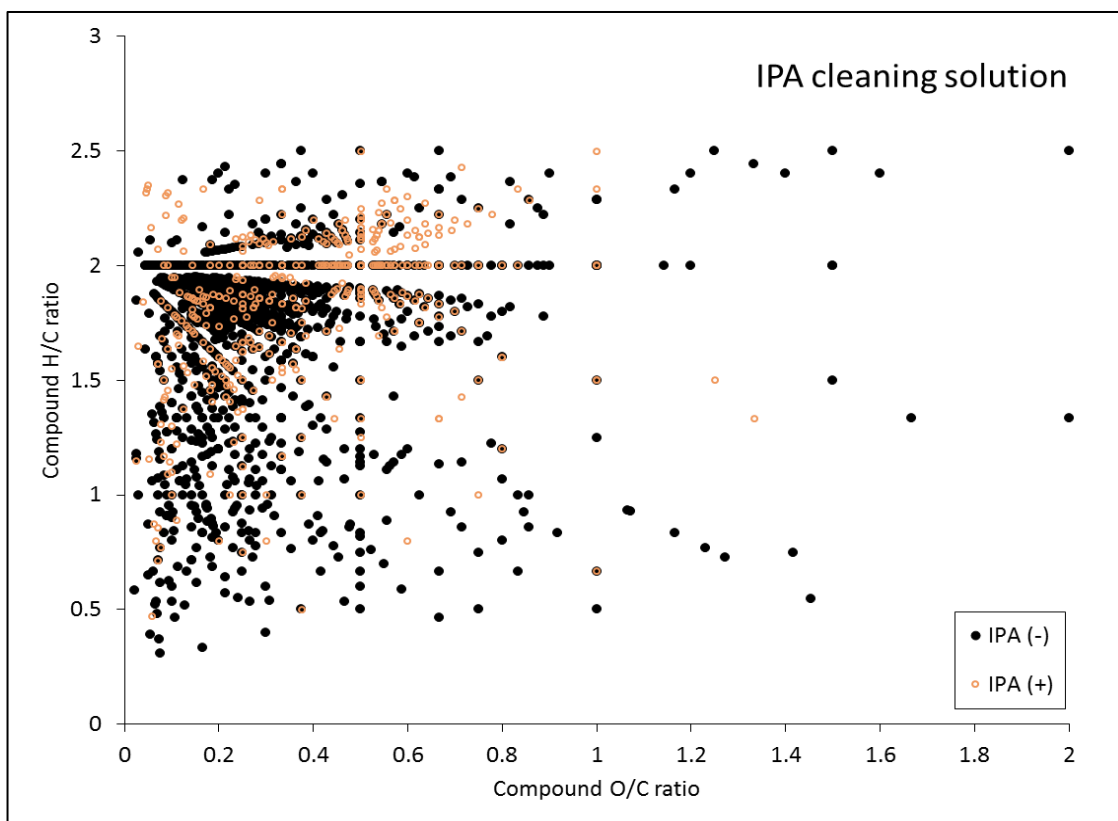
Figure 7: Van Krevelen plots for IPA cleaning solution

3.2 Ice core drilling fluid diffusion

Initial LC-MS analyses showed none of the target analytes of this study were present in the samples of ESTISOL 240- coated Milli-Q core, although they may be present below detection limits. However many other organic compounds are found in the samples based on direct injection MS analyses, and these allow us to test the diffusion potential of organic compounds through the core surface.

To test for a diffusion pattern through outer ice layers using other organic compounds present, the total list of outputted compounds from the Mathematica code was sorted based on relative intensity of the output signal. The 30 compounds with highest relatively intensity were selected, and plotted as either 'present' or 'absent' in each individual ice layer through the core section by matching to the compound lists for each individual 2mm layer. The diffusion pattern for each of these 30 compounds is shown in Figure 8. For simplicity, the compound has been

assigned a label number 1-30 instead of its assigned formula. The figure clearly shows the dominant presence of all compounds on the very outer layer of ice, between 0-2 mm, but there is no significant trend found in diffusion pattern persisting from outer to inner core up to 2 cm.

To assess the relative contributions of each contaminant source to the presence of the compounds in the outer core layers, total formula lists outputted previously for each of ESTISOL 240, non-EVA layflat bag, and IPA solution were searched for formula matches to the 30 compounds. In total, 13 of the compounds found in the ice analyses were also found in the non-EVA layflat, two in the IPA solution and only one in the ESTISOL 240 drill fluid (Table 3). Therefore, the strongest contribution to identified contaminants appears to come from the core storage layflat bags. Among the 13 compounds highlighted in this category, there are some compounds which show presence through the whole outer 2 cm surface, which may be a concern if this suggests diffusion through this whole section. However the majority of compounds proposedly from this source are more sporadically identified, showing no diffusion pattern, or sitting only on the outer surface. This study focuses on these overall patterns as a first investigation of contamination potential, however more detailed studies would be required in the future to investigate these more pervasive compounds.

The compound $C_4H_5O_5S$ is the only one to be associated with a drilling fluid source, identified as compound one in Figure 8, does not have a consistent pattern of diffusion from outer to inner ice, the possibility that it may be a contamination which also comes from an additional source cannot be ruled out (rather than being unique to the drilling fluid).

Compounds identified from the IPA solution (numbered 17 and 21 in Figure 8) were found only on the very outer surface of the core between 0-2 mm, in agreement with this being the only part of the core coming in to contact with laboratory surfaces cleaned with the solution. However one of the two compounds was also shown to be present in layflat bags. There was no trend in the contribution of compounds by layflat bags and their pervasiveness through core layers.

There were 10 compounds of the 30 which had no matches to tested contaminant sources; there are other potential sources of organic compounds in the proxy ice core for example Milli-Q water itself, the atmosphere of the lab where the cores are made, as well as from general handling from contact with laboratory gloves and surfaces.

This study does not test Milli-Q water as a contaminant source, since each Milli-Q system is unique depending on environment of use and maintenance and the aim of this study was to be

able to make a more general recommendation of contamination potential from each media. However consideration of Milli-Q sources is particularly important for compounds which are present throughout the whole outer surface tested in this study; these are most likely to come from the Milli-Q used to make the proxy-core. However, if it was found these compounds were not from the Milli-Q system, there may be cause to suggest that contaminants are showing potential to diffuse from the outer surface through at least 2cm of ice. Nonetheless the focus of this study remains on the overall pattern observed in compounds.

Contaminants which appear more sporadically through the core layers are most likely to come from sources in the lab processing or analytical steps, since these may be variable sample to sample. For example, although cleaning protocols are followed, each sample is in a different vial and transferred using a different pipette, etc. This highlights the importance of stringent cleaning protocols to unify all samples measured, since these compounds are so sensitive to any contamination even at very low levels.
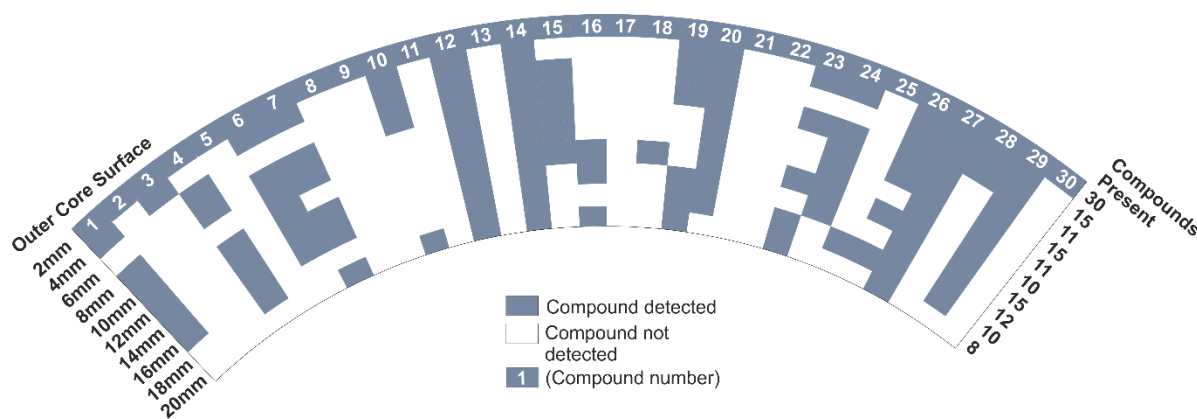
Figure 8: Illustration of contaminants through the outer 2cm of core for the 30 highest intensity organic compounds detected in the outermost core ice sample (0-2mm), and their presence or absence through progressively more inward layers. Each compound, 1-30, is shown as an individual core strip and its presence or absence in the ice shown by blue or no shading respectively. The total number of compounds present in each successively inward 2mm section of ice is shown as a total count. Information on the compounds formulas and their prospective sources can be found in Table 3.

Table 3: Table of assigned formulas for each of the 30 diffusion test compounds (as numbered in Figure 8) and suggested source for each based on the presence of the compound in the potential contaminating media applied to the core.

Compound	Formula	Non-EVA Layflat	ESTISOL 240 Drill Fluid	IPA
1	$C_4 H_5 O_5 S$	✓	✓	
2	$C_7 H_5 N_2 S_2$	✓		
3	$C_{22} H_{42} O N$	✓		
4	$C_{15} H_5 O_{11} S_2$			
5	$C_{17} H_{32} N_5 S$			
6	$C_4 H_7 O_4 S$	✓		
7	$C_8 H_{11} O_6 N_2 S$			
8	$C_{16} H_{13} O_6$			
9	$C_{12} H_{24} O_2 N$			
10	$C_{13} H_3 O_8 S_2$			
11	$C_{32} H_{39} N_2$			
12	$C_{24} H_{33} O_6$	✓		
13	$C_4 H_7 S_2$			
14	$C_7 H_9 O_3 S$	✓		
15	$C_5 H_7 O_6 S$	✓		
16	$C_{14} H_{29} O_8$			
17	$C_9 H_{21} O_3 N_2$			✓
18	$C_{17} H_{31} O_2 N_2$			
19	$C_{22} H_{40} O N$	✓		
20	$C_{10} H_{15} O_5 S$			
21	C22 H43 O8	✓		✓
22	$C_{14} H_{17} S_2$	✓		
23	$C_8 H_4 O N_5 S$			
24	$C_9 H_{15} O_6 S$			
25	$C_{16} H_{35} N_4$			
26	$C_{20} H_{38} O N$	✓		
27	$C_{15} H_7 S$			
28	$C_{18} H_{34} O N$			
29	$C_9 H_{13} O_4 S$	✓		
30	$C_{12} H_{22} O_2 N$	✓		

4 Conclusions

Drilling fluids Exxsol^{TM} D60, ESTISOL 140, ESTISOL 240, and COASOL, IPA solution, EVA coated layflat bags and non-EVA layflat bags were tested for their potential to contaminate this studies target analytes. Results show that all media contain a large amount of potential organic contaminants, especially layflat bags and especially for fatty acid compounds. Different batches of the same drilling fluid may also vary in composition, and therefore quantification of contamination potential of particular compounds is needed on a case-by-case basis. A very few SOA compounds tentatively identified in the contaminating media were found to be matched to the list of target analytes, resulting in the need to assess how much of a threat is posed to ice analyses when these compounds have contacted the outer core surface.

Diffusion tests of ESTISOL 240 through a core stored in a non-EVA layflat bag and processed on IPA-cleaned surfaces showed that surface contamination was limited to only the outer few mm of solid (i.e. not fractured and not firn) ice, and there was no significant pattern of diffusion within the outer 2cm of the core. However, there remains a risk of contamination for all ice-surfaces exposed to contaminating media or indeed the general environment.

Therefore contamination-limiting protocols are outlined:

Ideally, ice cores should be stored wrapped in aluminium foil directly from the field-drilling site. During cold-room processing of the core, worktop surfaces should also be covered in aluminium foil, while band-saw blades should be thoroughly cleaned using high-purity grade solvents. Contact with the drill itself and the possible need for drill fluid application for deep-cores is unavoidable, but can be moderated for in future sample preparation by removal of the outer ice layer. Outer ice core sections are more appropriate for analyses such as isotopes which have very limited contamination potential, but if only an outer ice section is available for organics analyses removal of the outer few mm of ice is a must. Once cut, sample surface should be scraped clean to remove just the very outer surface which may have contacted work-surfaces, eliminating carryover of contamination from outer core sections which have also contacted the surface. This should be done using metal or ceramic utensils pre-cleaned using high-purity grade solvents. Samples should be directly stored in sealed glass vials with PTFE lined caps, pre-cleaned by baking and/or thorough solvent washing. Ideal sample processing conditions would include a clean-room for organics, but this a rare facility in current ice core laboratories. Working under a fume-hood or laminar flow bench in an inorganics clean-room

may still provide a lower contamination environment than a general lab, but keeping sample vials sealed and limiting processing steps is beneficial. Procedural blanks are required for each individual processing step, and for the following instrumental analyses, allowing accurate quantification of contamination.

The aim of this study was to give an overview of contamination potential from a variety of source-media. This is just a starting point to clarify that results of sample testing, as later shown in Chapters 4 and 5, would be valid following the above guidelines. However, more detailed, compound specific investigation would be required in the future leading from this investigation including testing on fracture and firn core sections.

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Chapter 3: Methodological development

Preface

Chapter 3 is a combination of two published, peer-reviewed, research papers:

King, A.C.F., Giorio, C., Wolff, E., Thomas, E., Karroca, O., Roverso, M., Schwikowski, M., Tapparo, A., Gambaro, A., Kalberer, M. 2019. A new method for the determination of primary and secondary terrestrial and marine biomarkers in ice cores using liquid chromatography high-resolution mass spectrometry. Talanta, 194, pp.233–242.

King, A.C.F., Giorio, C., Wolff, E., Thomas, E., Roverso, M., Schwikowski, M., Tapparo, A., Bogialli, S. Kalberer, M. 2019. Direct injection liquid chromatography high-resolution mass spectrometry for determination of primary and secondary terrestrial and marine biomarkers in ice cores. Analytical Chemistry, 91(8), pp.5051-5057

The choice of column and method of chromatographic separation using liquid chromatography, with post-column injection, was developed by Dr Chiara Giorio preceding this PhD project, and the section in this chapter reporting this method development (section 3.1) was written by Dr Chiara Giorio (originally in King et al. 2019a), as well as the paragraph describing matrix effects of the direct injection samples (section 3.7, originally in King et al. 2019b). It is necessary to include these sections to maintain the full story of the method optimisation both preceding and during this PhD project. Testing of the SPE method was carried out alongside a visiting PhD student project whom I supervised (Ornela Karroca, University of Venice), helping to decide which optimisation steps to perform and carrying out some of the SPE experiments.

All other method development work, data analyses, and writing of the chapter was carried out by Amy King.

Chapter 3: Table of Contents

1. Introduction

A small number of organic compounds have already been investigated and shown to give robust environmental records, including biomass burning markers (Kehrwald et al. 2012; Kawamura et al. 2012), anthropogenic pollutants such as persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) (Jaffrezo et al. 1994; Gabrielli & Vallelonga 2015), and the sea-ice proxy methanesulfonic acid (MSA) (Abram et al. 2013). Thus we can say with confidence that there are proven robust analytical methods for organic compounds in ice, as well as a chance to obtain robust palaeoclimate records from them. Non-anthropogenic organic compounds sourced from both the terrestrial and marine biosphere have been proposed to be similarly useful (Giorio et al. 2018) though in general are not as well developed in either analytical quantification, or our understanding of any available records. Therefore, analytical quantification has so far been done by a range of experimental methods and on varying lists of compounds from several distinct compound groups.

As introduced in Chapter 1, the aim of this project is to target two main organic compound groups in the development of an analytical method, fatty acids and terpene oxidation products, alongside a biomass burning marker, and a primary biogenic (Table 1). The method is to use high performance liquid chromatography – mass spectrometry systems (HPLC-MS) to provide a unified analytical method of quantification for this broad list of organic compounds in ice samples, with the aim of applying this method to ice samples in following Chapters.

Some compounds from our target list have been detected in snow throughout polar and low-latitude mountainous regions and with records dating back over many centuries. The most successful examples include the detection of lipid compounds in ice layers dating back 450 years at Site J, Greenland (Kawamura et al. 1996), oxidation products of isoprene and monoterpenes in ice up to 350 years old in Alaska (Pokhrel et al. 2015b), and an annually resolved record of carboxylic acids and inorganic ions between 1942-1993 from Grenzgletscher (Monte Rosa Massif) in the southern Swiss Alps (Müller-Tautges et al. 2016). One thing these studies have in common is compound concentrations at parts per billion (ppb, equivalent to µg/L) levels or well below, more commonly parts per trillion (ppt, equivalent to ng/L), leading us to the requirement to preconcentrate samples to allow reliable detection and quantification.

Rotary evaporation preconcentration has been previously applied to both the SOA and fatty acid compound groups including compounds considered in this project. Pokhrel et al. (2015a)

quantified fatty acids in rotary evaporated Alaskan ice samples using gas chromatography-mass spectrometry (GC-MS), with average concentrations of individual compounds ranging between 0.09 and 20.3 ppb (Limit of Detection (LOD - the lowest concentration of a component that can be reliably detected with a given analytical method, or three times the standard deviation of the blank) 0.001 ppb, percentage recovery not reported). Kawamura (1993) achieved LODs of 0.05 ppb for oxocarboxylic acids, and measured dicarbonyls at concentrations of 0.25-1.72 ppb in snow and aerosol samples. In examples of SOA compounds, Pokhrel et al. (2015b) and Fu et al. (2016) rotary evaporated ice from Alaska and Kamchatka respectively, with GC-MS analyses detecting isoprene and monoterpene SOA compounds at 6.99±17 to 692±702 ppb in the Alaskan and 0.05−18.4 ppb in the Kamchatkan (percentage recovery was not reported) ice.

Stir bar sorptive extraction (SBSE) has also been used to preconcentrate snow and ice samples. Muller-Tautges *et al.* (Müller-Tautges et al. 2014) used a polydimethylsiloxane (PDMS) coated bar for extraction of α-dicarbonyls (glyoxal and methylglyoxal). Using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), they report LODs of 0.242 and 0.213 ppb for glyoxal and methylglyoxal respectively, and recoveries of 78.9±5.6 % for glyoxal and 82.7±7.5 % for methylglyoxal.

Solid phase extraction (SPE) is a method which has been often applied to environmental samples for the analyses of organic compounds, however has not previously been used for any of the organics targeted in this project in snow and/or ice samples.

This chapter attempts to optimise an array of these preconcentration techniques, to find those best suiting the long compound list proposed (Table 1) and the following HPLC-MS analyses. Following on from this, testing is carried out attempting to eliminate the need for the preconcentration stage in analyses for i.e. direct injection - HPLC-MS. While preconcentration is still needed in many cases due to the very low levels of organic compounds in polar and alpine ice samples (typically parts per trillion (ppt) – parts per billion (ppb)), some samples closer to source location may contain higher compound concentrations detectable without requiring such a step. Alternatively, new instrumentation certainly presents the opportunity to analyse samples at detection levels as low as ppt, thus removing the need for preconcentration. The elimination of this a preconcentration step would be beneficial for two several reasons; reducing the processing steps of samples reduces the possibility for introduction of contamination, especially in the case of fatty acids where background contamination is generally high compared to SOA compounds. Additionally, for some compounds

preconcentration may be ineffective due to very low recovery from the available methods. Direct injection, if suitable detection limits can be achieved, opens up these additional compounds to ice core analyses, and therefore offers an enhanced suite of compounds for paleo-environmental reconstruction. Finally, the required sample volume for direct injection is also much smaller, in this case approximately 100 µL per sample rather than 10 mL for a sample requiring preconcentration, thus improving the depth and time resolution that can be attained from the ice core. As an example, this will often allow seasonally-resolved samples to be analysed, as opposed to annual or multi-annual records, which will be invaluable to develop an understanding of the processes and sources these novel organic paleo-environmental markers represent. This may also be particularly useful when evolving the method to analyse much older ice than currently tested, where annual ice layers are much thinner, due to ice flow, than those in younger, shallower counterparts. As a long-term perspective, methods requiring low sample volume may be amenable to adaptation for coupling with continuous flow analysis systems.

Adaptation of methods towards those not requiring preconcentration has been previously successfully applied to levoglucosan, an organic compound produced by combustion of cellulose and used to indicate past biomass burning trends from ice core analyses. In order to both circumnavigate the need for preconcentration and to avoid more time consuming GC-MS methods, Gambaro et al. (2008) developed the first method of direct injection HPLC-triple quadrupole tandem mass spectrometry (HPLC/ESI-MS/MS) for quantification of levoglucosan in Antarctic ice samples, where concentrations are expected to be very low. They achieved detection limits as low as 0.003 ppb in samples as small as 1 mL, reproducible at 20-50%, while lowering analysis time and contamination risk, demonstrating the potential benefits of this process.

Table 1: Target compound list for this project, by compound group and in order of increasing number of carbon atoms.

Source	Compound name	Neutral Formula
Isoprene-derived SOA	Meso-erythritol*	$C_4H_{10}O_4$
Isoprene-derived SOA	Methyl tetrols	$C_5H_{12}O_4$
Monoterpene-derived SOA	Pimelic acid*	$C_7H_{12}O_4$
Monoterpene-derived SOA	1,2,4-butanetricarboxylic acid (BTCA)*	$C_7H_{10}O_6$
Monoterpene-derived SOA	3-methyl-1,2,3-butanetricarboxylic acid (MBTCA)	$C_8H_{12}O_6$
Monoterpene-derived SOA	Terebic acid	$C_7H_{10}O_4$
Monoterpene-derived SOA	Pinolic acid	$C_{10}H_{18}O_3$
Monoterpene-derived SOA	Cis-pinonic acid	$C_{10}H_{16}O_3$
Monoterpene-derived SOA	Keto-pinic acid	$C_{10}H_{14}O_3$
Sesquiterpene-derived SOA	β-caryophyllinic acid	$C_{14}H_{22}O_4$
Sesquiterpene-derived SOA	β-caryophyllonic acid	$C_{15}H_{24}O_3$
Sesquiterpene-derived SOA	β-nocaryophyllonic acid	$C_{14}H_{22}O_4$
Biomass burning	Levoglucosan	$C_6H_{10}O_5$
Biogenic SOA	D-malic acid	$C_4H_6O_5$
Primary biogenic	Salicylic acid	$C_7H_6O_3$
Low molecular weight fatty acids (LFA) (<C24); marine / microbial sources	Lauric acid	$C_{12}H_{24}O_2$
	Myristic acid	$C_{14}H_{28}O_2$
	Heptadecanoic acid	$C_{17}H_{34}O_2$
	Oleic acid	$C_{18}H_{34}O_2$
	Nonadecanoic acid	$C_{19}H_{38}O_2$
	Arachidonic acid	$C_{20}H_{32}O_2$
	Behenic acid	$C_{22}H_{44}O_2$
	Tricosanoic acid	$C_{23}H_{46}O_2$
High molecular weight fatty acids (HFA) (>C24); terrestrial biomass	Heptacosanoic acid	$C_{27}H_{54}O_2$
	Octacosanoic acid	$C_{28}H_{56}O_2$
	Melissic acid	$C_{30}H_{60}O_2$

*surrogate standards (analytes chemically similar to those being extracted where actual standard not available).

2. Materials and methods

2.1. Preconcentration – HPLC-MS

Sample analyses, after preconcentration in a rotary evaporator, was carried out using high performance liquid chromatography (HPLC) electrospray ionisation (ESI) high-resolution mass spectrometry (HRMS) with a post-column injection of ammonium hydroxide in methanol. Methods have been optimised for analytes in Table 1 and the optimisations steps leading to this final methodology are described in section 3.

2.1.1. Chemicals and reagents

Dichloromethane (>99.9%, Optima^{TM}, HPLC/MS, Fisher Chemical), and acetonitrile (>99.9%, Optima™ HPLC/MS, Fisher Chemical) were used for preparation of the bulk standard solutions. Standard solutions of each analyte were prepared at a concentration of 100 ppm in acetonitrile for methyl tetrols (synthesised standard), meso-erythritol (≥99%, Sigma-Aldrich®), levoglucosan (99%, Sigma-Aldrich®), ketopinic acid (99%, Sigma-Aldrich®), pinolic acid (Sigma-Aldrich®, analytical grade), terebic acid (Sigma-Aldrich®, analytical grade), MBTCA (synthesised standard), BTCA (99%, Sigma-Aldrich®), cis-pinonic acid (98%, Sigma-Aldrich®), D-malic acid (HPLC/GC suitable, Supelco), salicylic acid (≥99%, Sigma-Aldrich®,), pimelic acid (98%, Sigma-Aldrich®), β-caryophyllinic acid (synthesised standard), β-caryophyllonic acid (synthesised standard), β-nocaryophyllonic acid (synthesised standard), oleic acid (>99%, Sigma-Aldrich®), arachidonic acid (95%, Sigma-Aldrich®), palmitic acid (≥99%, Fluka^{TM}), heptadecanoic acid (≥98%, Sigma-Aldrich®), lauric acid (97.9%, European Directorate for the Quality of Medicines & HealthCare), myristic acid (≥99.5%, Fluka^{TM}), d10-pimelic acid (99%, Sigma-Aldrich®) and d3-malic acid (98%, Sigma-Aldrich®), and in dichloromethane for behenic acid (≥99%, Fluka^{TM}), melissic acid (≥98%, Sigma-Aldrich®), tricosanoic acid (>99%, Sigma-Aldrich®), heptacosanoic acid (≥97%, Sigma-Aldrich®), octacosanoic acid (≥98%, Sigma-Aldrich®), nonadecanoic acid (≥99.5%, Fluka^{TM}) and d31-palmitic acid (99%, Sigma-Aldrich®). Five of the compounds on our list are not commercially available standards and were therefore specifically synthesised and provided by other labs; MBTCA from the lab of Magda Claeys (University of Antwerp, Belgium), methyl tetrols from the lab of Jean-Louis Clement (Aix-Marseille Universite, France), and β-caryophyllonic, β-caryophyllinic, and β-nocaryophyllonic acids from the lab of Thorsten

Hoffman (University of Mainz, Germany). Standard solutions were then combined into a diluted standard mixture of all analytes at a concentration of 1 ppm in acetonitrile. All standards were stored at -18°C.

Methanol (>99.9%, Optima™ UHPLC/MS, Fisher Chemical), water (>99.9%, Optima™ UHPLC/MS, Fisher Chemical), and acetonitrile (>99.9%, Optima™ HPLC/MS, Fisher Chemical) were used as eluents. Ammonium hydroxide (25% in water, LC-MS grade, Honeywell Fluka™), ammonium formate (≥99%, Sigma-Aldrich®), ammonium acetate (≥98%, Sigma-Aldrich®), sodium acetate (≥99%, Sigma-Aldrich®), ammonium fluoride (≥99.99%, Sigma-Aldrich®), and formic acid (98%, LC-MS grade, Honeywell Fluka™) were tested as eluent additives.

2.1.2. Cleaning procedures and solvent purification

All glassware was baked in a furnace at 450°C for 8hrs following the method of Müller-Tautges *et al.* (2014). Solvents, used as eluents and for preparation of the diluted standard solutions, were additionally cleaned by ozonation (see section 3.3 for further discussion). The set up used a stream of air (Zero grade, BOC) at 0.2 L/min run through a glass tube containing a UV lamp (185/254 nm, Appleton Woods), which created air at high concentrations of ozone (ca. 290 ppm). This air was bubbled directly through the solvents using a pre-cleaned glass pipette, for 1 hr per 1 L of solvent. Solvents were then sonicated for 15 minutes to remove residual ozone from the solvent.

2.1.3. Ice core sample preparation

Ice samples from the Belukha glacier (Russian Altai Mountains) ice core were provided by the Paul Scherrer Institut, Switzerland, for which details on drilling, transportation and cutting can be found in (Olivier et al. 2003; Eichler et al. 2009). Additionally for organics samples, cut using the band-saw to remove any outer ice surfaces, the sample surfaces were scraped using a clean metal blade and placed directly inside amber glass vials with PTFE lined caps. Cut samples were transported onward to Cambridge frozen, and stored at -25°C until melting (in sealed glass vials inside a clean-room at approx. 16°C), preconcentration and analyses. A total of 18 samples were tested representing ice from a range of ice core ages, accounting for

differences in ice chemistry and physical ice properties which may affect analyses. These were 12 samples from 1866-1869, and 6 samples from 1821-1823.

Samples were preconcentrated in a rotary evaporator; this followed testing of a range of methods using stir-bar preconcentration, SPE or rotary evaporation. Optimisation and the choice of final parameters are discussed in section 3.4.

10 mL of each sample was evaporated to dryness after addition of d3-malic acid, d10-pimelic acid, and d31-palmitic acid at a concentration of 10 ppb in a 50 mL round-bottom flask. Evaporation was done with a water bath temperature of 30°C, a rotator speed of 60 rpm, and a vacuum pressure of 100 mbar. 3 mL of methanol was added to the flask and sonicated for 5 mins. The methanolic extract was transferred into a 4 mL vial and evaporated down to 0.5 mL under a gentle flow of N_2 at room temperature of approximately 18°C. 0.1 mL of methanolic extract was then transferred into a glass HPLC vial for analyses while the remaining 0.4 mL were kept at -18°C for eventual future analyses.

2.1.4. Instrumental analyses

Preconcentrated sample analyses used a HPLC-ESI-HRMS with an Accela system HPLC (Thermo Scientific, Bremen, Germany) coupled to an LTQ Velos Orbitrap (Thermo Scientific, Bremen, Germany). A Waters XBridge™ C18 (3.5 μm, 3.0x150 mm) column was used for chromatographic separation of the analytes. Mobile phases were (A) water with 0.5 mM NH_3 and (B) methanol with 0.5 mM NH_3. Separation was done at room temperature (~20°C), with a flow rate of 250 μL/min. Elution gradient was: 0–3 min 0% B, 3–4 min linear gradient from 0% to 30% B, 4–9 min 30% B, 9–10 min linear gradient from 30% to 100% B, 10–25 min 100% B, 25–26 min linear gradient from 100% to 0% B, 26–35 min 0% B. In addition, a post-column injection of methanol with 5 mM NH_3 was added at 100 μL/min. Injection volume was 20 μL. All analytes were quantified in negative ionisation using the following ESI source parameters: 400°C source temperature, 40 arbitrary units (a.u.) sheath gas flow rate, 20 a.u. auxiliary gas flow rate, 3.5 kV needle voltage, 350°C transfer capillary temperature, S-Lens RF Level 50%. MS spectra were collected in full scan, with a resolution of 100 000 at m/z 400, in the mass range m/z 80–600 and in MS/MS for all target compounds with a collision-induced dissociation (CID) energy of 30 (normalized collision energy). The mass spectrometer was calibrated routinely to within an accuracy of ± 2 ppm, using Pierce LTQ Velos ESI Positive Ion Calibration Solution and a Pierce ESI Negative Ion Calibration Solution (Thermo

Scientific, Bremen, Germany). The instrument was calibrated daily using standard solutions in the range 1-100 ppb in methanol prepared by diluting the 1 ppm stock standard mixture. d3-malic acid, d10-pimelic acid and d31-palmitic acid at a concentration of 10 ppb were used as internal standards. Quality check standards at a concentration of 10 ppb have also been analysed every 10 samples.

2.2. Direct Injection – HPLC-MS

Sample analyses were carried out by direct injection ultra-high performance liquid chromatography (UHPLC) electrospray ionisation (ESI) high-resolution mass spectrometry (HRMS) with a post-column injection of ammonium hydroxide in methanol.

2.2.1. Standard solutions, eluents and cleaning procedures

Chemicals and reagents, and the applied cleaning procedures, were the same as those used for the preconcentration HPLC-MS method. Bulk standard solutions were prepared in dichloromethane (>99.9%, Optima^{TM}, HPLC/MS, Fisher Chemical), and acetonitrile (>99.9%, Optima™ HPLC/MS, Fisher Chemical), and then combined into a diluted standard mixture of all analytes at a concentration of 1 ppm in acetonitrile. Final standards for instrument calibration, quantification of detection limits, and quantification of matrix effects were made at concentrations of 10 ppt, 100 ppt, 1 ppb, 10 ppb and 100 ppb by dilutions with water (>99.9%, Optima™ UHPLC/MS, Fisher Chemical).

2.2.2. Ice core sample preparation

Ice samples analysed were the same as those used for the preconcentrated method, and were cut and stored in the exact same way. After storage, samples were melted in sealed vials inside a class 100 clean-room, at approximately 16°C. Each sample represented 10 cm ice core resolution, equivalent to sub-annual resolution. 1 mL of the well mixed sample was transferred to a glass LC-MS vial and spiked with 10 ppb deuterated standards for immediate analysis, rather than for preconcentration.

2.2.3. Instrumental analyses

Analyses were carried out using an UltiMate3000 UHPLC coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap MS at the Department of Chemical Sciences, University of Padua, Italy. This more sensitive instrument is used compared to that of the methodological development of the preconcentration – HPLC-MS method. This instrument lowered detection limits to the range of ppt for many compounds, in comparison to the HPLC-ESI-HRMS (with Accela system HPLC (Thermo Scientific, Bremen, Germany) coupled to an LTQ Velos Orbitrap (Thermo Scientific, Bremen, Germany) at the University of Cambridge, UK, used in the previous study which did not achieve detection limits below ppb concentrations (See section 3.6). The new instrument also reliably reproduced sample concentrations of preconcentrated samples in an interlaboratory comparison with the previously used instrument. Given that concentrations of compounds detected in preconcentrated samples were in the order of ppb, this more sensitive instrument should allow detection not only of these compounds without preconcentration but may allow detection of previously undetected compounds.

The optimised settings of the instrument were the same as for the LTQ Orbitrap Velos. Calibration for quantification of target analytes was carried out at the start of each sample series, for which analyses took approximately 60 continuous hours, using standard solutions of 10 ppt, 100 ppt, 1 ppb, 10 ppb and 100 ppb. Deuterated internal standards d3-malic acid, d10-pimelic acid and d31-palmitic acid at a concentration of 10 ppb were used as internal standards to adjust concentrations accounting for methodological and instrumental variability. Quality check standards solutions at a concentration of 10 ppb have also been analysed every 10 samples to ensure no changes in detection sensitivity throughout the sequence of analysis.

3. Results and Discussion

The aim of the study was to develop a single analytical method for the quantification of both primary, e.g. long chain fatty acids, and secondary, e.g. oxidation products of isoprene, monoterpenes and sesquiterpenes, sourced biomarkers in ice cores using HPLC-MS. Optimisation of the chromatography and mass spectrometry was done using the HPLC-ESI-HRMS with an Accela system HPLC (Thermo Scientific, Bremen, Germany) coupled to an LTQ Velos Orbitrap (Thermo Scientific, Bremen, Germany) used for preconcentrated sample

analyses, the parameters of which were then carrier over to the UltiMate3000 UHPLC coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap MS for direct injection sample analyses.

3.1. Optimisation of the chromatographic separation

The optimisation of the chromatographic separation aimed at finding a good compromise in terms of retention and sensitivity between low molecular weight and high molecular weight compounds. Different chromatographic columns have been tested: two long C18 columns (Waters Atlantis® T3 and Waters Xbridge™, 3.5 μm, 3.0x150 mm), a short C18 column (Phenomenex Synergi™ Hydro-RP, 4.0 μm, 4.6x50 mm), a C3 column (Agilent ZORBAX SB-C3, 3.5 μm, 3.0x100 mm), and a pentafluorophenyl (PFP) column (Phenomenex Kinetex® PFP, 2.6 μm, 2.1x100 mm). The HPLC columns have been tested with different eluent compositions using a gradient elution with water as eluent A and an organic phase constituting of either acetonitrile, methanol or a mixture of methanol and isopropyl alcohol (90:10) as eluent B. In addition, different combinations of additives have been tested on both the water and the organic phase to improve separation and instrumental response: formic acid (0.01% and 0.1%), ammonium formate (5 mM), ammonium acetate (5 mM), sodium acetate (5 μM), ammonium fluoride (1 mM) and ammonium hydroxide (0.1, 0.5, 1 and 5 mM). A list of the different conditions tested, including different combinations of chromatographic columns, eluents and additives, is reported in Table 2. The effects of different eluents tested on the separation, peak shape and sensitivity towards the target analytes are schematically shown in Figure 1.
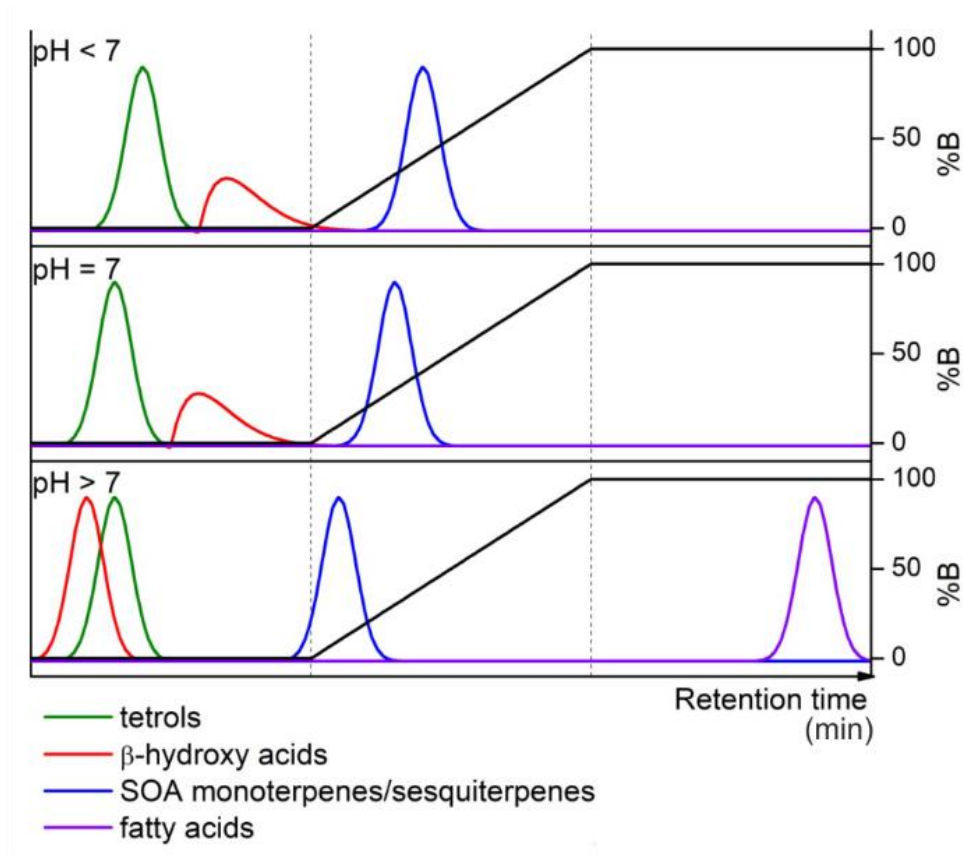
Figure 1: Example chromatograms summarising the observed effects of the pH of the eluents on the elution time, peak shape and sensitivity of the HPLC-ESI-HRMS method for the determination of terrestrial and marine biomarkers both primarily and secondarily sourced. Theoretical chromatograms are shown to more concisely indicate the effects over groups of compounds, as opposed to actual chromatograms for each compound for each method. "%B" indicates the percentage concentration of organic phase in the eluent, indicated by the black line.

Table 2. List of all chromatographic conditions tested for the chromatographic separation and determination of the target analytes including column types, eluents, additives and post-column injection conditions.

Column name	Column type	Eluent A	Eluent B	Post-column injection
Atlantis® T3	Long C18	Water	Acetonitrile	
Atlantis® T3	Long C18	Water + 5 μM CH$_3$COONa	Acetonitrile + 5 μM CH$_3$COONa	
Atlantis® T3	Long C18	Water + 5 mM HCOONH$_4$	Acetonitrile + 5 mM HCOONH$_4$	
Atlantis® T3	Long C18	Water	Methanol	
Atlantis® T3	Long C18	Water + 5 μM CH$_3$COONa	Methanol + 5 μM CH$_3$COONa	
Atlantis® T3	Long C18	Water + 5 mM HCOONH$_4$	Methanol + 5 mM HCOONH$_4$	
Atlantis® T3	Long C18	Water + 5 mM NH$_4$OH	Methanol + 5 mM NH$_4$OH	
Atlantis® T3	Long C18	Water	Methanol + 5 mM NH$_4$OH	
Atlantis® T3	Long C18	Water + 0.1% HCOOH	Methanol + 0.1% HCOOH	
Atlantis® T3	Long C18	Water	Methanol/isopropyl alcohol (90:10)	NH$_4$OH 1.4 mL in 200 mL water, 10 μL/min
Atlantis® T3	Long C18	Water + 0.01% HCOOH	Methanol/isopropyl alcohol (90:10) + 0.5 mM NH$_4$OH	NH$_4$OH 400 μL in 100 mL methanol, 100 μL/min
Xbridge™	Long C18	Water	Methanol + 1 mM NH$_4$OH	
Xbridge™	Long C18	Water	Methanol/isopropyl alcohol (90:10)	
Xbridge™	Long C18	Water	Methanol/isopropyl alcohol (90:10) + 0.1 mM NH$_4$OH	
Xbridge™	Long C18	Water	Methanol/isopropyl alcohol (90:10) + 0.5 mM NH$_4$OH	
Xbridge™	Long C18	Water + 0.01% HCOOH	Methanol/isopropyl alcohol (90:10) + 0.5 mM NH$_4$OH	
Xbridge™	Long C18	Water + 0.01% HCOOH	Methanol/isopropyl alcohol (90:10) + 0.5 mM NH$_4$OH	NH$_4$OH 400 μL in 100 mL methanol, 100 μL/min
Xbridge™	Long C18	Water + 0.5 mM NH$_4$OH	Methanol + 0.5 mM NH$_4$OH	
Xbridge™	Long C18	Water + 0.5 mM NH$_4$OH	Methanol + 0.5 mM NH$_4$OH	NH$_4$OH 40μL/400μL/800μL in 100mL methanol, 100 μL/min
Xbridge™	Long C18	Water + 1 mM NH$_4$F	Methanol + 1 mM NH$_4$F	
Synergi™	Short C18	Water	Methanol	NH$_4$OH 400 μL in 100 mL methanol, 100 μL/min
Zorbax	C3	Water	Methanol	NH$_4$OH 400 μL in 100 mL methanol, 100 μL/min

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Column name	Column type	Eluent A	Eluent B	Post-column injection
Cont.				
Kinetex®	PFP	Water + 5 mM CH_3COONH_4	Methanol + 5 mM CH_3COONH_4	
Kinetex®	PFP	Water + 0.5 mM NH_4OH	Methanol + 0.5 mM NH_4OH	

Elution of long-chain fatty acids proved to be challenging due to their high affinity for all the stationary phases. For example, retention time of melissic acid ($C_{30}H_{60}O_2$) was >60 mins for both long C18 columns with neutral eluents (flow rate 250 µL/min). There was not any significant improvement in this regard by using a short C18 column while the C3 and PFP columns provided shorter retention times (<60 mins at 250 µL/min). Methanol provided slightly shorter retention times, more symmetric and sharper peaks for long chain fatty acids compared with acetonitrile. The use of a mixture of methanol and isopropyl alcohol substantially, but not sufficiently, reduced retention times of long chain fatty acids. The best results in terms of instrumental response and sufficiently short retention times have been obtained using ammonium hydroxide as an additive in the organic phase. Ammonium hydroxide can successfully deprotonate the fatty acids decreasing their affinity for the stationary phase.

Concerning low molecular weight compounds, the two long C18 columns provided the best chromatographic separation with all eluent compositions tested. However, the use of ammonium hydroxide as an additive shortens their retention times so that the smallest compounds are eluted close to the dead time. A combination of acidic eluent A (with 0.01% and 0.1% formic acid) and basic eluent B (with 0.1-5 mM ammonium hydroxide) was tested to overcome this issue while maintaining short retention times and a good instrumental response for fatty acids. While chromatographic separation improved for most of the low molecular weight compounds, the most acidic compounds, like the β-hydroxy acid (malic acid) and the tricarboxylic acids (BTCA and MBTCA), presented extensive peak broadening due to the establishment of an equilibrium between the protonated and neutral forms, or the neutral and deprotonated forms, which substantially decreased sensitivity for those compounds. The same applies with the other eluent additives tested which provided a neutral pH eluent. Using ammonium hydroxide at a concentration of 0.5 mM in both eluent A and B provides a good compromise between retention of low molecular weight compounds and sensitivity (sharp peaks) for β-hydroxy acids and tricarboxylic acids.

Finally, gradient elution has been optimised for chromatographic separation of low molecular weight compounds in conjunction with analyses time (dependent on the elution of fatty acids). Retention times of all analytes with the optimised gradient elution are demonstrated in the example chromatogram for a 100 ppb standard in Figure 2.
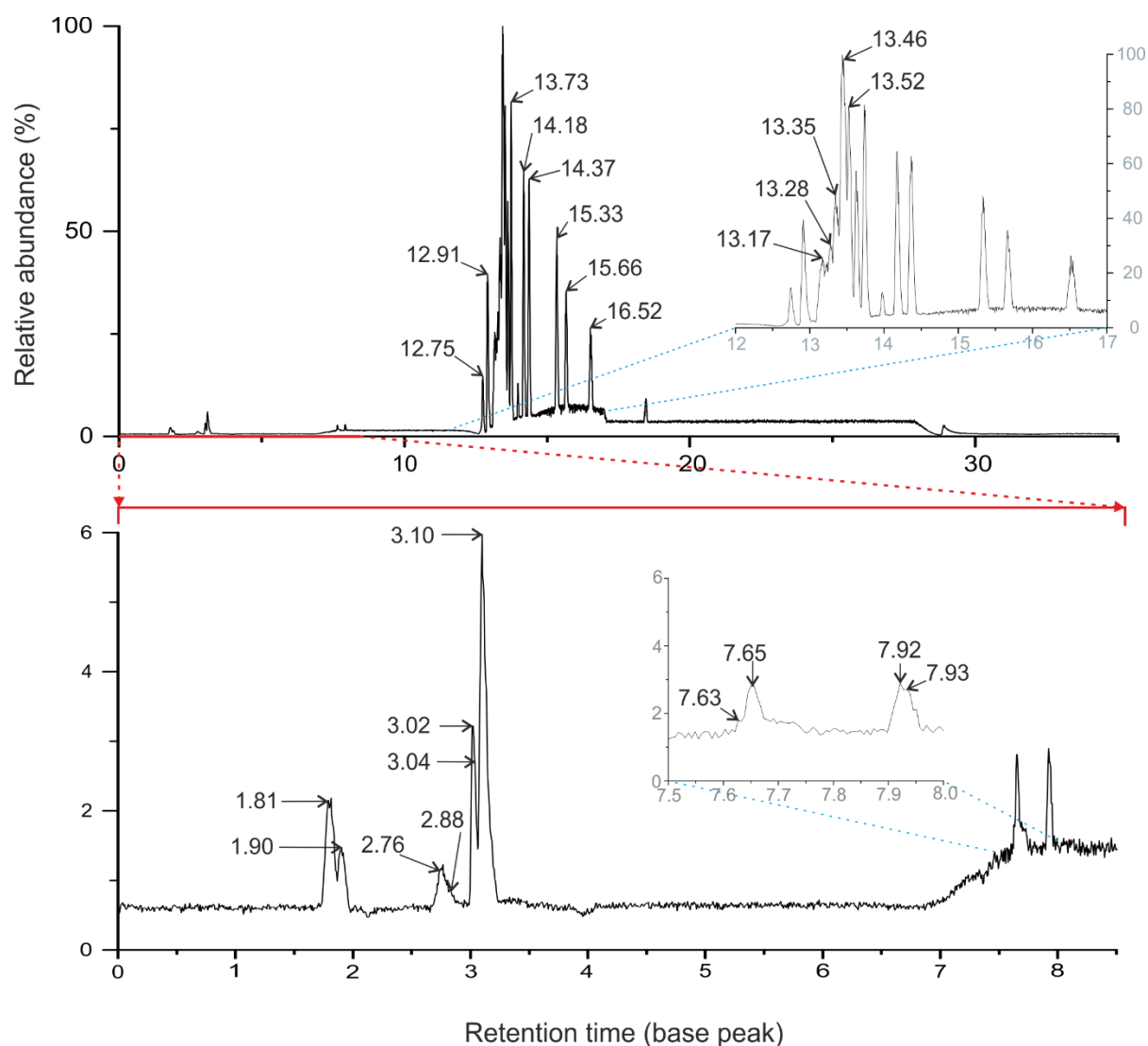
Figure 2: Example chromatogram in base peak for a 100 ppb standard solution with the fully optimised method of chromatographic separation and instrumental response. Compounds are as follows by increasing retention time: 1.81 (BTCA), 1.81 (MBTCA), 1.90 (D-malic acid), 1.90 (Pimelic acid), 2.76 (Levoglucosan), 2.88 (Meso-erythritol), 3.02 (Terebic acid), 3.04 (Methyl tetrols), 3.10 (Pinolic acid), 7.63 (cis-pinonic acid), 7.65 (Salicylic acid), 7.92 (Keto-pinic acid), 7.93 (β-caryophyllinic acid), 12.75 (β-nocaryophyllonic acid), 12.91 (β-caryophyllonic acid), 13.17 (Lauric acid), 13.28 (Myristic acid), 13.35 (Arachidonic acid), 13.46 (Oleic acid), 13.52 (Heptadecanoic acid), 13.73 (Nonadecanoic acid), 14.18 (Behenic acid), 14.37 (Tricosanoic acid), 15.33 (Heptacosanoic acid), 15.66 (Octacosanoic acid), 16.52 (Melissic acid).

3.2. Optimisation of the instrumental response

Most of the target analytes are organic acids, and so are better ionised in negative mode. In one particular case, the isoprene-derived methyl tetrols and the surrogate standard meso-erythritol, the analyte could be ionised in both positive and negative polarity. Positive ionisation as protonated (with formic acid additive), adduct with ammonium (with ammonium formate and acetate additives) and adduct with sodium (with sodium acetate additive) molecular ions was compared with negative ionisation as deprotonated molecular ions (with ammonium hydroxide or ammonium fluoride additives). The best performances for all compounds were obtained using ammonium hydroxide as an additive in negative ionisation. High concentrations of ammonium hydroxide are necessary to ensure good sensitivity for those compounds; however, it also reduces chromatographic separation. In order to increase the instrumental response for meso-erythritol and methyl tetrols in particular, while maintaining a good chromatographic separation, post-column injections of ammonium hydroxide solutions were tested. Solutions of 5 mM, 50 mM, 100 mM and 200 mM ammonium hydroxide in either water or methanol at a flow rate of 10-100 μL/min were tested. The best results, with a sensitivity increase by a factor of five for the two compounds, have been obtained using a 5 mM ammonium hydroxide solution in methanol at a flow rate of 100 μL/min and this is therefore the conditions chosen as most optimised. Increasing the ammonium hydroxide concentration further did not make any improvement. The post-column injection also provided a higher sensitivity for the other analytes, especially those being eluted at the beginning of the chromatographic run at 100% A eluent composition.

Optimisation of source parameters with the final chromatographic method was done by changing source temperature between 50 to 400°C, capillary temperature between 300-350°C, sheath gas flow rate between 40-60 a.u., RF Lens between 10-100% and needle voltage between 3-4 kV. The best instrumental response for all analytes were obtained using 400°C source temperature, 350°C capillary temperature, 40 a.u. sheath gas flow rate, RF lens of 50% and 3.5 kV needle voltage. Auxiliary gas flow rate was kept at 20 a.u. while the sweep gas was not used.

Sample injection volume was also tested between 1-100 μL. A final injection volume of 20 μL was used as it provided a good compromise between maximising injected quantity for better sensitivity at low concentrations, and providing sharp enough chromatographic peaks in the concentration range tested.

3.3. Optimisation of the decontamination procedures

Ozonation of both UHPLC water and UHPLC methanol considerably reduced, but did not totally eliminate, background contamination of unsaturated fatty acids to the extent that calibration curves could be generated for all fatty acids on our list down to 1 ppb concentrations. Peak area of the unsaturated fatty acid oleic acid, for example, reduced from 5284408 in non-ozonated solvents to 1249219 in ozonated solvents, the latter indicating background contamination more than four times lower than for non-ozonated solvent. A risk in this process is the introduction of contamination for other compound from the ozonation apparatus or air used in the process. However a comparison of background contaminations in ozonated and non-ozonated solvents for all target analytes showed that ozonated solvent did not introduce or increase contaminations for any other compound.

The remaining contamination in the water blanks may come from sample preparation, the solvent used to make the stock-standard solution, or the instrument during sample analyses. It is worth noticing that the use of ozonated solvents causes a shift in the elution of most of the analytes, especially fatty acids, to higher retention times.

In an attempt to decrease background contamination of some fatty acids (e.g. lauric and myristic acids) two techniques were tested: (i) adding a C18 SPE cartridge mounted on the water line (before the pump) in order to trap in the cartridge the fatty acids eventually present in water, and (ii) using an on-line trap (chromatographic) column (Salihovic et al. 2013) mounted between the mixer and the injector in order to separate chromatographically the background contamination of fatty acids in the eluents from the analyte and contamination present in the samples. The SPE cartridge did not make any improvement since it is probably quickly overloaded with the contamination and does not retain further contaminants. The use of a trap column caused a substantial background reduction of lauric, myristic and palmitic acid however this was accompanied by a loss of sensitivity for other target analytes. While it is difficult to explain the loss of sensitivity, it was decided to sacrifice those three analytes in order to maintain a good sensitivity overall.

3.4. Optimisation of the preconcentration method

3.4.1. Stir-Bar (SBSE) preconcentration

The polydimethylsiloxane (PDMS) stir bar used in previous studies (Lacorte et al. 2009; Müller-Tautges et al. 2014) (GERSTEL Twister®) enables extraction of organic compounds from a liquid matrix. The bars are also available with a second solid phase type: the EG/Silicon stir bar is a combination of PDMS / Ethylene glycol (EG). Both PDMS and EG stir bars were tested here.

The variables in the process which were optimised are as follows: stir bar solid phase (EG or PDMS), stir time (10 or 22 hrs), solvent for desorption of compounds from the bar (methanol or acetonitrile with additions of ammonium hydroxide), sonication time for desorption (15 minutes or 1 hr). There were further tests adjusting the pH of the sample (pH 3.5 or 5) to protonate the most acidic analytes and increase their affinity for the stationary phase for initial capture, and adding salts to the sample (sodium chloride, sodium sulphate) to decrease the solubility of non-electrolytes and increase their transfer to the stationary (organic) phase (salting-out effect), but these did not improve extraction of the compounds and were not used further. All tests were performed on a 10 mL sample of 10 ppb bulk standard solution of all compounds, concentrating down to 1 mL of sample for injection in to the HPLC-MS at a final theoretic maximum concentration of 100 ppb if recovery was 100%. Factors which were kept constant throughout the tests were the stir rate of the magnetic plate (700 rpm) and the temperature of solvents, which were at the lab temperature of 18°C.

The final, most optimised (i.e. highest average recoveries of all compounds), stir bar method stirred the PDMS bar in the liquid sample at 700rpm for 20hours, and then desorbed the compounds in to 1mL of methanol+0.5mM ammonium hydroxide by sonication for 15 minutes. This is illustrated graphically in Figure 3.
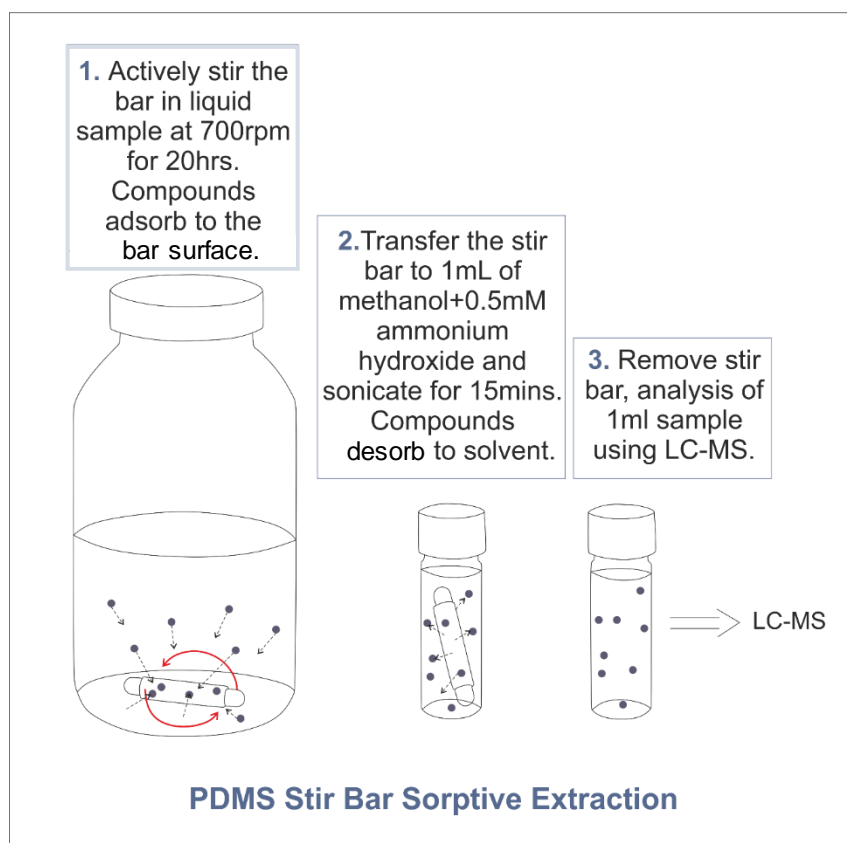
Figure 3. Processing steps illustrated for the most effective stir bar method.

3.4.2. Solid Phase Extraction (SPE) preconcentration

A C18 cartridge (Perkin Elmer), and two new cartridges not previously used for organic analyses in snow or ice: HyperSep™ SAX (Thermo Fisher Scientific), a strong anion exchange sorbent for extraction of weak acids) and Strata-X® X-A (Phenomenex®), a strong anion-exchange functionalized polymeric sorbent, were tested.

A number of factors can be adjusted throughout the SPE method. This includes changing the counter ion (in this case, from chloride to formate), adjusting the acidity of the samples themselves (either acidified or basified from original pH) to change affinity of the compounds to the cartridge, changing solvents or solutions used to wash the cartridge to reduce loss of target compounds at this stage (either 25 mM ammonium acetate in water or 0.25% ammonium hydroxide in water for the first wash, and methanol for the second wash), and changing the number of washing stages (via elimination of the second wash stage). Further tested were the solvents or solutions used to elute the compounds, to improve recovery rate of total compounds

from the cartridge (either 5% formic acid in methanol, ammonium hydroxide in water at solutions of 0.25%, 2%, and 5%, 1.2% hydrogen chloride in methanol, 20 mM potassium hydroxide (KOH) in water).

The processing steps of the optimised SPE method are shown in Figure 4. Factors that were constant throughout the tests were as follows; all cartridges were 1 mL in size with 100 mg sorbent mass. Manufacturer guidelines stipulate using 1 mL of solvents and solutions at stages 1, 2, 4 and 5 for this size and mass of cartridge. All samples were 10 mL of 50 ppb bulk standard, concentrated to 1 mL corresponding to a final concentration of 500 ppb if recovery is fully successful. Conditioning stages were always 1 mL of UHPLC water followed by 1 mL of UHPLC methanol. Because cartridge tops are open, all tests were performed under a fume hood and the cartridges covered over with foil between additions of liquid to the cartridges, to limit contamination.

The final method, considered most fully optimised due to best overall compounds recoveries, conditioned the HyperSep™ SAX cartridge with 1 mL of water and 1 mL of methanol, changed the counter ion to formate using 1 mL of 2% formic acid followed by 1 mL water and 1 mL water at pH 7, loaded 1 mL of liquid sample, washed the cartridge with 1 mL of 25 mM ammonium acetate solution at pH 8, and eluted the compounds with 1 mL of a 50/50 water/methanol solution with 5% ammonium hydroxide.
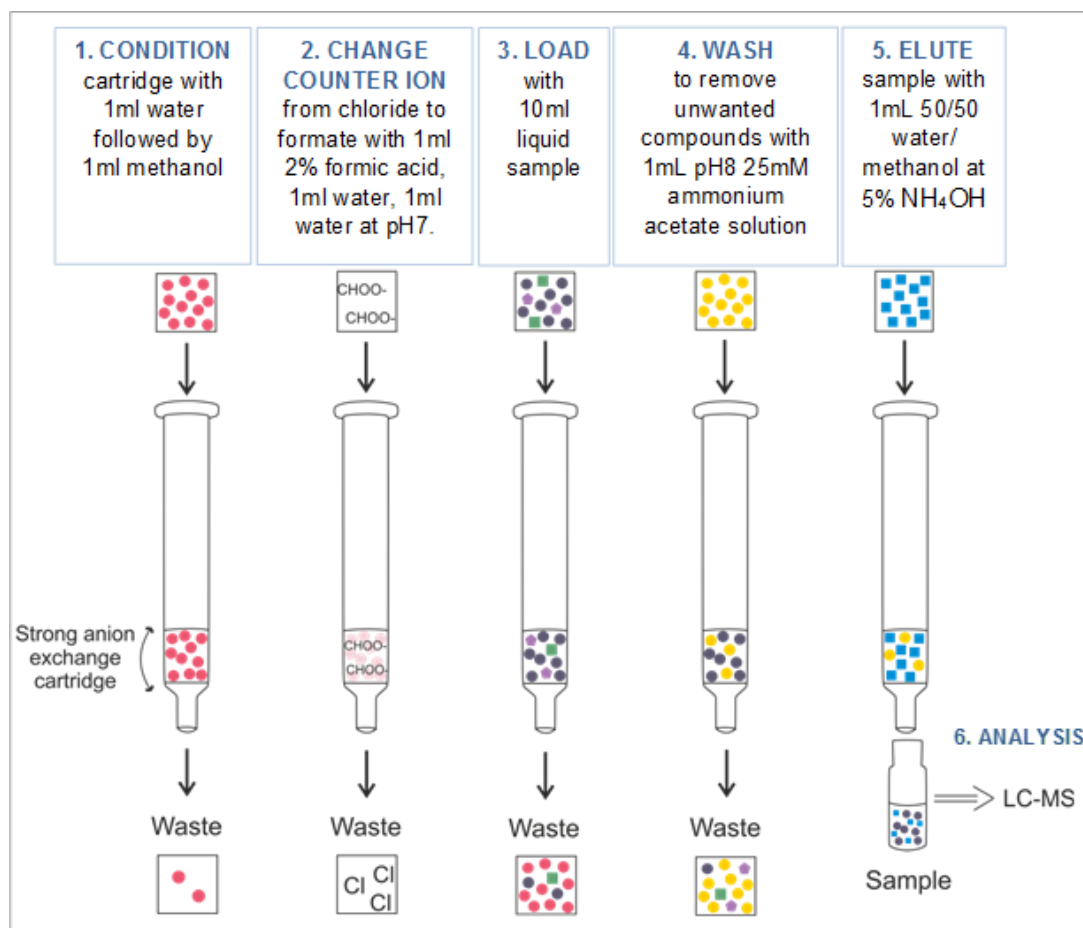
Figure 4. Sample processing steps of the fully optimised SPE method. Different colour/shape symbols represent the different media/compounds introduced or lost by each stage of processing.

3.4.3. Rotary evaporation preconcentration

Figure 5 illustrates the processing steps associated with rotary evaporation preconcentration, described for the most fully optimised method (i.e. the method with the highest recoveries of compounds).

Variables tested in method optimisation were the addition of KOH to the samples to adjust pH, the volume of solvent used to re-dissolve compounds in step 2 (1-4 mL), whether to stir or sonicate this solvent to extract the dried analytes from the glass wall, and how many samples to run on the rotary evaporator at the same time (one or four flasks). This last point arises

because it is possible to fit an attachment to the rotator to allow up to four individual evaporator flasks to be run at the same time.

Factors that were kept constant were as follows; water bath temperature was 30°C, rotator speed 60 rpm, and vacuum pressure of 100 mbar. Compounds were eluted from the flask with high purity methanol. All samples were 10 mL of 10 ppb bulk standard solution. The resulting 0.5 mL sample corresponds to a final concentration for analyses of 200 ppb if recovery was fully successful. The above combination of factors gave a sample evaporation time (i.e. step 1) of ~45 minutes. The final method, considered fully optimised, is as presented in Figure 5.
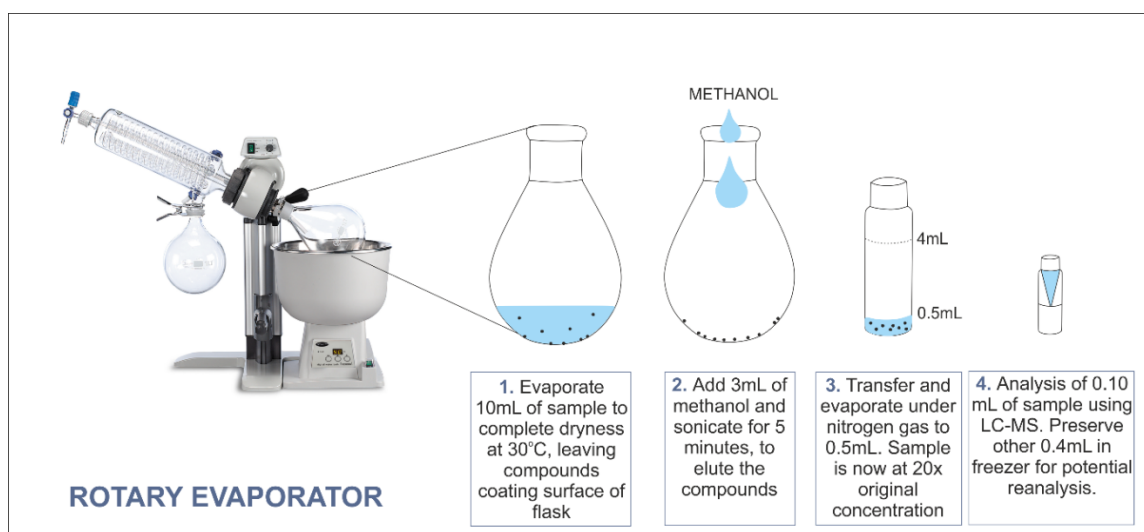
Figure 5: Sample preparation stages of the optimised rotary evaporator process resulting in concentration of analytes by a factor of 20.

3.4.4. Comparison of the preconcentration

The results presented here refer to the optimised version (based on the entire compound list) of each of the methods as presented in figures 3-5: SBSE, SPE and rotary evaporation. Results are presented in Figures 6 and 7.

Stir bar preconcentration was not successful for SOA compounds, with 0% recovery. The technique performed better for fatty acids, with recovery of 60% on average. It was the most successful of all techniques for the longer chain fatty acids on our list, heptacosanoic acid, octacosanoic acid, and melissic acid, with recoveries of 68%, 91%, and 104% respectively.

This is considerably higher than those obtained with the SPE and rotary evaporation techniques.

SPE achieved highly variable results test-to-test, with methods improving some compound recoveries often being at the expense of other compounds. For example, a test in which elution used 50/50 water/methanol with 2% NH_4OH (herein referred to as Test A), instead of 5% in the chosen 'most fully optimised' test (referred to herein as Test B), while keeping all other variables the same, gave higher recoveries of terebic acid, pinolic acid, keto-pinic acid, oleic acid, heptadecanoic acid, nonadecanoic acid, and behenic acid and tricosanoic acid. However, Test A performed very poorly for the smallest SOA compounds BTCA, MBTCA and pimelic acid, as well as all β-sesquiterpene SOA compounds (Figure 6). Because the aim is to achieve a method which targets all the compounds groups on the list, the Test A method was not therefore chosen as more optimised.
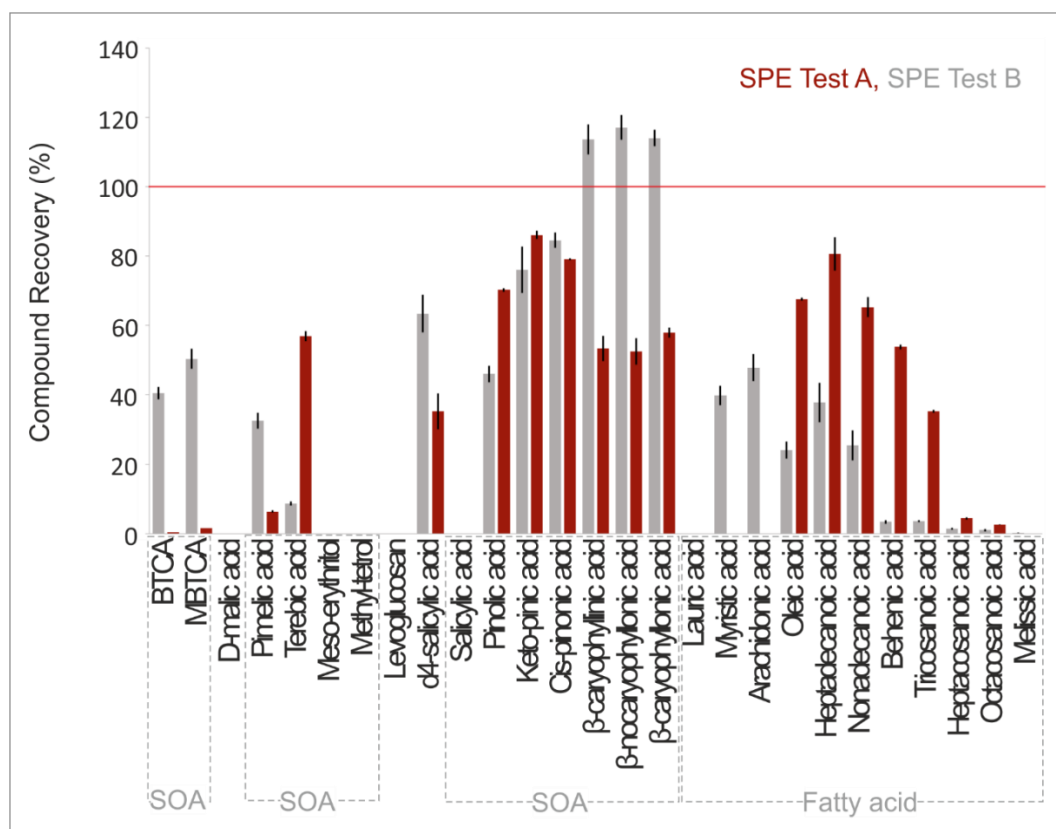
Figure 6: Compound recovery comparison of the fully optimised SPE method. The processing steps of the SPE is the same for both tests changing only in the final elution, which used 5% NH4OH in 50/50 methanol/water solution in Test B and 2% NH4OH 50/50 solution in Test A. The tests demonstrate improved recoveries of some compounds, in this case fatty acids, comes always at the expense of reduced recoveries of others and thus full optimisation was difficult for all compounds. It should be noted that lauric acid, myristic acid and arachidonic acid could not be compared in this case because of contamination affecting the calibration curve in Test A.

Considering the SPE optimisations steps, it can be concluded that exchanging the counter ion from chloride to formate and using ammonium acetate 5 mM solution at the wash stage strongly improved retention of our target compounds on the cartridge, with the chosen elution method exerting the most control on the overall success. Using NH4OH in solution with either methanol or water, at concentrations 0.25-5%, gave the best results compared to alternative elutions, such as formic acid in methanol, as basic solutions are good eluents for anion-exchange cartridges. An elution solution of 2-5% NH4OH in water was the most successful for SOA compounds. The highest recovery elution solution for fatty acids was 2-5% NH4OH in

methanol, which gave higher fatty acid recoveries in tests leading up to the most optimised final test, but as previously shown in Figure 6, this gave lower recoveries for SOA compounds. The 5% NH_4OH in 50/50 methanol/water solution was the optimal balance between the two, recovering fatty acids at acceptable levels while not compromising SOA recovery in comparison to elution in water.

The fully optimised method, Test B, was the highest of any method for recoveries of our smallest SOA compounds and was particularly successful for sesquiterpene oxidation products (recovering ≥100%) and moderately successful for some SOA compounds and shorter-chain fatty acids, with recoveries between ~30—50%. However SPE did not perform well for the alcohols in our target list, meso-erythritol, methyl tetrols and levoglucosan, with the latter showing very low recovery levels (only measurable in 2 of the total 18 iterations of the SPE method which were tested), and the others in none (including the most optimised choice). Collection of the 'waste' at each stage of the SPE method (see Figure 4) revealed that the loss of these compounds was entirely at the load stage, meaning none of the compound concentration was retained by the cartridge. This was not improved from test-to-test by changing of the counter ion, and suggests that the SAX cartridge is too polar and thus not appropriate for these compounds.

When considering the initial SPE tests between the different types of sorbent mass in the cartridge, the results overall promoted the use of the SAX cartridge since the load phase showed loss of the least number of compounds. The C18 cartridge, although showing some loss of methyl tetrols, meso-erythritol and levoglucosan at the load stage, also showed loss at the wash stage, suggesting these compounds were retained to some extent by this cartridge and with further optimisation may show successful recoveries for these specific compounds. This is for future consideration if these are specific target compounds.

Considering this 'balancing act' between methods and compound recoveries, it was decided that although recovery was well below 100% for many compounds, the method had probably reached its optimum output if the goal was to target such a diverse list of compounds of varying molecular sizes, structures and chemical-physical properties such as polarity. Such a list makes it difficult to find an all-inclusive technique at each stage of the SPE method, using one type of cartridge, and the 'success' of the method must therefore be adjusted in expectation.
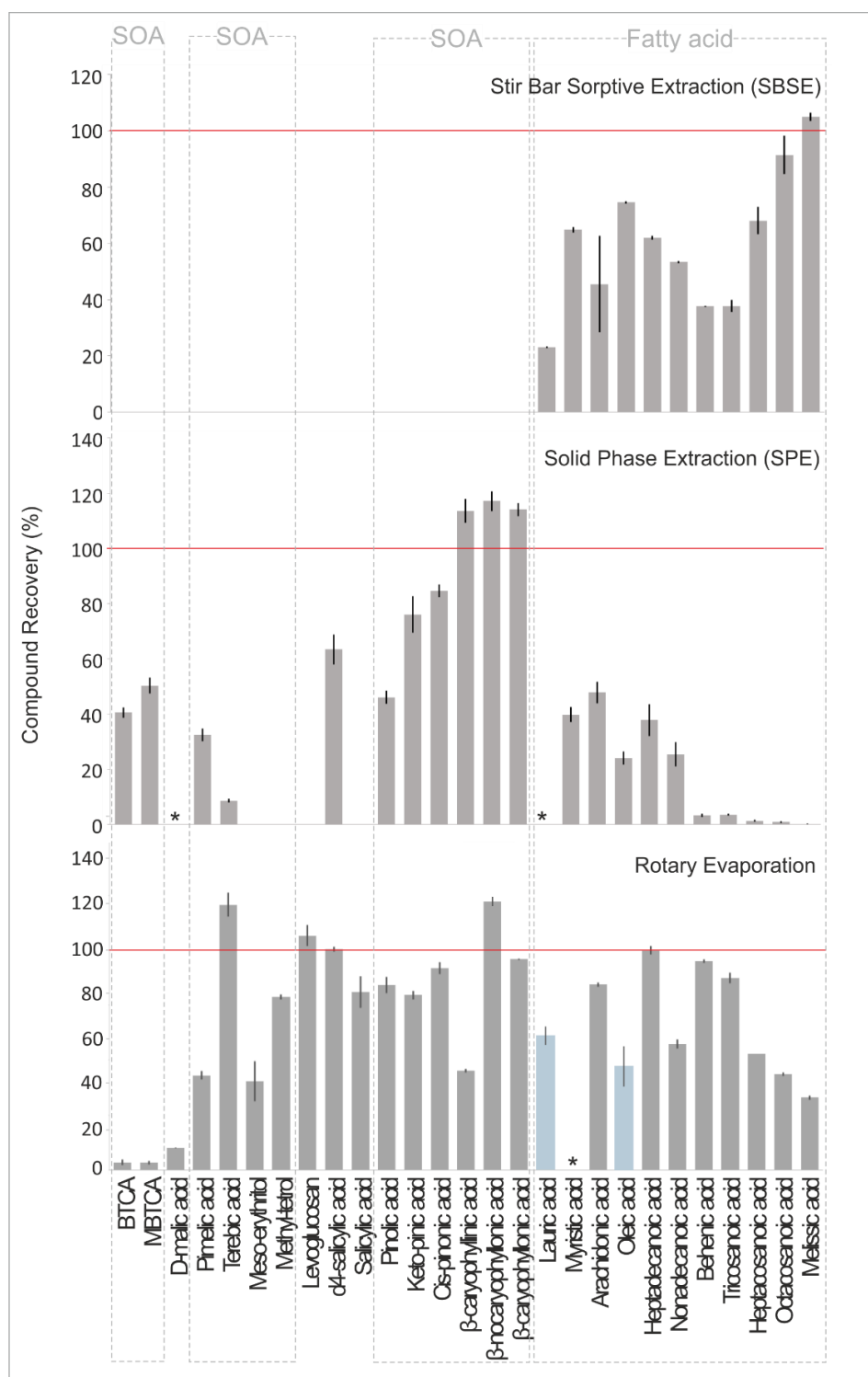
Figure 7: Comparative compound recoveries for each of the most fully optimised preconcentration techniques. Dashed red lines represent 100% recovery, while blue bars differentiate compounds recovered only after ozonation of solvents to reduce background contamination (tested for rotary evaporation only). Asterisks represent compounds that were recovered, but contamination was too high to obtain a reliable calibration curve.

Rotary evaporation was the most successful method of preconcentration, being the only method to display recovery to some extent of all compounds. In Figure 7, the exception to this is lauric acid, myristic acid and oleic acid because of very high background contamination which prevented generation of a calibration curve at ppb concentration levels. This was improved with ozonation of the solvents (see also the "Optimisation of the decontamination procedures" section, 3.3).

In more general terms, it was observed that only one sample could be run at a time, as the multiple-vial attachment of the rotary evaporator caused cross-contamination between samples. The greater the solvent volume used to elute the compounds from the dried rotary evaporator vial, the greater the compound recovery; this is because it increased the coverage of the solvent over the vial surface during sonication to include the entire surface which the liquid sample was in contact with during evaporation. The maximum increase in solvent volume required was 3 mL, since this covered the whole inner-vial surface upon rotation of the vial within the sonicator.

The lowest recoveries overall (<10%) were for the smallest compounds (lowest C-numbers) on the compound list: BTCA, MBTCA, and D-malic acid. This is perhaps due to their higher vapour pressures, meaning they are more easily lost at the evaporation stage than the majority of the other compounds tested which have lower vapour pressures. The exception to this is meso-erythritol, which has higher vapour pressure and yet shows higher recovery. Recoveries of other compounds were 33-100%, with average recovery of 80% overall; 86% for SOA compounds (not including BTCA, MBTCA and D-malic acid) and 69% for fatty acids. Considering this overall recovery, it is the best method of preconcentration for the compound list as a whole. It is therefore the method carried forward for sample preparation before further optimisation work. The best preconcentration method for each individual compound can be found summarised in Table 3, showing how rotary evaporation dominates as the best choice for many compounds, but how other methods are better in some cases. This is discussed further in the conclusions of this chapter (section 4).

Table 3: List of the most successful preconcentration method for each individual compound, based on highest percentage recovery.

Compound name	Highest recovery preconcentration method	Percentage recovery ± standard deviation	
Meso-erythritol	Rotary Evaporation*	41±18	
Methyl tetrols	Rotary Evaporation*	79±2	
Pimelic acid	Rotary Evaporation/SPE (B)	43±3.9/ 32±1.8	
BTCA	SPE (B)	41±0.1	
MBTCA	SPE (B)	50±0.1	
Terebic acid	Rotary Evaporation	120±11.0	
Pinolic acid	Rotary Evaporation	84±7.4	
Cis-pinonic acid	Rotary Evaporation/SPE (A/B)	92±5.5/	79±11.3/ 85±1.1
Keto-pinic acid	Rotary Evaporation/SPE (A/B)	80± 3.9/	86±33.1/ 76±5.9
β-caryophyllinic acid	SPE (B)	113±18.3	
β-caryophyllonic acid	Rotary Evaporation	95±0.6	
β-nocaryophyllonic acid	Rotary Evaporation/SPE (B)	120±3.3/ 117±19.2	
Levoglucosan	Rotary Evaporation*	106±9.7	
D-malic acid	Rotary Evaporation*	10±0.5	
Salicylic acid	Rotary Evaporation*	81±7.3	
Lauric acid	Rotary Evaporation	61±10.5	
Myristic acid	SBSE	65±5.2	
Heptadecanoic acid	Rotary Evaporation	100±3.9	
Oleic acid	SPE (A)/SBSE	68±11.9/ 75±1.7	
Nonadecanoic acid	Rotary Evaporation/SBSE	57±4.2/ 54±1.7	
Arachidonic acid	Rotary Evaporation	84±2.0	
Behenic acid	Rotary Evaporation	95±1.7	
Tricosanoic acid	Rotary Evaporation	87±4.8	
Heptacosanoic acid	SBSE	68±24.3	
Octacosanoic acid	SBSE	91±33.8	
Melissic acid	SBSE	105±7.1	

*The only method above 0% recovery for this compound

3.5. Optimisation of the direct injection – HPLC-MS method

Some instrumental optimisation parameters developed on the LTQ Orbitrap Velos were re-tested to ensure the methodology was appropriate for the second instrument (i.e. the Q Exactive^{TM} Orbitrap MS). This particularly included steps in reducing background contamination, which can be different for individual compounds depending on the instrument and lab environment being used.

The repeated tests were: testing of non-ozonated and ozonated solvents, testing of the inclusion of a post-column injection, and the application of MS-MS analyses to ensure correct identification of peaks in the mass spectra.

On average, the application of a post column injection of 5mM NH_4OH in methanol increased peak areas by 1.5 to 2 times that of the peak areas without a post-column injection. The use of ozonated solvents was again shown to be effective at reducing background contamination of unsaturated fatty acids which break down during ozonolysis; in non-ozonated solvents these compounds were present at contamination levels of ≥ 10 ppb, while ozonated solvents allowed detection at as low as 10 ppt.

Instrumental analyses showed that the retention time of some compounds shifted when comparing preconcentration/direct injection analyses. The values can be compared between Table 4 (for preconcentrated samples) and Table 5 (for direct injection samples). The shift is because the solvent of the final sample (and standard solutions) is different in the two cases; in the preconcentrated samples the solvent is methanol, used to re-dissolve the compounds from the rotary evaporation vial. In direct injection, the solvent is the snow melt water of the sample or LC-MS water for the standard solutions. In general, the retention times of SOA compounds are slightly shorter while retention times of fatty acids are longer for samples and standard solutions in water compared with methanol.

3.6. Validation of the preconcentration – HPLC-MS method

Instrumental LODs were evaluated on standard solutions using the Hubaux-Vos method, following IUPAC recommendations (Hubaux & Vos 1970; Currie 1995). Limits of quantifications (LOQs) were evaluated as 10/3*LODs. Sensitivity (slope of the calibration line)

and linearity range were tested on standard solutions. Linearity was tested up to a concentration of 100 ppb using both the r-Pearson correlation test and the F-test to compare linear and quadratic fits. Results showed a good linearity in the tested range. Method/instrumental repeatability has been evaluated in real ice core samples. Validation parameters are reported and described in Table 4.

Matrix effects were tested by comparing the slopes of two calibration lines; one for standard solutions in water and one for standard additions (of the same concentrations) to an ice core sample. Results of the *t*-test showed that there are no statistically significant differences between the two slopes at 95% confidence level.

3.6.1. Interlaboratory comparison for preconcentrated samples

Ice samples from the Belukha glacier ice core were prepared for analyses using the fully optimised rotary evaporation preconcentration method. The final sample was split for replicate analyses on two HPLC-MS instruments; the first was the same HPLC-LTQ Velos Orbitrap used for methodological development at the Department of Chemistry, University of Cambridge, UK, and the second a UHPLC UltiMate3000 coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap MS at the Department of Chemical Sciences, University of Padua, Italy. Both instruments used the same optimised settings developed previously, and were tested for limits of detection using replicate calibration standards. Overall, the Q Exactive showed detection limits down to ppt levels for SOA compounds, while the LTQ Velos Orbitrap did not achieve detection below ppb. The Q Exactive also gave lower detection limits for most fatty acids, mostly in the ppb, rather than ppt, range.

The same compounds were detected in both sample analyses of the Belukha ice core; D-malic acid, Terebic acid, Methyl tetrols, Keto-pinic acid, Pimelic acid, cis-pinonic acid, Heptacosanoic acid, Octacosanoic acid, and Melissic acid, with the addition of MBTCA on the Q Exactive which was below detection limits on the LTQ Velos Orbitrap. Results are shown as reproducibility-between-instruments values in Table 4, which are R^2 values from linear regression lines of scatterplots comparing the data series for each compound from the two different instruments. Scatterplots are shown in Figures 8 and 9 all compounds. For particular compounds the concentrations in individual samples varied by typically a factor 10 between seasons, indicating that the reproducibility achieved here is sufficient to clearly observe the seasonal trends on different instruments. One compound, heptacosanoic acid, showed very

poor reproducibility with an R^2 value of 0.32. This was due to high background contamination levels, combined with low compound concentrations in samples.

Table 4: Compound specific limit of detection achieved using a linear calibration method, of standard values 1, 10 and 100ppb, listed in order of lowest to highest detection limit for the Cambridge instrument. Also presented are retention time, limit of quantification, limit of detection for the comparative instrument in Padua, instrument repeatability (i.e. variability between repeat injections of the same sample in to the same instrument), method repeatability (variability between different samples prepared using the same method and analysed on one instrument), reproducibility (difference in results between the same samples analysed on two different instruments, given as R^2 of a linear regression line between the two sets of sample concentrations) and recovery (the percentage of the compound recovered from analyses compared to that which was present in the original sample before processing, as determined using standards of known input values). As is expected, RSD values of the method and instrumental repeatability increased greatly as concentrations lowered towards detection limits for all compounds, and the presented values therefore exclude values at 1ppb concentration so as not to be disproportionately weighted to these high errors. N/D = not detected.

Compound	Retention time (min) [Cambridge]	LOD (ppb) [Cambridge]	LOQ (ppb) [Cambridge]	LOD (ppb) [Padua]	Instrumental Repeatability (% RSD) [Cambridge]	Method Repeatability (% RSD) [Cambridge]	Reproducibility (R^2) [Cambridge]	Recovery (%) [Cambridge]
Nonadecanoic acid	13.73	2.00	6.67	0.29	0.94	9.50	N/D	57
Pimelic acid	1.90	2.32	7.73	0.04	1.47	2.02	0.87	43
β-nocaryophyllonic acid	12.75	2.52	8.40	0.25	3.18	2.67	N/D	122
D-malic acid	1.90	2.61	8.70	0.09	2.68	2.31	0.92	10
Keto-pinic acid	7.92	2.62	8.73	0.03	3.46	13.14	0.90	80
MBTCA	1.81	2.68	8.93	0.04	1.37	3.92	N/D	3
β-caryophyllonic acid	12.91	2.73	9.10	0.11	2.39	11.69	N/D	95
β-caryophyllinic acid	7.93	2.91	9.70	0.07	3.05	11.27	N/D	45
BTCA	1.81	3.09	10.30	0.08	2.31	5.77	N/D	3

Compound	Retention time (min) [Cambridge]	LOD (ppb) [Cambridge]	LOQ (ppb) [Cambridge]	LOD (ppb) [Padua]	Instrumental Repeatability (% RSD) [Cambridge]	Method Repeatability (% RSD) [Cambridge]	Reproducibility (R^2) [Cambridge]	Recovery (%) [Cambridge]
Cntd.								
Lauric acid	13.17	4.47	14.90	13.72	2.43	6.23	N/D	62
Methyl tetrols	3.04	4.57	15.23	0.55	3.26	10.62	0.94	79
Arachidonic acid	13.25	4.69	15.63	0.05	3.51	10.96	N/D	84
Tricosanoic acid	14.37	4.73	15.77	0.25	2.53	6.21	N/D	87
Terebic acid	3.02	5.65	18.83	0.09	4.99	7.27	0.85	120
Behenic acid	14.18	5.93	19.77	0.33	4.46	5.28	N/D	95
Meso-erythritol	2.88	5.94	19.80	3.16	17.74	15.49	N/D	40
Heptadecanoic acid	13.52	6.27	20.90	2.33	1.00	7.06	N/D	100
Pinolic acid	3.10	8.38	27.93	0.06	7.15	10.20	N/D	84
Cis-pinonic acid	7.63	8.94	29.80	1.00	7.80	10.96	0.83	92
Salicylic acid	7.65	10.23	34.10	0.35	9.09	11.27	N/D	81
Octacosanoic acid	15.66	11.73	39.10	0.32	1.35	12.10	0.74	44
Heptacosanoic acid	15.33	12.21	40.70	0.49	1.99	9.09	0.32	53
Melissic acid	16.52	17.03	56.77	6.04	0.28	17.03	0.67	33
Levoglucosan	2.76	17.53	58.43	100	27.72	10.29	N/D	106
Myristic acid	13.28	19.14	63.80	11.88	6.59	8.70	N/D	N/A
Oleic acid	13.46	20.13	67.10	2.11	2.91	12.22	N/D	54
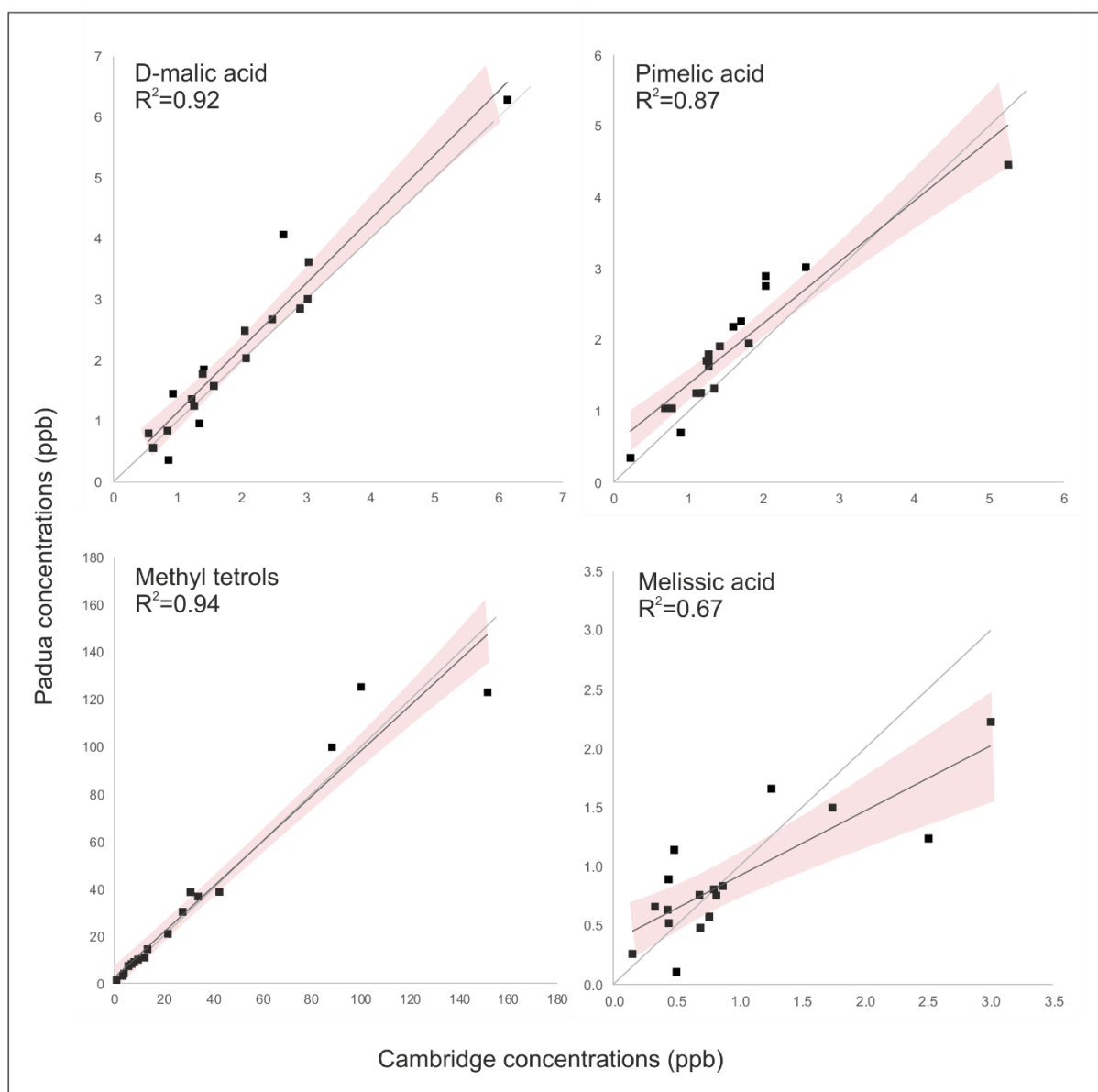
Figure 8: Scatterplots of comparison compound concentrations from replicate sample analyses on two different Orbitrap HPLC-MS instruments. The plots demonstrate good reproducibility of both concentrations and trends based on R^2 values of linear regression lines (black). 1:1 lines are presented in light grey for comparison, and error bands at 95% confidence intervals shown in pink. Plots for the remaining five compounds which were detected in samples are shown in Figure 9.
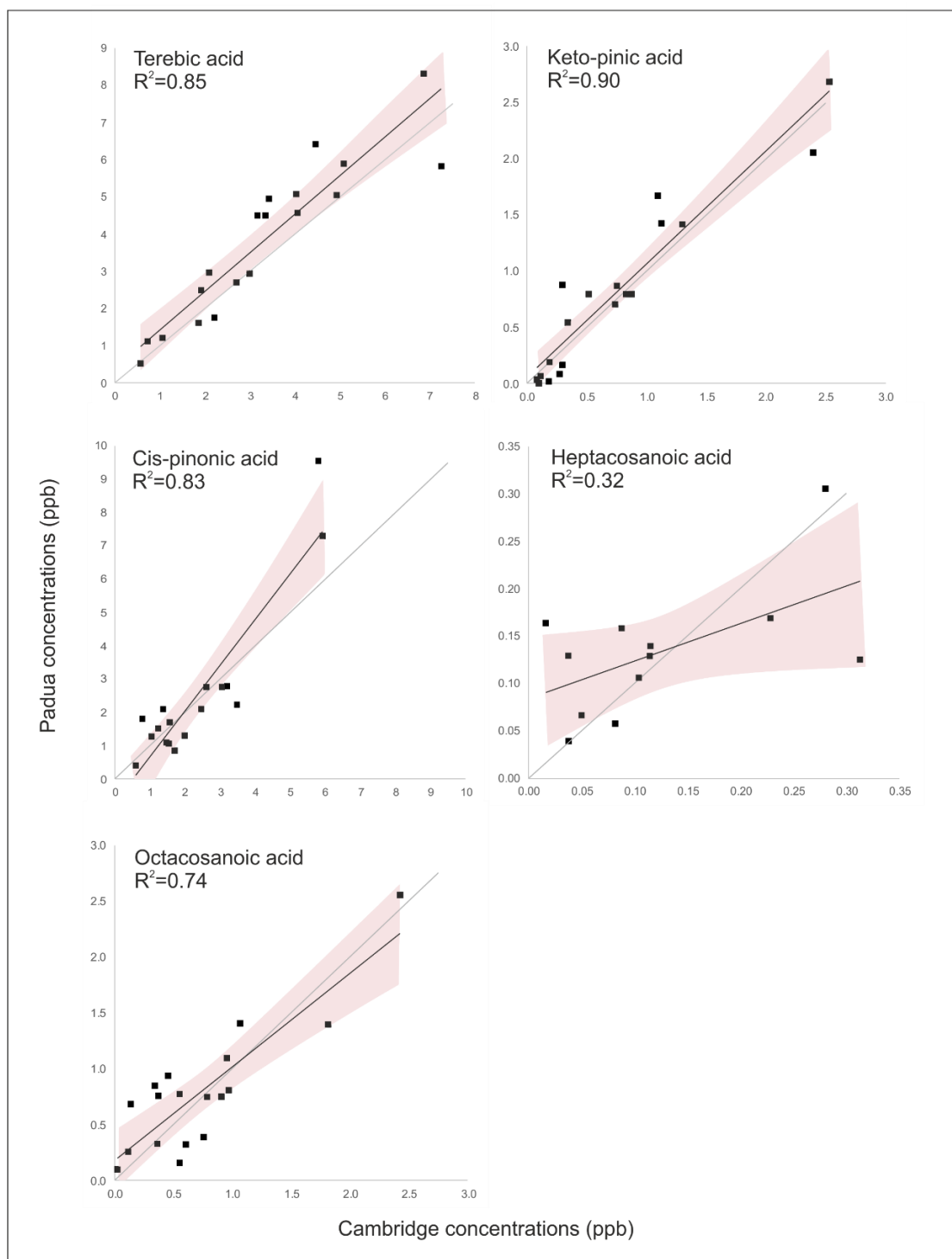
Figure 9: Scatterplots of comparison compound concentrations from replicate sample analyses on two different Orbitrap HPLC-MS instruments. The plots demonstrate good reproducibility of both concentrations and trends based on R^2 values of linear regression lines (black). 1:1 lines are presented in light grey for comparison, and error bands at 95% confidence intervals shown in pink. Compounds shown are all remaining compounds detected in samples and not shown in Figure 8.

3.7. Validation of the direct injection – HPLC-MS method

Instrumental LODs were evaluated on standard solutions prepared in water to match the matrix of the ice samples. Calculation used the Hubaux-Vos method, following IUPAC recommendations (Hubaux & Vos 1970; Currie 1995). Limits of quantifications (LOQs) are x̄ + (10*SD) where x̄ is the mean blank level. Sensitivity (slope of the calibration line) and linearity range were tested using both the r-Pearson correlation test and the F-test to compare linear and quadratic fits. Results showed a good linearity in the tested range (10 ppt-100 ppb) for all compounds. Method/instrumental repeatability was evaluated in real ice core samples. Validation parameters are reported in Table 5.

Matrix effects of direct-injection samples were tested by comparing the linear calibration lines of two different sets of prepared standards, each analysed in triplicate; one set of 1 ppb, 10 ppb, and 100 ppb concentrations diluted with water (external calibration), and another of the same concentrations diluted with ice-sample melt made by pooling together aliquots of the different Belukha ice samples (internal calibration). Comparison of the slopes of the lines, using a t-test, was used to evaluate the difference in values quantified between the two standard types. This approach was used instead of the post-column infusion and post-extraction addition protocols (Zhou et al. 2017) due to unavailability of blank samples (i.e. melted ice samples free from target analytes). Results show (Table 5) the presence of a small but significant matrix effect for most of the analytes. Analytes with lower background contaminations are generally also less affected by matrix effects while compounds with higher background contaminations are more affected by matrix effects (e.g. fatty acids). Isotopically labelled (deuterated) standards do not compensate for matrix effects, probably due to slight differences in lipophilicity and ion suppression effects, as observed in previous studies (Nicolò et al. 2017; Wang et al. 2007).

Table 5: Parameters of methodological validation of the direct injection HPLC-MS analyses, which are presented in order of increasing LOD. Also presented are LOQ, retention time, repeatability (presented as residual standard deviation from three repeat injections of calibration samples each of 10 ppt, 100 ppt, 1 ppb, 10 ppb and 100 ppb), method comparison (presented as R^2 values of a linear trend line of preconcentrated versus direct injection samples, see also Figure 10) and matrix effects (presented as the change in calibration slope between the standards diluted in ice sample melt compared to those diluted in water). NA=not applicable.

Compound	LOD (ppb)	LOQ (ppb)	Retention time (min)	Instrumental Repeatability (% RSD)	Intralaboratory comparison (R^2)	Matrix effect (% ±% RSD)
BTCA*	0.01	0.03	1.70	5	NA	13.5±9.1[a]
MBTCA**	0.02	0.06	1.70	5	NA	5.7±9.2[a]
Keto-pinic acid	0.02	0.07	7.85	7	0.68	4.9±4.8[a]
β-caryophyllinic acid	0.02	0.08	7.79	6	NA	5.6±4.3[a]
D-malic acid	0.04	0.13	1.76	4	0.75	3.9±6.8[a]
β-caryophyllonic acid	0.10	0.32	13.12	6	NA	-2.0±3.5[a]
Methyl tetrols	0.13	0.43	3.57	4	0.92	11.4±2.3[b]
Terebic acid	0.14	0.46	3.22	3	0.64	-9.4±5.5[a]
Pimelic acid	0.22	0.74	1.79	5	0.50	-4.2±8.4[a]
Cis-pinonic acid	0.35	1.16	7.61	6	NA	4.3±6.9[a]
Arachidonic acid	0.44	1.46	14.09	9	NA	1.1±3.1[b]
Pinolic acid	0.59	1.96	7.40	12	NA	-5.5±8.0[a]
Meso-erythritol	2.57	8.62	2.93	17	NA	9.9±3.8[b]
β-nocaryophyllonic acid	3.02	10.06	12.88	5	NA	6.8±8.6[a]
Tricosanoic acid	3.82	12.74	19.27	6	NA	16.8±5.3[b]
Salicylic acid	5.44	18.15	7.61	12	NA	7.5±6.0[a]
Behenic acid	5.68	18.93	18.19	5	NA	20.6±2.9[b]
Melissic acid	6.08	20.28	28.22	10	NA	18±53[b]
Nonadecanoic acid	6.32	21.07	15.91	12	NA	30±23[b]
Heptacosanoic acid	6.97	23.19	25.29	7	NA	3.0±3.4[b]
Octacosanoic acid	9.99	33.28	27.46	8	NA	11.7±6.5[b]
Lauric acid	10.91	36.35	13.56	5	NA	15.6±6.6[b]
Heptadecanoic acid	12.83	42.76	14.92	5	NA	10±27[b]
Myristic acid	15.74	52.46	13.94	6	NA	8.0±7.6[b]
Oleic acid	15.75	52.49	14.60	3	NA	-9±15[b]

[a] Evaluated in the concentration range 0-10 ppb; [b] Evaluated in the concentration range 0-100 ppb. * Butane-1,2,3,4-tetracarboxylic acid. ** 3-methyl-1,2,3-butanetricarboxylic acid.

3.7.1. Method comparison; preconcentrated versus direct injection samples

A method comparison was done to assess the accuracy of the direct injection UHPLC-ESI-HRMS method, comparing the ice samples from the Belukha glacier ice core measured both with preconcentration – HPLC-MS method (already analysed on the Q Exactive for the interlaboratory comparison in section 3.6.1) and the direct injection – HPLC-MS method. An inter-laboratory comparison has already been carried out showing that sample concentrations measured on the LTQ Orbitrap are reliably reproduced on the Q Exactive, and therefore our sample concentrations of the preconcentrated method are accurate and may be reliably compared to the direct injection samples.

Compounds detected in the preconcentrated Belukha samples were as follows; D-malic acid, terebic acid, methyl tetrols, pimelic acid, keto-pinic acid, cis-pinonic acid, heptacosanoic acid, octacosanoic acid, and melissic acid. MBTCA was detected in only a few samples above detection limits. In the direct injection method compounds detected were MBTCA, D-malic acid, terebic acid, methyl tetrols, pimelic acid and keto-pinic acid. BCTA and cis-pinonic acid were detected in some of the direct injection samples, but in others was below LODs suggesting these compounds are borderline compared to the detection capabilities without preconcentration. In comparison, the direct injection promoted BTCA and MBTCA detection, as recovery percentage for both compounds in preconcentrated samples was only 3%, the lowest value observed for all compounds, which results in values falling below LOD in these samples. Without this drawback, the direct injection method successfully detects MBTCA in all samples well above LODs.

All of the fatty acids detected with the preconcentration technique were below detection limits in the direct injection samples; this is because background contamination levels were high in these experiments, and consequently so are LODs.

The results of the comparison between the preconcentrated and direct injection samples are shown in Figure 10, as scatterplots representing the reproducibility of final concentration values in the samples. The scatterplots show good linearity for all compounds, indicating that trends in the sample timeseries are reliably reproduced. For some compounds, the linear trend lines deviate from the 1:1 ratio line, for example terebic acid. This difference is not accounted for by matrix effects evaluated using a test ice-sample melt. However, each individual ice sample would be characterized by a different matrix composition, which may affect quantification differently from one sample to another. In each case, the deviation from the 1:1

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ratio line suggests either a lower-than-expected sample concentration in the direct injection samples, or higher-than-expected concentration in the preconcentrated samples. This may be because preconcentrated samples are finally analysed in methanol, used to re-dissolve the samples from the dried vial following rotary evaporation, whereas direct injection samples are measured in the original snow melt. It would be expected that methanol is an overall cleaner sample as the lower solubility discourages the presence of inorganics in the sample which may otherwise interfere with the ionisation of the analytes in the ESI source. Ideally, matrix effects could be accounted for by using an internal calibration. However, this is not a viable alternative for this application due to limited amount of sample available for analyses.

The observed offset, where large enough to be significant such as for terebic acid, may be quantified and accounted for in future sample analyses.
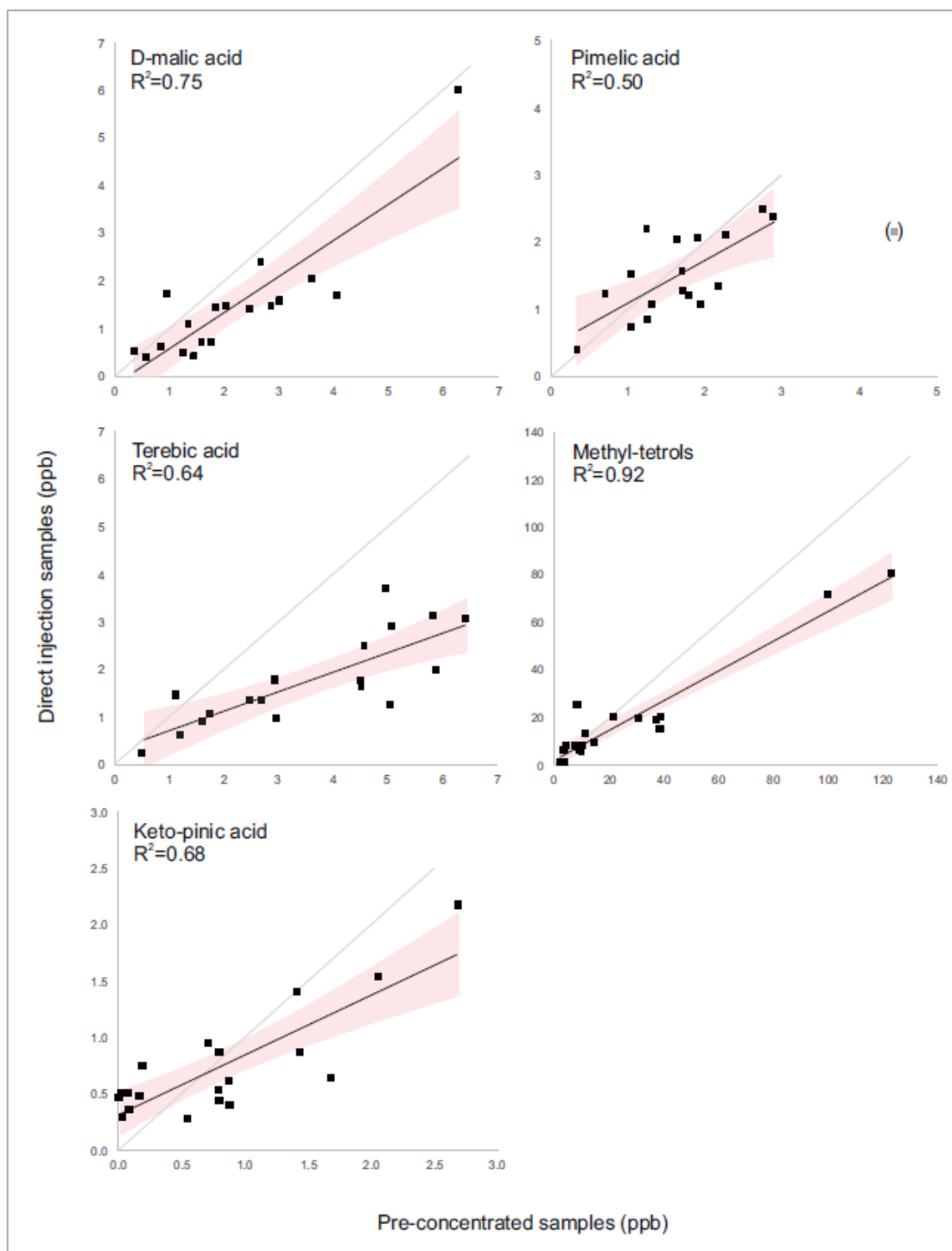
Figure 10: Scatterplots representing comparisons between final sample concentrations of each of direct injection and preconcentration methods of analyses of replicate environmental samples. Linear trendlines and associated R^2 values are presented to assess reproducibility, and error bands at 95% confidence intervals shown in pink. The bracketed outlying point in the pimelic acid plot is shown but not included in the trend line and R^2 value. Compounds shown are all those with a complete dataset from both preconcentrated and direct injection samples (i.e. no sample concentrations below detection limits).

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4. Conclusions

This chapter presents an optimised HPLC-MS analytical method, including preconcentration steps, for the detection and quantification of fatty acids and secondary organic aerosol components in ice cores as markers of terrestrial and marine activity. The method is shown to provide reproducible results for concentrations of organic markers in ice core samples in the range of ppt-ppb concentrations. The optimised preconcentration – HPLC-MS method achieved repeatability of 9% (RSD) averaged for all compounds.

The study tested and compared three preconcentration techniques with the aim of choosing the best method for the compound list as a whole, representing a wide range of organic compounds detectable in snow and ice. The chosen method was rotary evaporation, with average recoveries of 80%. However, optimising one technique for all compounds was challenging and different techniques were more successful for individual compounds.

For future analyses, the recommendation would be to reduce the target list following an initial broad investigation in to the sample content, to allow specific preconcentration techniques to be applied to those markers. Alternatively if a more extensive list of compounds is maintained and where enough sample volume is available, to divide each sample between multiple preconcentration methods. As an example, for very long chain fatty acids, specifically those considered to be indicators of terrestrial source location (heptacosanoic acid, octacosanoic acid, and melissic acid), stir bars would give the best recoveries. For the very smallest SOA compounds (BTCA, MBTCA, and D-malic acid) solid phase extraction is recommended, which would also give reasonable results for other SOA compounds such as sesquiterpene SOAs, but not in combination with fatty acids. If the intention was specifically to target isoprene SOAs (methyl tetrols and meso-erythritol) or levoglucosan, rotary evaporation is the only successful preconcentration method out of those applied here, and could be combined with good recoveries of fatty acids in the same analyses.

This study further presents testing of direct injection – HPLC-MS analyses, i.e. eliminating the preconcentration step of sample preparation. This method is beneficial in reducing the required sample volume and the potential for contamination generated by sample preconcentration steps, as well as total analyses time per sample, but presents challenges in detecting low concentration compounds in the sample. The method gives average instrumental repeatability

of 7% (RSD) averaged for all compounds. Small, but significant, matrix effects (~10% on average) were determined.

This direct injection analytical method is particularly suitable for SOA compounds which showed low recoveries in preconcentrated samples, e.g. MBTCA, and which are clearly above detection limits only with direct injection analyses. Other SOA compounds detected in preconcentrated samples were detected with similar sensitivity in direct injection samples. Many of the studied tracers showed good reproducibility in final sample concentrations in both analytical methods, while others showed a lower-than-expected concentration in direct injection samples compared with pre-concentrated samples. This can be accounted for by differences in sample matrices or ionisation efficiency in samples analysed with the two techniques, and can be adjusted for in final sample concentrations.

Direct injection is less suitable for fatty acid compounds; their high background contamination results in high detection limits. Detection limits for these compounds require new, tailored, cleaning protocols to reduce background contaminations in the solvents and in the instrument itself before direct injection analyses.

At this early stage of the development of these novel organic markers in ice, the aim is to investigate the presence of as many organic compounds as possible in a single sample preparation step in ice samples across multiple locations. The rotary evaporation method combined with the optimised HPLC-MS methodology allows the maximum potential for compound recovery, with low error for methodological repeatability and good reproducibility when applied to analyses in different mass spectrometers. The optimised rotary evaporation preconcentration – HPLC-MS method presented in this chapter therefore provides maximum potential for the identification of new records of organic compounds in ice, and is carried forward to sample analyses in Chapters 4 and 5.

5. Acknowledgements

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Chapter 4: Organic compounds in a sub-Antarctic ice core

Preface

The Bouvet Island ice core was drilled and processed by Amy King as part of the sub-Antarctic ice core expedition (SubICE). Major ion analyses of the Bouvet ice core samples was performed by lab technicians at the British Antarctic Survey. Statistical analyses used this pre-acquired data but was performed by Amy King. All organics sample analyses, statistical analyses and interpretation was carried out by Amy King.

Overview

This chapter present results from the organics analyses of the first ever ice core drilled on sub-Antarctic island Bouvet, in a climatologically important but understudied region. It brings together analyses of organic compounds as developed in Chapter 3, more familiar organic compounds in the ice core, and commonly measured inorganic ions. The fatty acid compound oleic acid shows a significant, positive correlation to sea ice concentration, alongside methanesulfonic acid (MSA). Both compounds are sourced from spring phytoplankton blooms, which are larger with greater amounts of sea ice in the preceding winter. Oxalate, formate and acetate are positively correlated to sea ice concentration in summer, but their source is uncertain and requires further investigation. Back trajectory analyses show that the transportation of compounds is dominated by westerly winds to the extent that there is little seasonal variation, and this is the major mechanism of supply of marine aerosol to the ice core site. This study demonstrates the application of organic compounds from the marine biosphere in generating multi-proxy sea ice records, which is critical in improving our understanding of past sea ice changes.

Chapter 4: Table of Contents

1. Introduction

Due to the current sparcity of palaeoclimate archives available in the sub-Antarctic it is one of the least studied climate regions on Earth (PAGES Consortium 2017). There are very few opportunities to recover terrestrial records from this region, and ice cores from remote ice-capped sub-Antarctic islands (SAIs) are rare. One of the more accessible Islands, South Georgia, has seen recent investigation in terms of reconnaissance for suitability of ice coring, and was found to show great potential in harboring annually resolved climate archives from this highly sensitive climate region (Mayewski et al. 2016). However, there are a number of other ice-capped islands circumnavigating the sub-Antarctic region including the Balleny Islands, Scott Island, Peter 1st Island, South Sandwich Islands and Bouvet (Figure 1), all of which have virtually no pre-existing ice core investigation. And yet, the sub-Antarctic is a region exhibiting many environmental factors that are important for global climate which could be captured in ice core records; for example it sits immediately within the westerly wind belt, at the interface between high and lower latitude climate, and is the region of maximum summer sea ice extent in the Southern Hemisphere. Subtle changes in all of these contribute substantially to climate feedbacks. Ice cores in the sub-Antarctic may provide the best direct record of these changing factors throughout climate history.
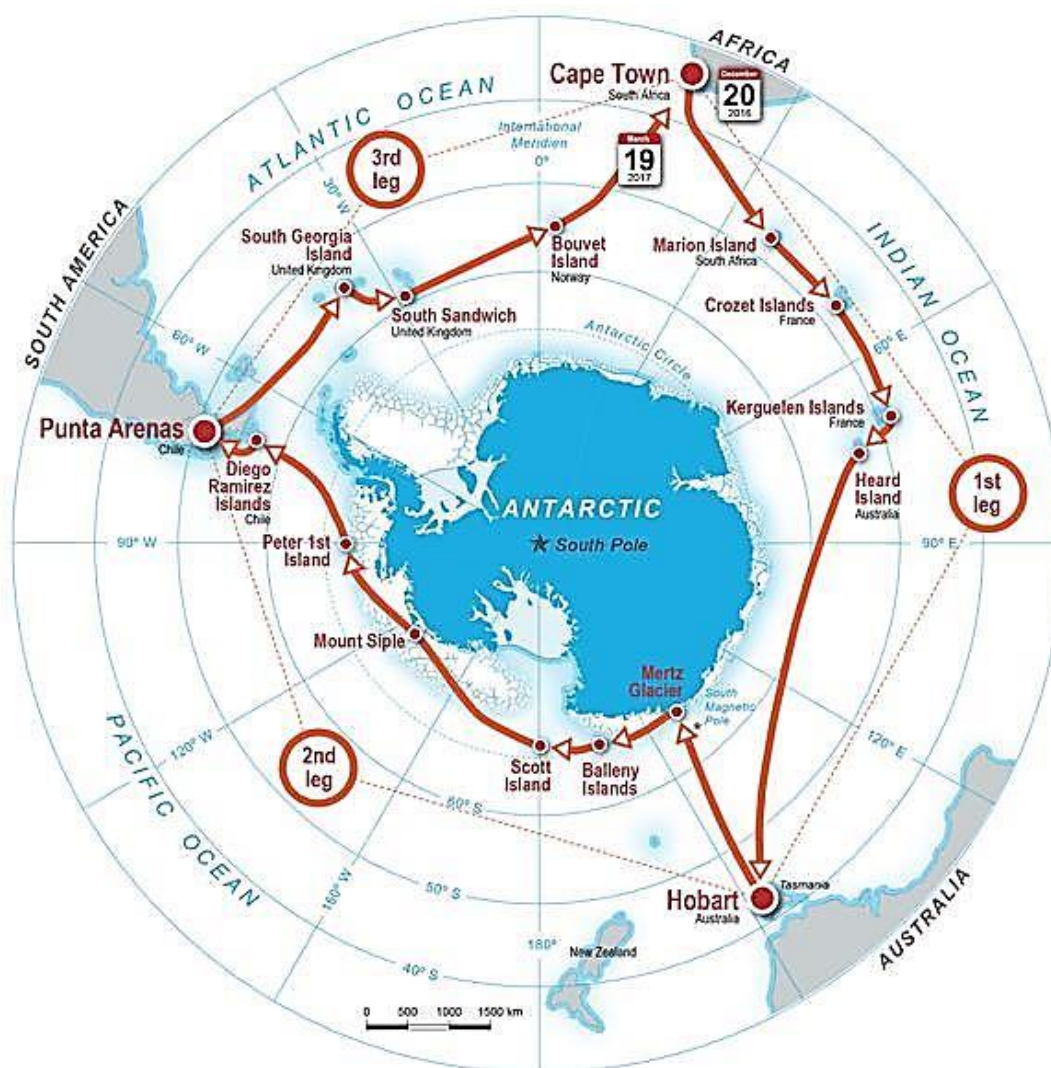
Figure 1: The sub-Antarctic islands, as shown on an illustration of the Antarctic Circumnavigation Expedition cruise track from which the Bouvet Island ice core was collected (from Walton and Thomas 2018).

Bouvet Island is a highly remote SAI, and therefore ideally placed to offer a dominantly marine aerosol-input study site. This will be complementary to the dominantly terrestrial aerosol-input ice core (from Belukha Glacier, Russian Altai Mountains) measured in Chapter 5, giving a clear view of compounds coming from the two sources and without the complication of dual sources for the same compounds. There is already evidence that organic compounds from the marine biosphere are robust environmental proxies in ice cores; methanesulfonic acid (MSA) is uniquely formed as an oxidation product of dimethylsulfide (DMS), which in turn is emitted

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by phytoplankton in the sea ice zone (Curran & Jones 2000) and provides arguably our best records of sea ice before the satellite era, something which inorganic compounds in ice cores do not capture. Strong correlations between MSA and sea ice extent have been observed at a number of ice core sites around the coast of Antarctica. This correlation may be positive, where greater sea ice extent in winter leads to a larger area of sea ice breakup during the spring, promoting larger phytoplankton blooming events (Curran 2003; Abram et al. 2010; Thomas & Abram 2016). A negative correlation may also be observed where MSA is produced in summer within the sea ice zone in open water polynyas (e.g. Sinclair et al. 2014). Thus, considerations of local sea ice conditions and transport processes are important in interpretation, and additional records of sea ice from new compounds would make our understanding more robust.

Dicarboxylic and monocarboxylic acids are also important contributors to global marine aerosol; Fu et al. (2013) found that dicarboxylic acids contribute on average ~ 15 % to total marine organic aerosol. Marine biogenic sources of these oxidation products are via photochemical degradation of biogenic matter in sea spray, and in-cloud oxidation processes (e.g. Kawamura et al. 2001; Rinaldi et al. 2011). Compounds such as oxalic acid, formic acid, and acetic acid, have been detected in remote locations along the coast of Antarctica in snow samples (Legrand & Saigne 1988) and in the remote marine boundary layer in aerosol samples including around coastal Antarctica (Legrand et al. 2004; Baboukas et al. 2000). Routinely employed ion chromatographic methods allow detection of the dicarboxylic acids oxalate, formate and acetate in ice core samples.

Earlier chapters in this thesis have outlined two groups of marine-sourced organic compounds which show promise as biomarkers in ice cores; fatty acids and secondary oxidation aerosols of terpenes. They survive long distance transport to high-latitude locations and resist degradation in older snow layers, and concentrations in ice samples have been related to environmental conditions. Existing ice core records of these compound groups include the 450 year record of lipid compounds in snow layers at Site J, Greenland (Kawamura et al. 1996a) and the 350 year record of both lipids and oxidation products of isoprene and monoterpenes in Alaska (Pokhrel, Kawamura, Ono, et al. 2015; Pokhrel, Kawamura, Seki, et al. 2015). These records showed marine emissions of these compounds may be linked to wind speeds and sea

surface temperature, and northern hemispheric temperature signals respectively, though interpretation may be complicated by terrestrial sources of these compounds.

There are currently no ice core records of any of these compounds from the SAIs. In this study, I aim to quantify the presence, and interpret the records of, organic compounds from both the fatty acid and terpene SOA components, oxalate, formate, acetate and MSA alongside more traditional ice core chemicals (Table 1) in the first ever ice core record from the remote SAI of Bouvet (Bouvetøya).

Table 1: Target compound list for this Bouvet Island ice core, by compound group and the range of concentrations detected (bold) in the Bouvet ice core (adapted from King et al. 2019).

Source	Compound name	Formula	Concentrations this study (ppb)
Isoprene SOA	**Meso-erythritol**	$C_4H_{10}O_4$	**0.1-0.7*[a]**
Isoprene SOA	Methyl tetrols	$C_5H_{12}O_4$	N.D.
Monoterpene SOA	**Pimelic acid**	$C_7H_{12}O_4$	**0.1-0.4*[a]**
Monoterpene SOA	1,2,4-butanetricarboxylic acid (BTCA)	$C_7H_{10}O_6$	N.D.
Monoterpene SOA	3-methyl-1,2,3-butanetricarboxylic acid (MBTCA)	$C_8H_{12}O_6$	N.D.
Monoterpene SOA	Terebic acid	$C_7H_{10}O_4$	N.D.
Monoterpene SOA	Pinolic acid	$C_{10}H_{18}O_3$	N.D.
Monoterpene SOA	*Cis*-pinonic acid	$C_{10}H_{16}O_3$	N.D.
Monoterpene SOA	Keto-pinic acid	$C_{10}H_{14}O_3$	N.D.
Sesquiterpene SOA	β-caryophyllinic acid	$C_{14}H_{22}O_4$	N.D.
Sesquiterpene SOA	β-caryophyllonic acid	$C_{15}H_{24}O_3$	N.D.
Sesquiterpene SOA	**β-nocaryophyllonic acid**	$C_{14}H_{22}O_4$	**0.2-1.7*[a]**
Biomass burning	Levoglucosan	$C_6H_{10}O_5$	N.D.
Dicarboxylic acid	**D-malic acid**	$C_4H_6O_5$	**0.2-2.5*[a]**
Hydroxyacid	Salicylic acid	$C_7H_6O_3$	N.D.

Source	Compound name	Formula	Concentrations this study (ppb)
Cntd.			
Low molecular weight fatty acids (LFA) (<C24); marine / microbial sources	Lauric acid	$C_{12}H_{24}O_2$	N.D.
	Myristic acid	$C_{14}H_{28}O_2$	N.D.
	Heptadecanoic acid	$C_{17}H_{34}O_2$	N.D.
	Oleic acid	$\mathbf{C_{18}H_{34}O_2}$	**2.4-7.6[a]**
	Nonadecanoic acid	$C_{19}H_{38}O_2$	N.D.
	Arachidonic acid	$C_{20}H_{32}O_2$	N.D.
	Behenic acid	$C_{22}H_{44}O_2$	N.D.
	Tricosanoic acid	$C_{23}H_{46}O_2$	N.D.
High molecular weight fatty acids (HFA) (>C24); terrestrial biomass	Heptacosanoic acid	$C_{27}H_{54}O_2$	N.D.
	Octacosanoic acid	$C_{28}H_{56}O_2$	N.D.
	Melissic acid	$C_{30}H_{60}O_2$	N.D.
Oxalic acid	**Oxalate**	$\mathbf{C_2O_4^-}$	**0.4-64.2[s]**
Formic acid	**Formate**	$\mathbf{CHO_2^-}$	**12.61-1167.4[s]**
Acetic acid	**Acetate**	$\mathbf{C_2H_3O_2^-}$	**6.6-1360.2[s]**
Dimethylsulfide (DMS)	**Methanesulfonic acid (MSA)**	$\mathbf{CH_4O_3S}$	**0.03-22.0[s]**
Anions	**Bromide**	$\mathbf{Br^-}$	**0.5-35.5[s]**
	Chloride	$\mathbf{Cl^-}$	**149.5-7014.1[s]**
	Sulfate	$\mathbf{SO_4^{2-}}$	**0.1-903.4[s]**
	Nitrate	$\mathbf{NO_3^-}$	**7.1-1729.4[s]**
Cations	**Ammonium**	$\mathbf{NH_4^+}$	**9.7-63.0[s]**
	Potassium	$\mathbf{K^+}$	**8.4-329.9[s]**
	Sodium	$\mathbf{Na^+}$	**77.8-4404.3[s]**
	Magnesium	$\mathbf{Mg^+}$	**6.4-332.4[s]**
	Calcium	$\mathbf{Ca^+}$	**9.3-608.2[s]**

N.D. = Not detected

* = Discontinuous time series

[a] = Values from annual resolution data

[s] = Values from seasonal resolution data

2. Methods

2.1 Ice core site and age scale

Bouvet Island (Bouvetøya) is a volcanic island at E3º-S54º, between the southern tips of South America and South Africa (Figure 2). The Bouvet ice core was collected as part of the SubICE (Sub-Antarctic Ice Core Expedition) project onboard the Antarctic Circumnavigation Expedition (ACE) in 2017 (Walton & Thomas 2018) . The drill site was on the eastern slope of the island at approximately 350 m altitude. This location slopes gently, without crevassing, and is the maximum altitude (desired in order to limit melt layers in the core from warmer temperatures at lower altitude) which was safely below the cloud-base throughout the drilling period. The core was drilled to a depth of 14.2m using a Kovacs Mark II Coring System, and transported frozen back to Cambridge, UK, for later cutting and analyses.
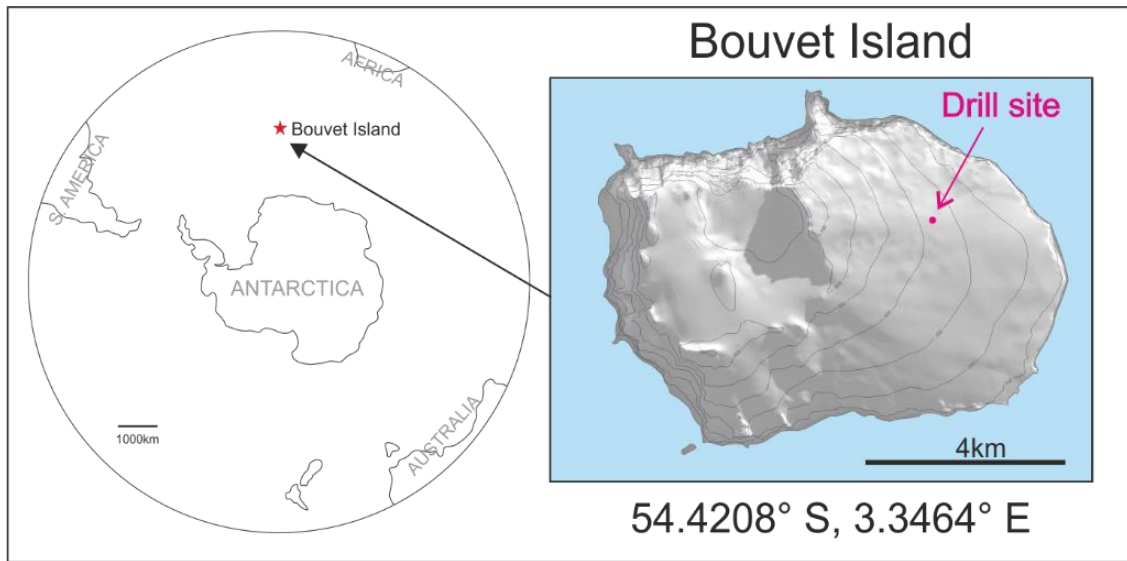
Figure 2: Location map of Bouvet Island and the ice core drilling site on the island.

The core allows high resolution sampling due to thick annual layers of approximately 85 cm core-length per year. Seasonal cycles in the isotope and major ion records can therefore be used to identify and count annual cycles. Dating of the Bouvet record was somewhat challenging; the age scale relied on a combination of isotopes and chemistry as no one record clearly dated

the entire core length. In the case of isotopes, which vary in conjunction with local temperature, the seasonal signal usually present in ice records may be subdued by the sub-Antarctic location. The sub-Antarctic does not see the same temperature differences as the Antarctic continent between summer and winter. Many major ions did not display clearly defined seasonal peaks and troughs; the records may be complicated by the combination of source and transport processes. The clearest records used for dating purposes were the deuterium isotope record, where values are lower in winter corresponding to lower temperatures, and where this was not clear to additionally use the calcium record which in some years did appear to show a summer-time peak. An example of this simple layer-counting method is shown in Figure 3. Each record had been smoothed over a three-point sliding data window to aid visual clarity in the plot. This interpretation was also guided by the predicted accumulation rate on Bouvet Island using ECMWF ERA5 reanalysis data (Dee et al. 2011), which is available for public use though the data for the Bouvet region had been previously obtained by staff at the British Antarctic Survey. Thus, if neither the isotopes nor chemistry provided a clear annual signal, a year-break could be placed based on annual layer thickness. The years were dated winter-to-winter, based on annual minimum concentrations, to encompass summer-time peaks in concentrations in to one whole sample. This method resulted in an age scale from 2001-2016 for the full core length. ERA5 suggests a yearly average accumulation of approximately 0.65m weq (metres of water equivalent, representative of ice thickness and ice density), and the final age scale for the Bouvet core results in an annual average of 0.59m weq.

The layer counting method, in particular when records are not clear, introduces some error in to the annual dating. In this case, some error is moderated for precisely since the dating is only annual resolution, so the exact year-boundary may move slightly and still fully incorporate the summer peaks in concentrations, and therefore represent the annual average concentration adequately. However the possibility that annual breaks could have been placed where they do not actually exist, or missed therefore combining years in to one, creates a larger error since this would affect correlations to other time series which rely on year-to-year correspondence. It is difficult to avoid this since the dating applied is the best possible with the available data.
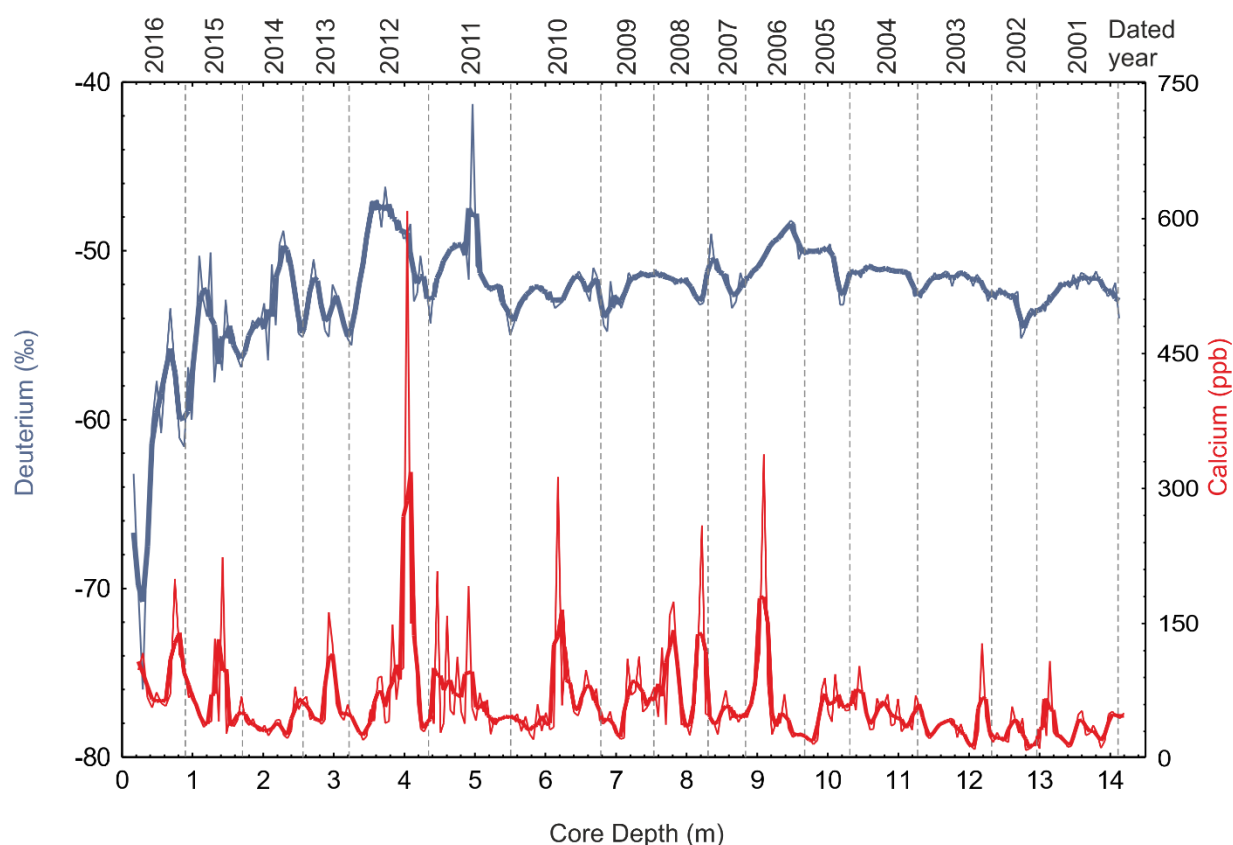
Figure 3: Plot of smoothed high-resolution deuterium and calcium records used for dating by annual layer counting, with assigned winter-to-winter annual layers (dashed lines) marked by date.

2.2 Sample preparation and analyses

All ice core cutting was done using a steel band-saw blade in a -25°C cold lab at the British Antarctic Survey, UK. Blade and working surfaces had been cleaned with isopropyl alcohol (IPA), and the cores portioned cross-sectionally in to multiple strips of ice for different analyses. All ice core strips destined for fatty acid and SOA analyses were cut at annual resolution at the winter-to-winter time scale identified in section 2.1 following clean-protocols described in Chapter 2; outer core surfaces were scraped clean using a steel scalpel, on surfaces covered in aluminium foil. Annual samples were placed in amber glass vials with PTFE lined caps. All glassware had been baked at 450 °C for 8 hrs (Müller-Tautges et al. 2014). Vials were transferred to the British Antarctic Survey class 100 clean-room, and samples left to melt at room temperature preceding sample preconcentration.

Samples were preconcentrated in a rotary evaporator inside the clean-room. Analyses were carried out using high performance liquid chromatography (HPLC) electrospray ionisation (ESI) high-resolution mass spectrometry (HRMS) with a post-column injection. All samples were analysed for the organic compounds listed in Table 1 following the method of King et al. (2019), as developed in Chapter 3, with the exception of oxalate, formate and acetate for which analyses are described herein;

Common major ions (Table 1) alongside MSA, oxalate, formate and acetate were measured as discreet samples of 5cm resolution, which had also been cut using a using a pre-cleaned steel band-saw blade in a -25°C cold lab at the British Antarctic Survey. Samples were placed inside plastic, capped, vials, which has been pre-cleaned by microwaving in milli-q water for 5 mintues and repeating 3 times. Sample were left to melt at room temperatures and analysed on a Dionex Integrion reagent free ion chromatograph in a class-100 cleanroom. Analyses and raw data processing were carried out by lab technicians at the British Antarctic Survey. These samples were later averaged to annual resolution (approximately January to December as for the other organics).

2.3 NAME (Numerical Atmospheric-dispersion Modelling Environment) back trajectory modelling

The NAME back trajectory model was run for 1-month periods for each of February and September in each year 2006-2016, which is the maximum time period available on the model with the applied meteorological data. These months were chosen because they are those in which a compound or compounds had the strongest correlations to sea ice concentration; as described later in sections 3.2/3.3 these were a positive correlation between sea ice concentration and oleic acid/MSA in September, and a positive correlation between sea ice concentration and oxalate in February. The model was set to run 5-day back trajectories (i.e. a maximum particle ages of 5 days estimated based on compound lifetimes, see Chapter 1) with a 24 hr sampling period producing one back trajectory output file per day of the month.

2.4 Statistical analyses

2.4.1 Sea ice correlations

Three sea ice datasets were used throughout analyses carried out herein; firstly correlations between compounds and sea ice concentration were run on the KNMI climate explorer using NSIDC Antarctic monthly average sea ice concentration data, constrained to the time period of the Bouvet Island ice core record (2001-2016). Sea ice concentrations were averaged over 3-month bins, one starting at each of the 12 months of the year. Each of the 12 bins was correlated with the annual average ice core record of the respective compound. Both sea ice and ice core data were linearly detrended prior to correlation. Correlations presented in this study are the 3-month average found to have the strongest correlation between sea ice concentrations and the respective compound record.

Secondly, regional 2001-2016 sea ice extent data, for correlations with chemical compounds, had been generated by staff at the British Antarctic Survey from NASA 1.1 monthly mean sea ice concentration values, calculating the area of all data points where sea ice was above a concentration threshold of 15% within the region. The region was defined as S45-S70, E310-E10, encompassing the area in which a positive correlation was observed between sea ice concentration and the respective organic compounds (Figures 5a, 5b and 7a) as well as the area shown to be supplying air masses to the ice core site in back trajectory analyses (Figure 4a-d). For the correlations in particular, the September average sea ice extent data was extracted from the monthly mean data, to use as the annual maximum (winter) sea ice extent. A geometric mean regression technique was used to calculate correlation coefficients between winter sea ice extent and organic compound concentration following the method of Abram et al. (2010) after Smith (2009). This accounts for measurement error in both the sea ice extent (i.e. from satellite measurements) and compound data series (i.e. in the annual bulking of samples relying on precise dating and cutting, and analytical error from both sample preparation and instrumental analyses).

Thirdly, median average sea ice extent, to display as a geographical line over the stereographic plots outputted from the KNMI climate explorer as above, was acquired as a polyline shapefile from the National Snow and Ice Data Centre (NSIDC) under the freely available Sea Ice Index dataset. This 1981-2010 median is only available representing the bulk period, which does not

exactly match the time-period represented by the core as in the other datasets but is still a good representation for its purposes.

2.4.2 Principle Components Analysis

Principal Components Analyses (PCA) with varimax rotation were carried out using annual average data for all compounds, using RStudio software (R version 3.5.1). PCA plots a dataset as one whole data matrix on a 3D coordinate based plot, and finds linear, orthogonal trendlines which explain the most variance in the data (also known as components). The first component, PC1, always explains most variance, PC2 the second most, and so on. Within these components, each variable of the data matrix input (in this case each compound) is given a 'loading' value, which is equivalent to the amount of the variance of the component that variable is contributing to. The overall aim is to group the input variables (or compounds) in to significant factors (components) which may be determined by the same variances in the input data (for example, compounds with the same emission sources).

Firstly, PCA input data requires pre-analyses normalisation, and all data was therefore log transformed to normalise data distribution. The reliability of the initial PCA output was checked using a resampling method, where a repeat PCA was run for the dataset removing each compound from the matrix one at a time. The variance explained by each output component (i.e. PC1, PC2 etc.) was compared for each repeat run. This determines whether one input compound is having a disproportionate impact on the output components. For the full data matrix PCA, principle components and variance explained were: PC1 61%, PC2 18%, PC3 8%, PC4 6%, all other components $\leq 2\%$. For all resampling PCAs, reduced by one compound at a time, PC1 65-59%, PC2 20-15%, PC3 8-6%, PC4 6-4%, all other components $\leq 2\%$. This demonstrates no extremes in component values are caused by the removal of any one compound, and therefore all are retained in the input data matrix.

The number of components to retain as significant was decided based on Kaisers Rule, also known as the 'Eigenvalue-greater-than-one rule'. There are many different methods that can be employed to determine component significance, but there is no particular consensus on the best one. The Eigenvalue method is one of the simplest and most widely used (Kanyongo 2017) which requires that eigenvalues, or simply the square of the standard deviation of each component, be greater than 1. This is because scores less than 1 imply component scores would

have negative reliability (Kaiser 1960), or otherwise described as scores less than 1 describe less variance than a single variable. Some studies have found the method can either over- or underestimate the number of components to be retained (Cliff 1988) but that reliability is significantly better when there are fewer than 15 variables and 250 samples (Cattel & Jaspers 1967) both of which are met for this study. Based on this, the PCA retains the first four components as significant, explaining 93% total variance of the data.

Finally, a varimax rotation was performed, which rotates the linear orthogonal trendlines of the significant components in the 3D data coordinate plot so that the variance in the loadings is maximized, i.e. the loadings will consist only of high value or low value (nearer zero) loadings and less in the middle ground, and each component will therefore be 'explained' by only a few variables (Abdi 2003). The aim of this step is not so much statistical, as total variance explained by each component will reduce slightly, but to aid interpretation by more clearly selecting the dominant loadings. Varimax rotation was used as it is an 'orthogonal' rotation, which keeps the linear lines orthogonal to one another and thereby does not allow components to be correlated, appropriate to highlighting distinct sources of the ice core compounds.

3. Results and discussion

Major ions (chloride, bromide, nitrate, sulfate, potassium, calcium, magnesium and ammonium, MSA, oxalate, formate and acetate were all detected in substantial concentrations in the Bouvet ice core (concentration ranges are listed in Table 1, and full annual resolution datasets in Appendix 1). From the list of fatty acid and terpene oxidation products tested one continuous record was achieved, for the fatty acid oleic acid. Analyses also detected the compounds D-malic acid, pimelic acid, meso-erythritol and β-nocaryophyllonic acid however the records were not continuous due to several of the samples being below background contamination levels of the cutting blank. This does not suggest that background contamination was very high, but rather the extremely low concentrations of the compounds present are very sensitive to even small background contamination levels. A higher preconcentration factor would not have given better results, since background contamination would have been similarly multiplied.

Many SOA compounds were not detected in any samples. Although terpenes, and in particular isoprene, are known to have marine sources, they are minor in comparison to the terrestrial

sources of these compounds (Hallquist et al. 2009). These results suggest terpenes do not have a substantial enough marine source to be transported, deposited and preserved in the ice at the concentrations required for the limits of detection of the method used.

It is not surprising that long chain fatty acids (C27-C30), which are indicative of terrestrial sources (Eglinton & Hamilton 1967) (as opposed to shorter chain fatty acids (C<27) indicative of marine sources), were not detected in the ice at such a remote marine location. The nearest large-scale sources of terrestrial organic compounds to Bouvet, i.e. forested areas of South America, are too distant for transport within the atmospheric lifetimes of the compounds, as shown by back trajectory analyses (Figure 4a-d).

High background contamination is often an issue for fatty acid analyses as they are very common compounds in the natural environment, and despite the stringent contamination-limiting protocols followed in this study all other fatty acids (except oleic acid) were below background contamination levels. However, King et al. (2019) discuss possibilities of improving the analytical method used by tailoring of the method solely towards fatty acids, rather than the long list targeted in this study. With the promising results shown for oleic acid, further optimisation of the method for this compound group is desirable and may show a broader suite of fatty acids from marine phytoplankton in sub-Antarctic and coastal-Antarctic ice core samples.
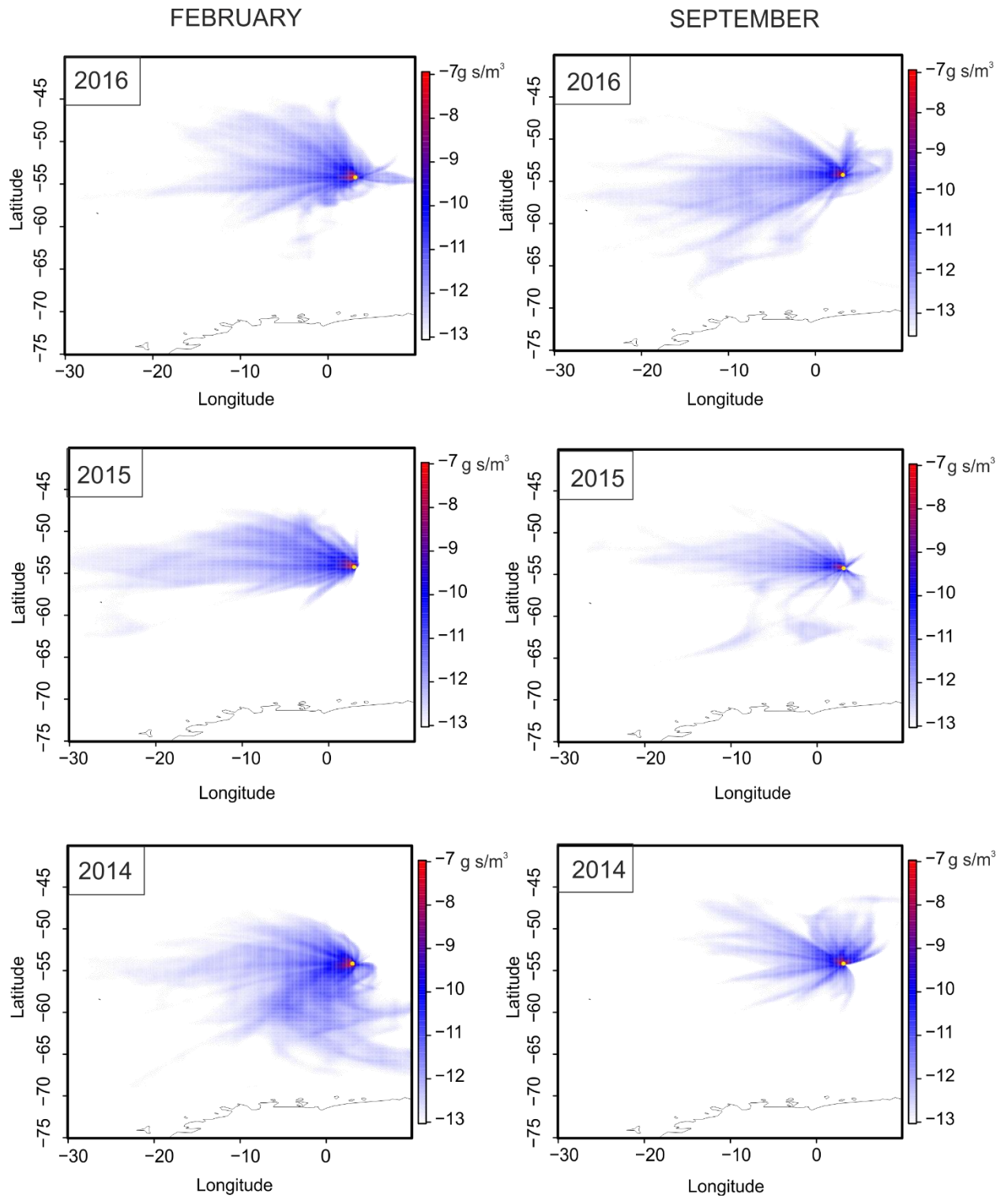
Figure 4(a): Back trajectory plots for one month average back trajectories for each of February and September 2016-2014. Scales are log values.
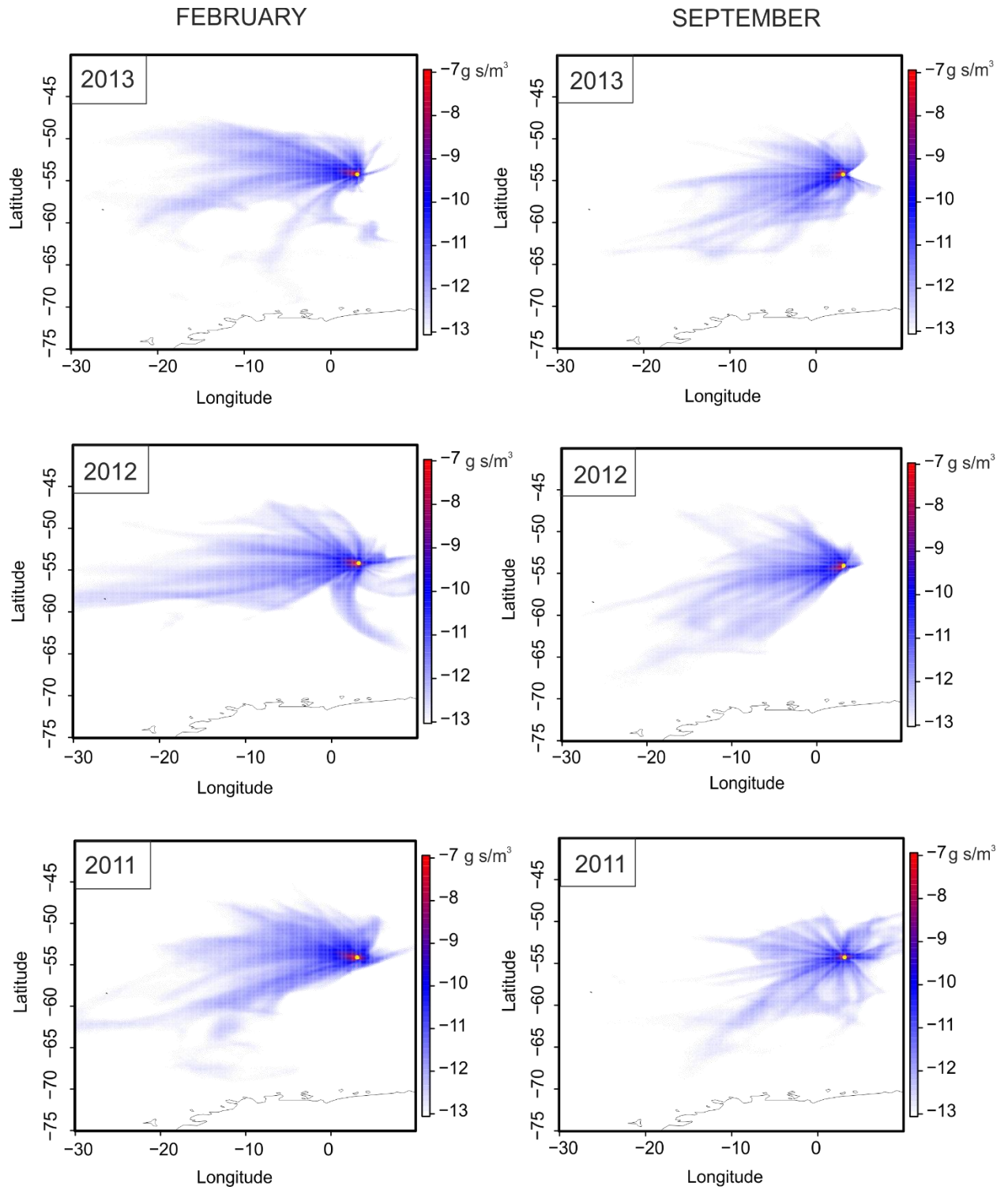
Figure 4(b): Back trajectory plots for one month average back trajectories for each of February and September 2016-2014. Scales are log values.
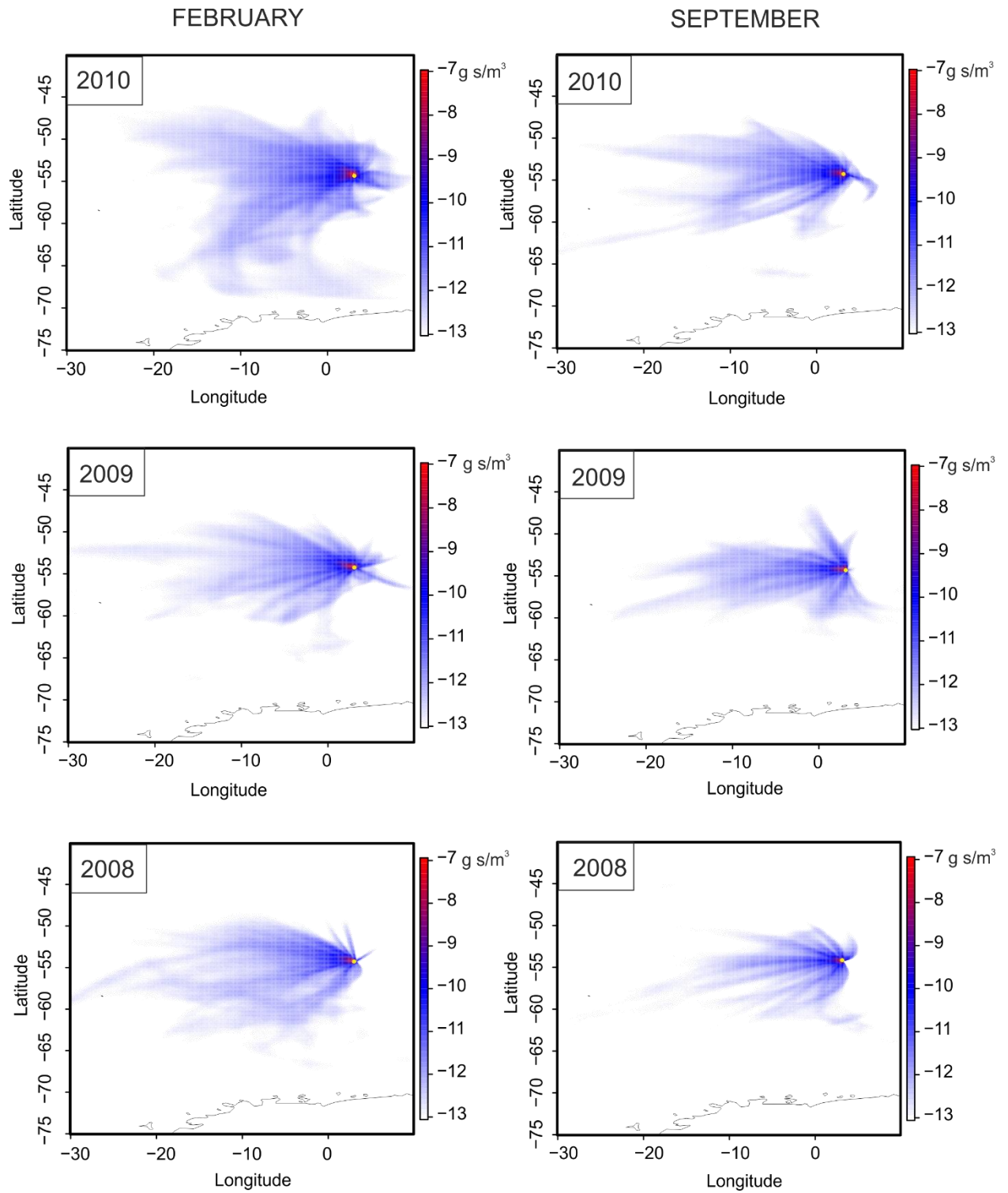
Figure 4(c): Back trajectory plots for one month average back trajectories for each of February and September 2016-2014. Scales are log values.
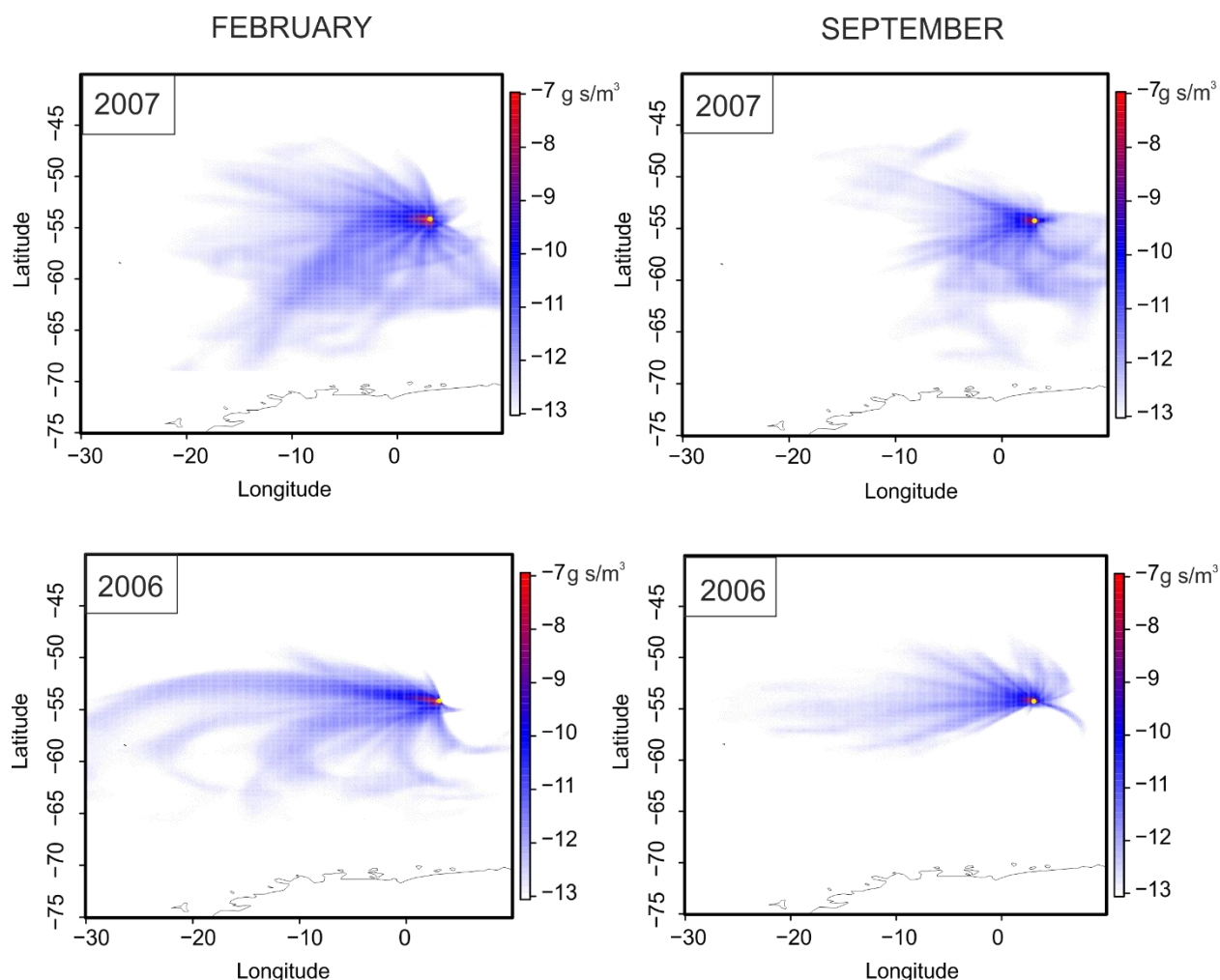
Figure 4(d): Back trajectory plots for one month average back trajectories for each of February and September 2016-2014. Scales are log values.

3.1 Back trajectory analyses

Before statistical investigation of the data, back trajectory plots are presented to guide an understanding of the sources of the compounds being interpreted. It should be noted here that although the scale is given in unit values (g s/m^3), this is not a quantitative assessment of the concentrations of the particles being supplied to Bouvet Island, only the amount of air parcels being received from an area. This is because the former would also require knowledge of the source emission of compounds. For example areas with a lower back trajectory value, or with relatively lower supply of air parcels, may in fact supply greater concentrations of a compound

to the ice core location than an area of greater back trajectory values if emissions of a compound in the former region were much greater, or vice versa.

A feature of all back trajectories, regardless of year or season, is that transport is dominated by westerly winds, as is expected for the region. There is no substantial difference observed between the February and September time plots within each year suggesting no significant seasonal variation in transport. This is also consistent throughout each year; a consistent source region means that the chemistry recorded in the ice core is sampling the changes within that one region, aiding environmental interpretation of the time series.

Despite this region experiencing strong wind conditions, transport distances are not great enough within the lifetime of the organic compounds considered (maximum age 5 days) to reach terrestrial sources. Therefore, the organic compounds detected in this study are assumed to be only from marine biogenic sources.

Further discussion of the back trajectories in relation to outcomes of the following statistical analyses is found in section 3.3.

3.2 Principal Components Analyses

As an initial test for relationships between compounds, a PCA was carried out for all major ions and organic compounds using annual resolution data (Appendix 1). The results of the PCA are displayed in Table 2. The principle component explaining most variance, PC1 (61%), shows strong contribution from the majority of compounds. This is common in ice core PCA and likely indicates that this is the transport component in the core, as transport is expected to contribute to the signal of most compounds to some extent. PC2 is the next strongest component (24%) and is dominated by the organics oxalate, formate and acetate, and ammonium and calcium. PC3 (8%) is dominated solely by nitrate, and PC4 (6%) by MSA and oleic acid.

Table 2: Results of Principal Components Analyses (PCA) with varimax rotation for all measured major ions and organic compounds in the Bouvet ice core. All compounds are inputted as annual averages for the time period 2001-2016. The four significant principle components based on the Kaiser Rule are shown. Loadings contributing most strongly to each components are emphasised in bold.

Component:	PC1	PC2	PC3	PC4
Variance explained:	61%	18%	8%	6%
Ammonium	**0.549**	**0.774**	-0.130	
Nitrate		0.130	**0.974**	
Sulfate	**0.943**	0.118	-0.166	0.103
Sodium	**0.913**	0.195	0.170	0.257
Chloride	**0.924**	0.226	0.143	0.224
Potassium	**0.962**	0.108	-0.136	
Magnesium	**0.836**	0.414		0.251
Calcium	0.432	**0.690**		0.400
Oxalate	0.349	**0.886**	0.149	
Formate		**0.963**		0.192
Acetate		**0.943**	0.135	0.170
MSA	0.251	0.487		**0.787**
Oleic acid	**0.654**			**0.693**
Bromide	**0.906**	0.240	0.206	0.233

There are two features of note to the organic compounds at this stage; firstly the grouping of the organics oxalate, formate and acetate with ammonium and to a lesser extent calcium in PC2. Secondly, the grouping of MSA and oleic acid in PC4. These associations are investigated further using the existing knowledge of the better understood compounds as a guide. Firstly, the association of oleic acid with the sea-ice proxy MSA.

3.3 MSA and oleic acid

The grouping of MSA and oleic acid in PC4 of the PCA was investigated by generating maps of spatial correlation coefficients of each compound to sea ice concentration in the region around Bouvet Island (Figure 5a and 5b).
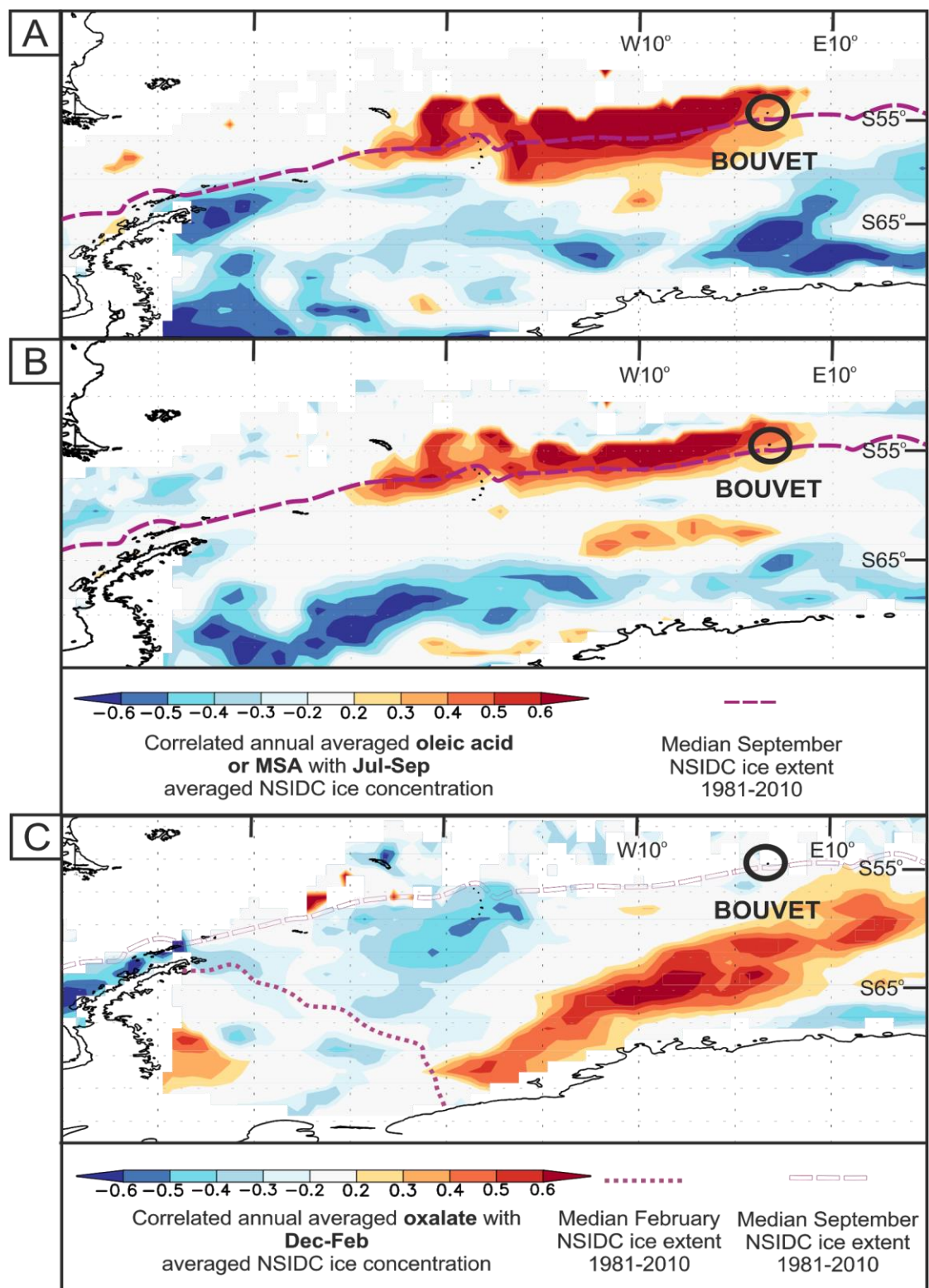
Figure 5: Maps of spatial correlation coefficients between (a) MSA, (b) oleic acid and (c) oxalate with sea ice concentration in the region. Only areas of significance are shaded (grey/red/blue) while areas of no significance remain white. Significance levels are p<0.05.

MSA shows a significant region of positive correlation with sea ice concentration surrounding Bouvet Island at the end of winter and the initiation of spring (Jul-Sep). This region lies along the sea ice margin during maximum winter sea ice extent. The correlation between oleic acid and sea ice concentration shows a very similar region of correlation during the same period. Tracing the correlation region and sea ice extent line over an example back trajectory plot (Figure 6) shows that the region coincides with transport from the west along the sea ice margin to the ice core site. This indicates a source for both oleic acid and MSA along this sea ice margin to the west of Bouvet Island.
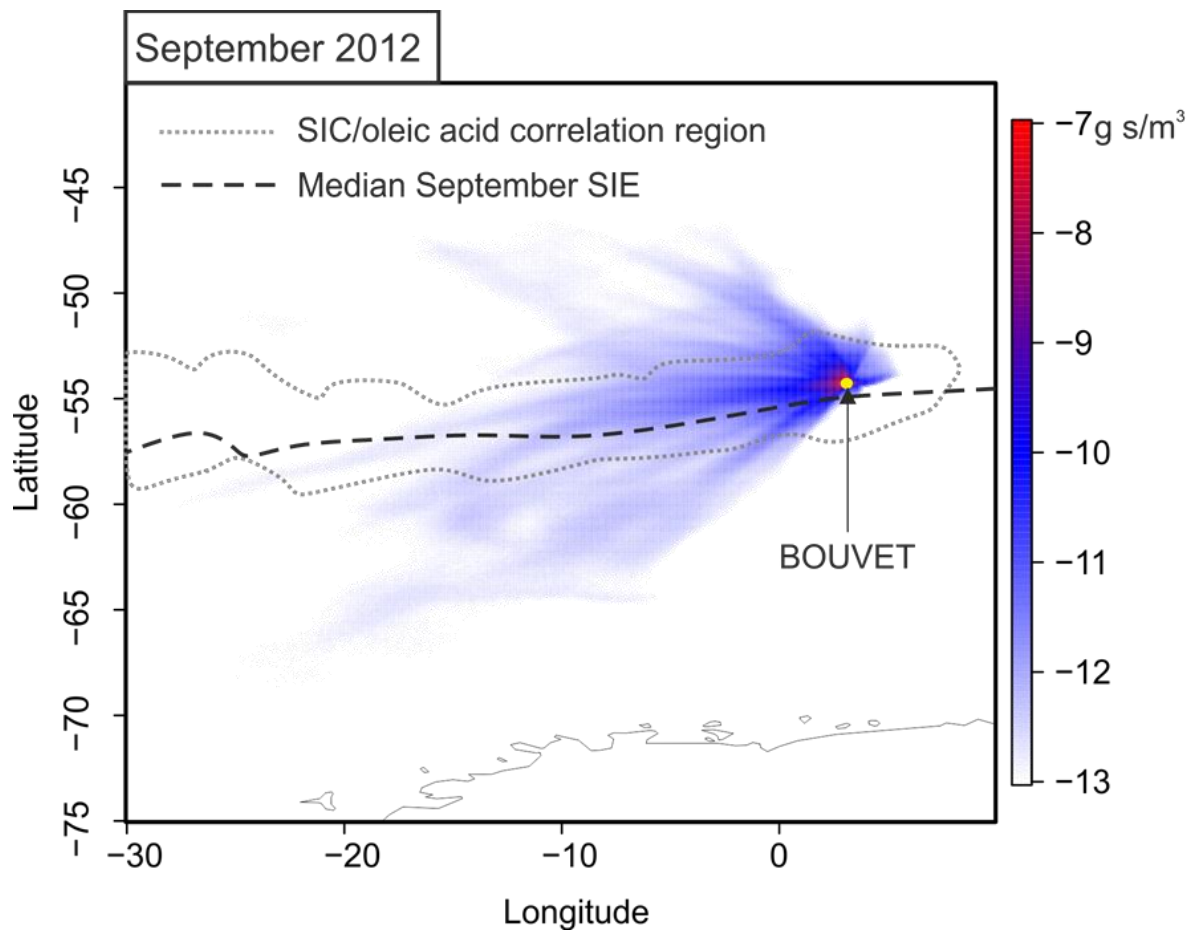
Figure 6: Graphic displaying median sea ice extent (SIE) and the outline of the oleic acid/sea ice concentration (SIC) correlation region as defined in Figure 5b, overlying a representative back trajectory plot from September 2012.

Considering the mechanisms proposed in previous studies where MSA has shown a positive correlation to winter sea ice extent: a positive correlation is created by phytoplankton which emit precursor gases to MSA in very large amounts along the sea ice margin, particularly during phytoplankton blooming events in sea ice break up during spring (Curran & Jones 2000; Abram et al. 2013). The greater sea ice extent during the winter, the larger the phytoplankton blooms the following spring. These blooms of phytoplankton may also enhance oleic acid concentrations; fatty acids are dominant components of phytoplankton, and blooming events of diatoms and dinoflagellates have been shown to be associated with large increases in medium chain fatty acids in the water column of which oleic acid was the dominant component (Kattner et al. 1983). In fact, association between phytoplankton and oleic acid is so strong that the compound, alongside other fatty acids, may be used as class-specific biomarkers of phytoplankton (Leveill et al. 1997; Sahu et al. 2013).

At Bouvet Island, a location on the edge of median maximum sea ice extent, sea ice extent variations in particular may to be important in controlling phytoplankton blooms in the area. In lower ice years, for example, the island may not actually be within the sea ice zone. To investigate this further, oleic acid, MSA and maximum annual sea ice extent were examined in time series record for the entire length of the Bouvet core (Figure 7). The linear regression correlation coefficient for oleic acid/MSA, r=0.79 (p=<0.01), reinforces a link between the two compounds. Correlation coefficients between the compounds and September sea ice extent averaged over the S45-S70, W50-E10 area are r=0.45 (p<0.05) for oleic acid/sea ice extent and r=0.40 MSA/sea ice extent but with p>0.05, showing positive correlations but with lower confidence in the case of MSA. In comparison, extracting the KNMI sea ice concentration data for September and averaging for the exact same geographical region produces correlation coefficients for oleic acid/sea ice concentration of r=0.20 (p>0.05) and for MSA/sea ice concentration of r=0.40 (p>0.05). Confidence levels may be reduced in this case because the area includes averaging over areas which include lower correlation values in the KNMI output plots. Nonetheless, these results show both sea ice extent and sea ice concentration may be related to concentrations of oleic acid and MSA.

While the long-term trend for sea ice over the record 2001-2016 does not substantially increase or decrease, concentrations of both oleic acid and MSA show a slight increase throughout the record. This may appear dominated by a sharp increase in concentration of both compounds in the surface layer (sample year 2016) but trend lines excluding this data point maintain a slightly reduced but still increasing trend 2001-2015. It has been shown previously that high diffusivity

of MSA in ice samples may lead to a loss of original concentrations of the compound deposited on the ice, but this will not affect the record of compound variability along an ice core record if storage of samples has been consistent (Roberts et al. 2009; Abram et al. 2008). There is also no evidence of degradation of fatty acids down an ice core at least over time scales of a few hundred year in previous studies (Kawamura et al. 1996). Therefore, it is likely that the overall trends in concentrations of MSA and oleic acid in the Bouvet ice core are representative of relative year-to-year values.
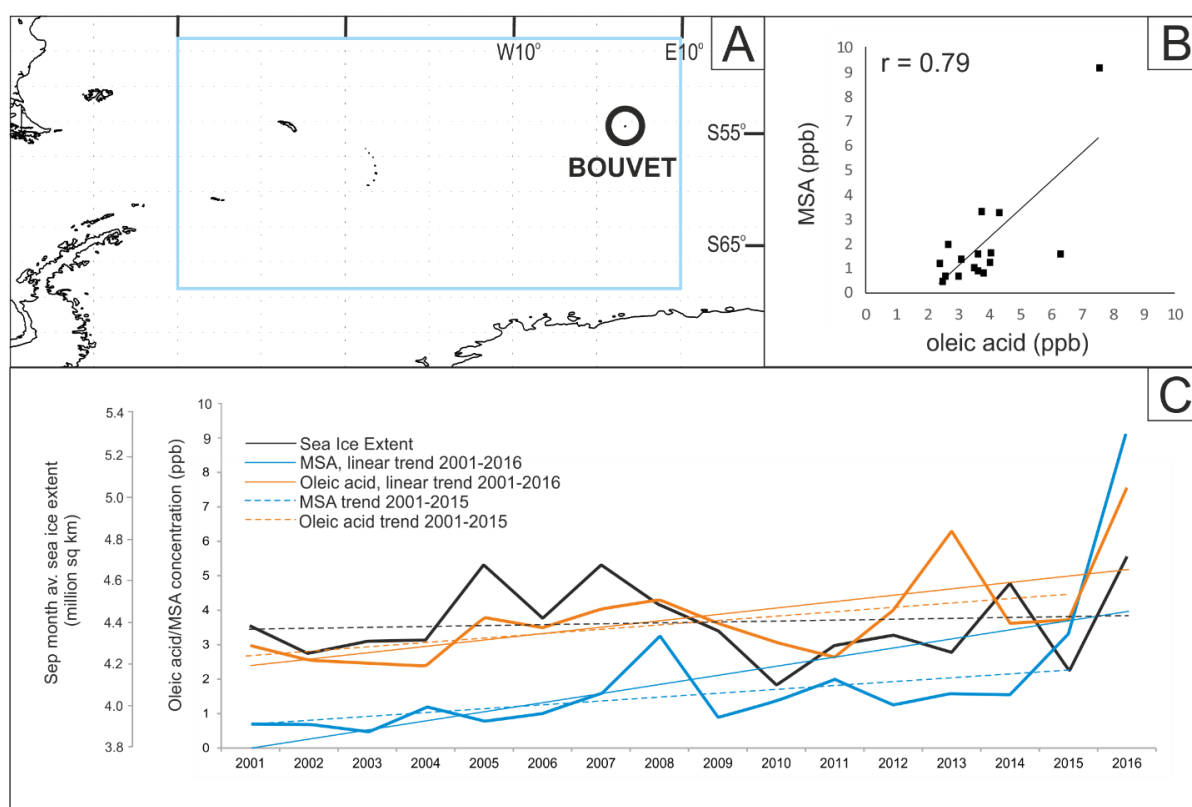
Figure 7: Comparisons between oleic acid, MSA and maximum (September) SIE in the region S45-S70, W50-E10, blue rectangle in (a). (b) Significant positive linear regression between oleic acid and MSA, r=0.79; p <0.01, and (c) time series plots of of SIE-oleic r=0.44; p = <0.05, SIE-MSA r=0.40; p = >0.05. Linear regression averages are shown by solid lines for each full time series and dashed lines for each compound time series excluding the high-concentration 2016 sample.

3.4 Oxalate, formate and acetate

Oxalate, formate and acetate showed a strong association in PC2 of the PCA. They are also shown to be associated with calcium and ammonium. These compounds show a region of positive correlation to sea ice concentration, much further south than that of MSA and oleic acid, for which a representative example for oxalate is shown in Figure 5c. The strongest correlation period for these compounds was December-February, therefore correlation is naturally further south as sea ice extent is lower (compared to the September MSA/Oleic correlation) during these months. Interpretation of this correlation is challenging; the sources of these compounds in particular in the marine biosphere are not well investigated, and in this study do not show strong associations with known sea ice markers in the PCA. They do show association with ammonium, likely from a marine biogenic source, as opposed to the compounds anthropogenic or terrestrial biosphere sources, at this remote marine location.

One previous study of snow and ice samples from coastal Antarctica attributed summer peaks in oxalate concentrations, alongside ammonium and calcium, to the presence of a large Adelie penguin population in the area from the end of October to March (Legrand et al. 1998). These compounds are all shown to be associated in PC2 of the PCA analyses. The amount of sea ice along the coast each year would certainly affect the activities and residing locations of penguin colonies. Back trajectories show that organic compounds in the Bouvet core are unlikely to come from as far away as the coast of the Antarctic continent where such colonies reside, however the correlation area itself is outside the reach of most back trajectories and thus this may not be a valid conclusion. Furthermore the transport mechanism for these compounds may not rely on air mass back trajectories but instead the travel of foraging penguins and sea birds from the sea ice margin towards Bouvet Island, acting as a direct input of these compounds on the island.

Alternatively, marine biogenic source of oxalic acid has been shown in a number of studies, with oxalate forming via degradation of organic precursors dicarboxylic acids and fatty acids, originally in phytoplankton and emitted to marine aerosol by sea-spray processes (Kawamura et al. 1996b; Miyazaki et al. 2010). In-cloud oxidation of glyoxal has been observed as a specific route for oxalic acid formation in marine clouds (Rinaldi et al. 2011), and as a result photochemical oxidation is an important mechanism to consider in resulting concentrations of oxalate (Kawamura et al. 2001). The combination of phytoplankton productivity and photochemical degradation is likely to be strongest in summer, providing a source for our

summer-time oxalate correlation. Since formate and acetate have also been observed in the marine boundary layer (Baboukas et al. 2000) and require oxidation for formation from their precursors, it could be assumed that similar production pathways exist for these compounds in marine aerosol as for oxalate. This is however only speculative and further studies of concentrations of these compounds both in marine aerosol and marine aerosol-dominated ice cores will be beneficial in aiding interpretation of what is an interesting positive sea-ice correlation.

4. Conclusions

A suite of marine-sourced organic compounds have been detected in the sub-Antarctic Bouvet Island ice core. Dominant transport of these compounds to the ice core site is via westerly winds throughout all seasons and years. MSA, a frequently applied sea ice marker, shows a positive correlation with sea ice concentration in the region of Bouvet Island July-September. The region of significant positive correlation sits along the margin of maximum sea ice extent and extends west from Bouvet Island due to dominant atmospheric transport from westerly winds. The correlation is explained by phytoplankton blooming events which are larger when the amount of sea ice in the source region was greater during the preceding winter, and occur along this margin of initial sea ice break up during spring. Strikingly, the organic compound oleic acid, a fatty acid constituent of phytoplankton, shows a strong positive correlation to MSA, and indeed also shows positive correlation to sea ice concentration during the same spring period, suggesting a potential new sea ice proxy. Oleic acid has also been strongly associated with phytoplankton sources.

Oxalate, formate and acetate are found to have positive correlations with sea ice concentration in the later season of summer, therefore leading to a correlation zone that is further south than that of MSA and oleic acid due to the lower sea ice extent at this time. Further investigation is required to help identify the source of these compounds.

This study highlights the application of organic compounds from ice core records as valuable marine biomarkers; indeed the organic records here contribute not only to the data-sparse sub-Antarctic region, but to building a suite of sea-ice proxies. It is hoped that the development of

these proxies and application to other sub-Antarctic, coastal Antarctic, and coastal Greenland cores will lead to a more robust record of local-to-regional scale sea ice changes in the past.

5. Acknowledgements

6. References

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Chapter 5: Organic compounds in the Belukha Glacier ice core

Preface

Major ion concentrations used in the chapter were provided by the research group of Margit Schwikowski at the Paul Scherrer Institut, Switzerland, who also provided the Belukha ice core samples for further organic compound analyses. Major ion data was used in statistical analyses which was carried out by Amy King. All other sample analyses, data exploration and interpretation was done by Amy King.

Overview

This chapter presents results of the organics-analyses of a selection of ice core samples from the Belukha Glacier (Russian Altai Mountains) ice core. It acts as a pilot study for the potential of the organic compounds in a terrestrial-aerosol dominated ice core location. Several organic compounds are detected for the first time in the Belukha Glacier ice core dating back to 1598. The majority are a suite of SOA compounds, which show seasonal cycles of very high concentrations in summer and lower concentrations in winter. This may be caused by seasonal changes in both transport processes and emissions. Variability between different time periods may indicate long term changes in source emission strength of SOA precursors such as isoprene and monoterpenes. The results highlight the potential of these compounds for use in future ice core analyses and for use as environmental proxies.

Chapter 5: Table of Contents

1. Introduction

Belukha Glacier is located in an alpine, landlocked region, presenting a record of atmospheric aerosol mostly, if not entirely, dominated by terrestrial sources. The ice core gives a record of these terrestrial sources without the complication of additional marine sources of the same target organic compounds, and is therefore a complementary and contrasting study to that of the marine-dominated sub-Antarctic Bouvet ice core presented in Chapter 4. Terrestrial sources of SOA compounds are shown to be more prolific compared to marine sources (Hallquist et al. 2009), and the terrestrial biosphere is also a considerable source of fatty acids. For these reasons, and combined with the fact that alpine glaciers are close to terrestrial source locations (in comparison to the sub-Antarctic especially), ice cores drilled here offer good potential for detection of many terrestrial biogenic compounds in substantial concentrations.

Chapter 3 has already shown that samples of Belukha ice, used in a test for method reproducibility, contain several of the organic compounds targeted in this study (Table 1). This chapter presents the full dataset of compounds detected throughout all tested ice samples. Pre-existing major ion records from the core are also presented. All records, combined, are used to present a preliminary environmental interpretation of the organic compounds detected in the Belukha ice core.

Table 1: Target organic compound list for this study, by compound group and in order of increasing number of carbon atoms, with detected (bold) concentrations in the Belukha ice samples. Major ion concentration ranges are for only those samples in which organics were also measured, for full core datasets see Olivier et al. 2003 and therein.

Source	Compound name	Formula	Concentrations in Belukha ice (ppb)
Isoprene SOA	Meso-erythritol	$C_4H_{10}O_4$	N.D.
Isoprene SOA	**Methyl tetrols**	$\mathbf{C_5H_{12}O_4}$	**0.3-151.7**
Monoterpene SOA	**Pimelic acid**	$\mathbf{C_7H_{12}O_4}$	**0.1-5.3**
Monoterpene SOA	1,2,4-butanetricarboxylic acid (BTCA)	$C_7H_{10}O_6$	N.D.
Monoterpene SOA	**3-methyl-1,2,3-butanetricarboxylic acid (MBTCA)**	$\mathbf{C_8H_{12}O_6}$	**0.6-6.2***
Monoterpene SOA	**Terebic acid**	$\mathbf{C_7H_{10}O_4}$	**0.6-11.1**
Monoterpene SOA	Pinolic acid	$C_{10}H_{18}O_3$	N.D.
Monoterpene SOA	***Cis*-pinonic acid**	$\mathbf{C_{10}H_{16}O_3}$	**0.2-6.7**
Monoterpene SOA	**Keto-pinic acid**	$\mathbf{C_{10}H_{14}O_3}$	**0.1-2.5**
Sesquiterpene SOA	β-caryophyllinic acid	$C_{14}H_{22}O_4$	N.D.
Sesquiterpene SOA	β-caryophyllonic acid	$C_{15}H_{24}O_3$	N.D.
Sesquiterpene SOA	β-nocaryophyllonic acid	$C_{14}H_{22}O_4$	N.D.
Biomass burning	Levoglucosan	$C_6H_{10}O_5$	N.D.
Biogenic SOA	**D-malic acid**	$\mathbf{C_4H_6O_5}$	**0.6-10.0**
Primary biogenic	Salicylic acid	$C_7H_6O_3$	N.D.
Low molecular weight fatty acids (LFA) (<C24); marine / microbial sources	Lauric acid	$C_{12}H_{24}O_2$	N.D.
	Myristic acid	$C_{14}H_{28}O_2$	N.D.
	Heptadecanoic acid	$C_{17}H_{34}O_2$	N.D.
	Oleic acid	$C_{18}H_{34}O_2$	N.D.
	Nonadecanoic acid	$C_{19}H_{38}O_2$	N.D.
	Arachidonic acid	$C_{20}H_{32}O_2$	N.D.
	Behenic acid	$C_{22}H_{44}O_2$	N.D.
	Tricosanoic acid	$C_{23}H_{46}O_2$	N.D.
High molecular weight fatty acids (HFA) (>C24); terrestrial biomass	Heptacosanoic acid	$C_{27}H_{54}O_2$	N.D.
	Octacosanoic acid	$C_{28}H_{56}O_2$	N.D.
	Melissic acid	$C_{30}H_{60}O_2$	N.D.
Major ions	**Ammonium**	$\mathbf{NH_4^+}$	**3.6-36.5**
	Calcium	$\mathbf{Ca^+}$	**0.5-28.2**
	Chloride	$\mathbf{Cl^-}$	**0.1-3.8**
	Magnesium	$\mathbf{Mg^+}$	**0.1-3.8**
	Nitrate	$\mathbf{NO_3^-}$	**1.1-12.6**
	Oxalate	$\mathbf{C_2O_4^-}$	**0.4-6.9**
	Potassium	$\mathbf{K^+}$	**0.1-1.5**

Source	Compound name	Formula	Concentrations in Belukha ice (ppb)
Cntd.			
	Sulfate	**SO$^{2-}_4$**	**1.0-31.7**
	Sodium	**Na$^+$**	**0.1-4.3**

N.D. = Not Detected

* = Discontinuous record, i.e. below detection limits in some samples.

2. Methods

2.1 Location

Belukha glacier is located on Belukha Mountain in the Russian Altai Mountains. The core was drilled by a research team from the Paul Scherrer Institut, Switzerland, in 2001 at 49°48'26''N, 86°34'43''E, 4062 m a.s.l. to a depth of 139m, and dated 1250 – 2001 (Olivier et al. 2003; Eichler et al. 2009).

Figure 1: Location of Belukha Glacier (red dot), in the Russian Altai Mountains.

2.2 Sample preparation

Core sections were sealed in polyethylene tubes in the field and transported frozen to Switzerland. All core sections were cut in a -20°C cold room at the Paul Scherrer Institut using a band-saw, pre-cleaned with isopropyl alcohol. Inner ice core sections were previously sampled for major ion analyses (Olivier et al. 2003) along the entire core length. Dating of the core had been completed using a combination of the decay of ^{210}Pb, annual layer counting using ammonium and oxygen isotopes, and known horizons (maximum of the nuclear weapon tests in 1963, Katmai eruption in 1912 and the year of maximum sulfate deposition from the Tambora eruption in 1816) acting as tie points for the layer counting method to (Olivier et al. 2006). Offset of assigned dates between these three methods was small and therefore the age scale is considered accurate.

This study samples discontinuous portions along the Belukha ice core length for analyses of organic compounds; from those sections with a high enough remaining sample volume to meet the requirements of the method, sections were chosen to represent distinct periods in time from both the pre- and post-industrial eras, allowing us to assess the extent to which concentrations and variability of each compound differed between these periods. The time periods studied were 1598-1601, 1826-1828, 1866-1869, and 1981-1983.

The ice strips for organic analyses were cut similarly to those for the ions, and additional clean protocols followed as suggested in Chapter 2. Ice surfaces were scraped after cutting using a cleaned metal scalpel on surfaces covered in aluminium foil. Each sample was placed directly inside pre-baked amber glass vials with PTFE lined caps. Samples were transported frozen to the British Antarctic Survey, Cambridge, UK, and stored at -25°C. Each sample represents 10 cm of ice core length, which is the minimum required to obtain a 10 ml melt volume for preconcentration, considering the cross-sectional size of the ice strip remaining. This results in a higher temporal resolution in the more modern samples.

Directly preceding preconcentration and analyses, samples were left to melt at room temperature (approx. 16°C) in their sealed vials inside a class 100 clean room. Samples were preconcentrated using rotary evaporation inside the clean room, following the method developed in Chapter 3, and transferred to pre-baked glass LC-MS vials for immediate analysis.

2.3 Analytical method

Major ions (ammonium, oxalate, calcium, magnesium, potassium, sodium, chloride, formate, nitrate, and sulfate) were analysed using standard ion chromatographic techniques as previously reported (Olivier et al. 2003).

Organic compounds were analysed using high performance liquid chromatography (HPLC) electrospray ionisation (ESI) high-resolution mass spectrometry (HRMS) with a post-column injection, on a HPLC-ESI-HRMS with an Accela system HPLC (Thermo Scientific, Bremen, Germany) coupled to an LTQ Velos Orbitrap (Thermo Scientific, Bremen, Germany) at the Department of Chemistry, University of Cambridge, UK, following the method of King *et al.* (2019) as developed in Chapter 3.

2.4 Statistical Analyses

Principle Components Analyses (PCA) were carried out using both the major ion and new organic compounds data. The intention is to assess the relationship between the new compounds and existing ones where an initial environmental interpretation has already been made. The PCA was run on a total of four versions of the total dataset, incorporating two comparative sets of the data. The first set of two PCA runs compares annual resolution and seasonal resolution records; one PCA was run on the data averaged across each time section to annual resolution (herein annual PCA), to study the long term input of the chemicals to the site, and one PCA was run on ice sections where original sample resolution allowed observation of seasonal signals i.e. for the time periods 1866-1869 and 1981-1983 (herein seasonal PCA). The second comparative set compares pre- and post-industrial records; sources of compounds and transport of them to Belukha could have changed over the industrial era, where the period 1981-1983 is clearly post-industrial, while the other periods are largely pre-industrial (e.g. Eichler *et al.* 2011). One PCA was performed on pre-industrial (1598-1602, 1821-1823, 1866-1869) data, at original sample resolution (herein pre-industrial PCA), and one PCA on post-industrial (1981-1983) data again at original sample resolution (herein post-industrial PCA). Original sample resolution concentration data for all organic compounds and major ions can be found in Appendix 2.

A more detailed description of the PCA method can be found in Chapter 4 (section 2.3.2). Briefly here, PCA with varimax rotation was carried out using the software RStudio (R version 3.5.1). Data was combined in to a data matrix and normalised by log-transformation before analyses. The validity of the results was checked using a resampling method for the annual and seasonal PCA runs, where a repeat PCA was run for the dataset removing each compound one at a time. For the annual PCA, principle components and variance explained were: PC1 39%, PC2 31%, PC3 11%, all other components <10%. For the annual resampled PCAs the range of values obtained were for PC1 37-43%, PC2 25-31%, PC3 9-12%, all other components <10%. For the seasonal PCA, principle components and variance explained were: PC1 52%, PC2 23%, PC3 10%, all other components <10%. For the seasonal resampled the range of PCAs was PC1 50-56%, PC2 19-25%, all other components <10%, and compounds contributing most significantly to each component remained the same throughout. This demonstrates no extremes in component values are caused by the removal of any one compound, and therefore all are retained in the input data matrix.

The number of components to retain as significant was determined using Kaisers Rule, also known as the 'Eigenvalue-greater-than-one rule'. For both the annual and seasonal PCAs this was the first three (PC1, PC2, PC3) components. These components respectively explain 80% of total variance in the annual PCA and 84% total variance in the seasonal PCA. The number of components retained for the pre-industrial PCA was also three, explaining 78% of total variance, and for the post-industrial PCA two components were retained explaining 85% of total variance. Varimax rotation was performed on each of the significant components, and the output for each is presented in Table 2 of the results section for all four PCA datasets.

2.5 NAME (Numerical Atmospheric-dispersion Modelling Environment) back trajectory modelling

The NAME back trajectory model was run for 3-month periods for each of winter (December-January-February) and summer (June-July-August) seasons in the years of 2015, 2013 and 2011. The met data on which the model relies, available 2006-2016, does not allow any direct comparison years to those of the Belukha ice core record, and so runs were chosen to represent a reasonable investigation of seasonal variability at the ice core location (as in observed

seasonal variations in compound concentrations in the measured organic records, see Figure 2 and results section for further discussion). The model was set to run 5-day back trajectories (i.e. a maximum air mass age of 5 days, the choice of which has been described in Chapter 1, section 1.7) with a 24 hr sampling period producing one back trajectory output file per day of the month. Data was averaged to present a single overall back trajectory representative of a full 3 month period.

3. Results and Discussion

3.1 Compound concentrations and time series

Multiple organic compounds are detected in the Belukha glacier ice core samples; these are the primary biogenic compound D-malic acid (0.6-10.0 ppb, average 2.2 ppb), the monoterpene SOA compounds pimelic acid (0.1-5.3 ppb, average 1.4 ppb), terebic acid (0.6-11.1 ppb, average 4.3 ppb), keto-pinic acid (0.1-2.5 ppb, average 0.5ppb) and cis-pinonic acid (0.2-6.7 ppb, average 2.6 ppb), and the isoprene SOA compound group methyl tetrols (0.3-151.7 ppb, average 20.1 pbb). Time series for these compounds are shown in Figure 2. The monoterpene SOA compound 3-methyl-1,2,3-butanetricarboxylic acid (MBTCA) is detected in the samples between 1598-1601 and 1981-1983 (0.6-6.2 ppb, average 2.2 ppb), but was not detected in the other time periods. It is likely that that compound does exist in these samples but below detectable concentrations, since in the samples that do show a record it is in low concentrations close to the detection limits. Because of the discontinuous record for MBTCA, this compound is not included in further data analyses. All other compounds from Table 1 were not quantifiable with the applied method. It cannot be said with certainty that these compounds would not be present if an analytical method allowed lower detection limits (i.e. in the range of ppt).

One compound expected to be present in the samples, based on previous investigations showing high charcoal concentrations in the core from biomass burning, especially in the period 1598-1602 (Eichler et al. 2011), is levoglucosan. However, this compound had a strong interference in the mass spectrum from a similar compound at the same retention time, meaning the peak was not clearly visible, if present.

Due to the sparsity of studies investigating organics in ice cores, there are only a very few direct comparisons obtainable for any compounds quantified in this study to concentrations of terrestrial biomarkers in other cores. The study of an Alaskan core dating back to 1660 by Pokhrel et al. (2015) detected average pinonic acid concentrations of 0.1 ppb, with maximum concentration of 0.3 ppb (average cis-pinonic acid in this study = 2.6 ppb). In a core from a Swiss alpine glacier dating back to 1940 (Müller-Tautges et al. 2016), average pimelic acid concentrations were 0.15 ppb, while in a Greenland core dating back to 1540 (Kawamura et al., 2001) average pimelic acid concentrations were 0.18 ppb (average pimelic acid this study 1.36 ppb). While these and other studies present additional compound records from the same SOA groups as this study, it is not realistic to directly compare values since each SOA precursor is unique as an aerosol in its sources and reaction pathways. Using these few examples as a guide it appears that compound concentrations in the Belukha core are higher than those previously detected in Alaska, Greenland and the Swiss Alps.

The length of time represented in each measured ice section, and the number of time periods covered, makes it hard to determine trends, but there are some interesting observations in the compound records that would recommend further study. Of the isoprene/monoterpene derived SOA compounds, methyl tetrols, pimelic acid, and keto-pinic acid all show lowest values in the earliest period (around 1600), and highest values in the 1866-1869 period, with a return to lower values in the 1980s section (Figures 2a and 2b). Cis-pinonic acid also shows lowest values around 1600, and high values in the 1860s section, but has high values in the section from the 1980s. Our isoprene marker, methyl tetrols, shows especially high concentrations in the 1860s compared to previous years, while the other three compounds, all monoterpene oxidation products, show a more subdued increase in this time period. The overall trend of increasing concentrations up to 1869 is accompanied by a gradual increase in temperatures in the local region of Belukha Glacier based on $\delta^{18}O$ temperature reconstructions (Eichler et al. 2009). This raises the question of whether the high values in the 1860s are indeed part of a longer trend, perhaps related to temperature. On the other hand, there may be high inter-decadal variability which we do not observe with this dataset. This can only be answered by analysing further sections of ice to fill in these gaps in the record.

Terebic acid and D-malic acid show different long-term trends to the other organics. In comparison to the other compounds these two organic compounds have rather similar concentrations in the different time periods, excluding a single high value for D-malic acid in 1599.

All new organic compounds detected display a strong sub-annual variability in their signals where sample resolution allowed, i.e. in the sample sections from 1866-1869 and 1981-1983. In particular, there are notably higher concentrations during summer, indicating this variability is a seasonal signal. This regular seasonal pattern is also seen in all major ions previously analysed, an example of which is given in Figure 3 (for major ions ammonium and calcium chosen to give an example of one compound which may also have terrestrial biogenic sources and one which does not, respectively). It has been suggested to result largely from seasonality of atmospheric transport at mid-latitudes (Olivier et al. 2006). A stronger transport signal in summer is due to stronger convection to the high-elevation glacier compared to winter conditions. However, for organics we may expect the seasonal signal to be more intensified due to the likely contribution of larger source emissions in summer; further work will be needed to untangle these two influences (transport and emission strength) however the following statistical testing begins to investigate this further.
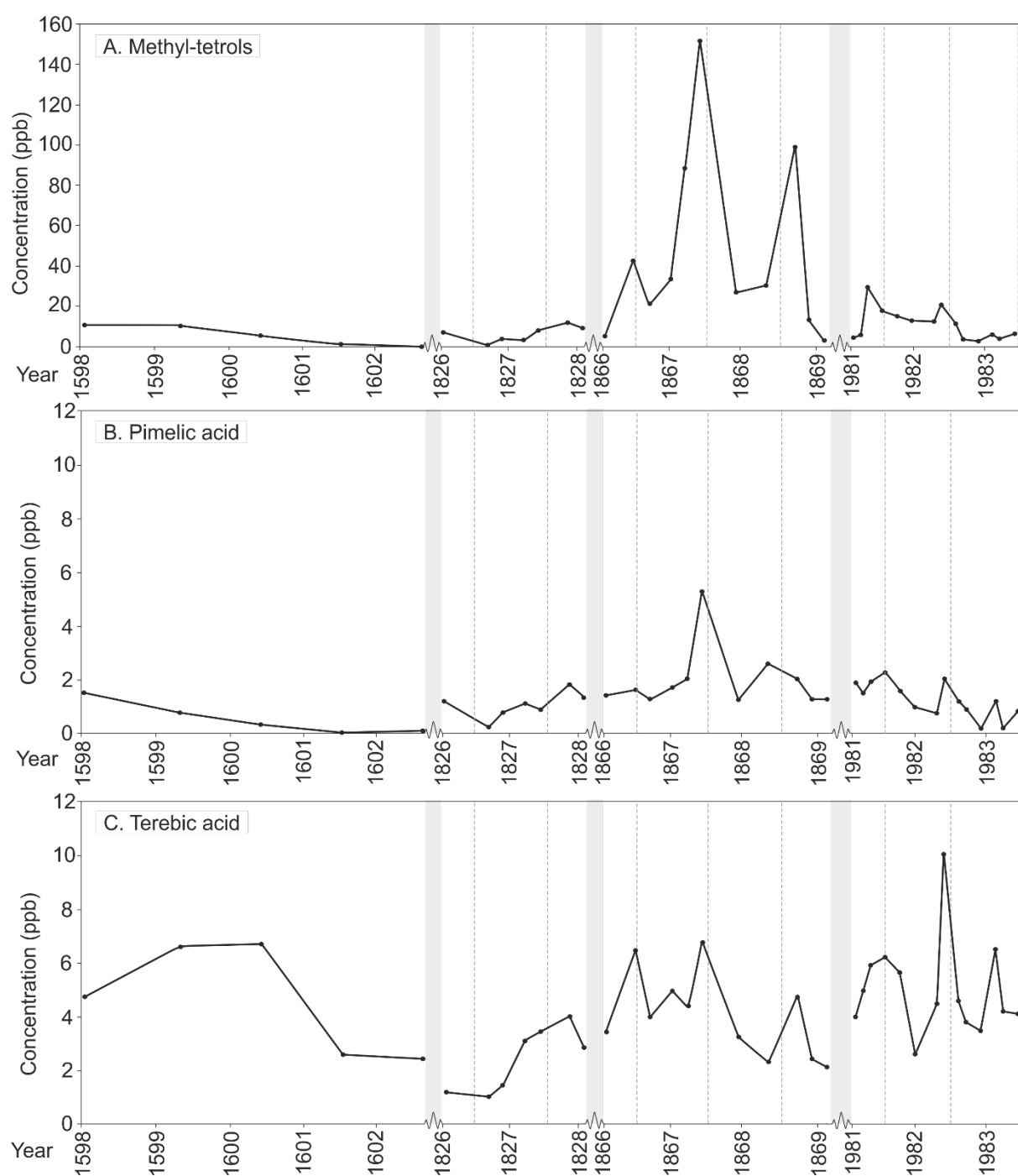
Figure 2a: Time series plots for (a) methyl tetrols, (b) pimelic acid and (c) terebic acid in the Belukha ice core. Note the decreasing sample resolution back in time. Dashed grey lines represent mid-year (summer) time points, and shaded grey boxes represent breaks in time. Time series of all other organic compounds are shown in Figure 2b.

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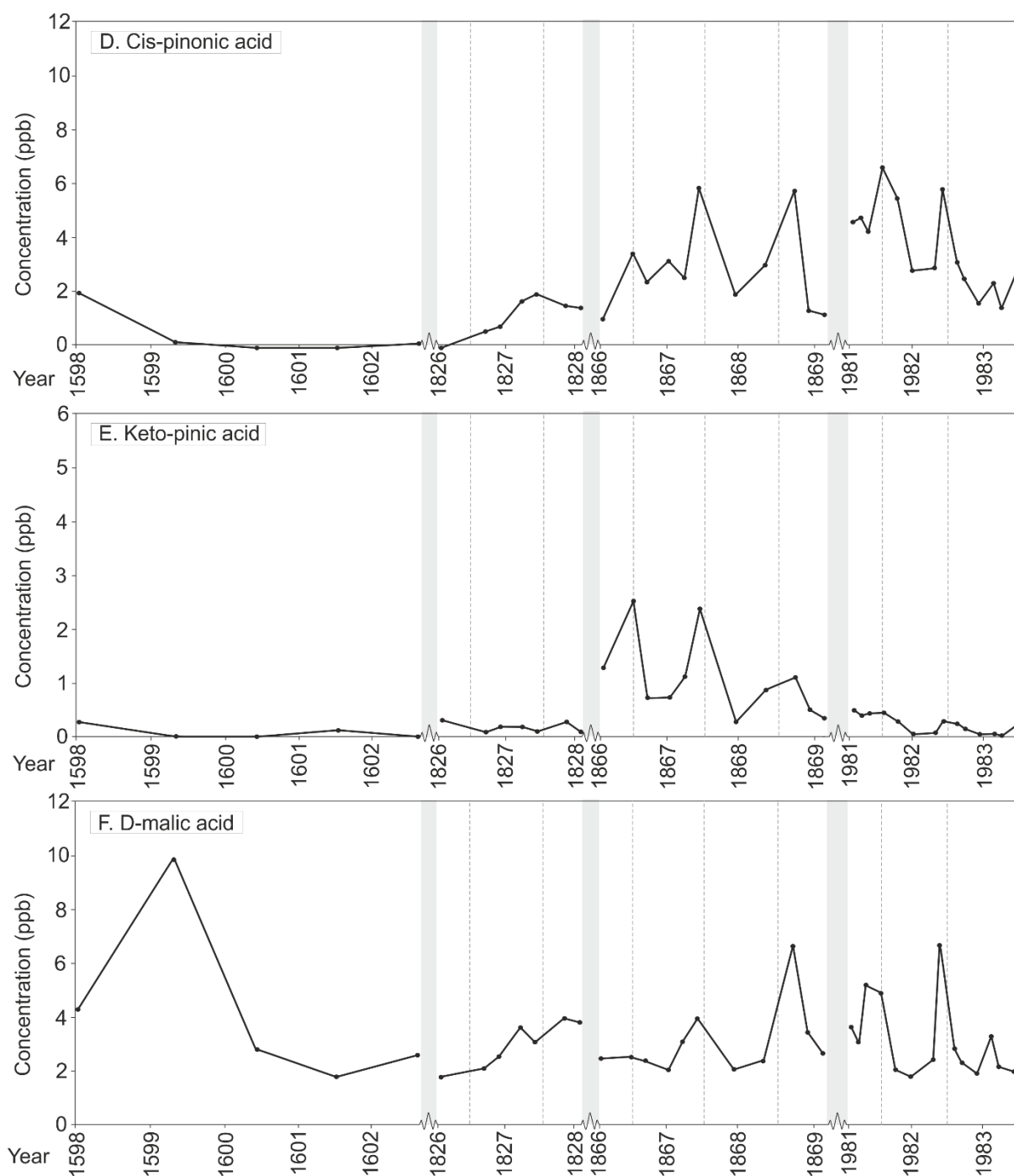
Figure 2b: Time series plots for (d) cis-pinonic acid, (e) keto-pinic acid and (f) D-malic acid in the Belukha ice core. Note the decreasing sample resolution back in time. Dashed grey lines represent mid-year (summer) time points, and shaded grey boxes represent breaks in time. Time series of all other organic compounds are shown in Figure 2a.
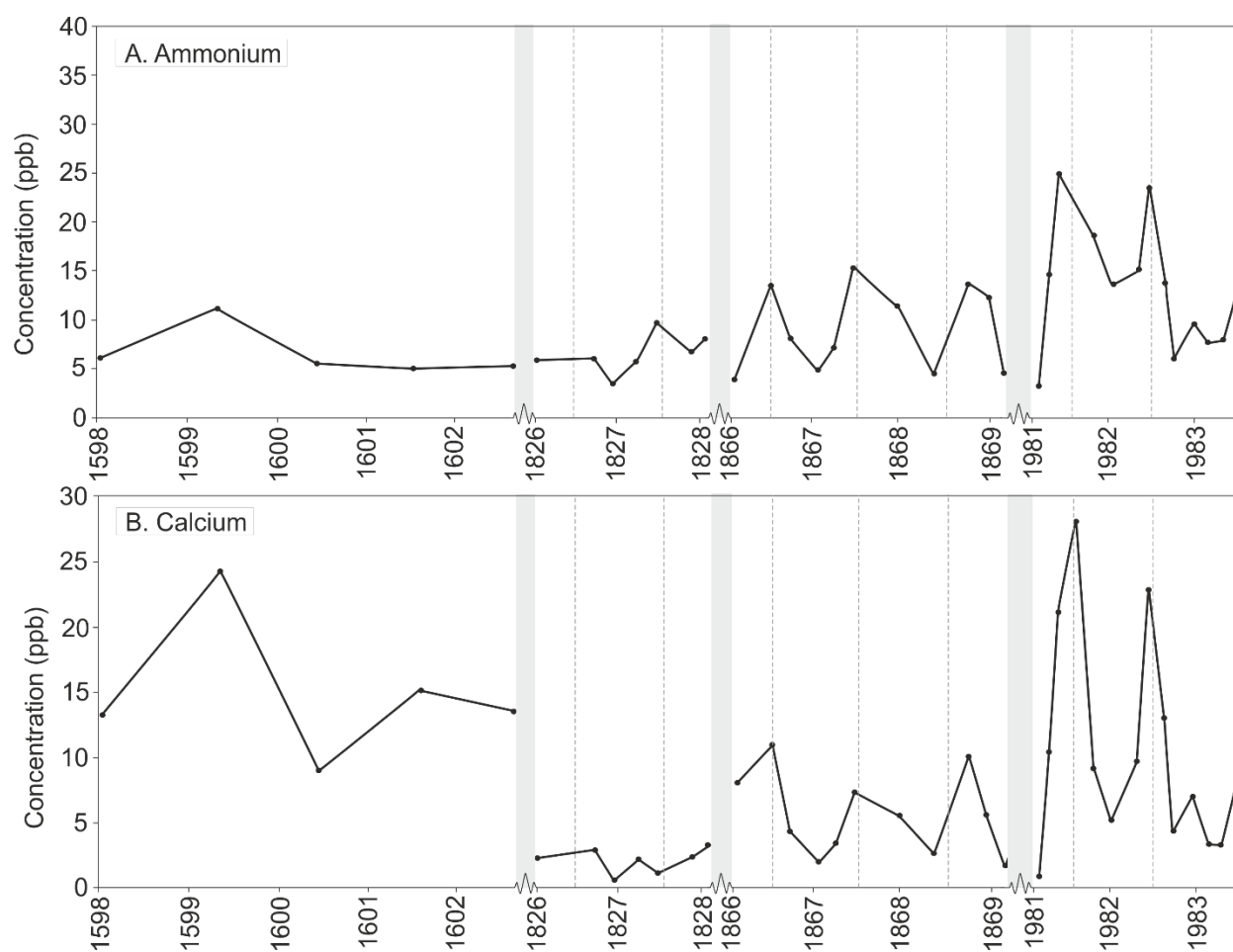
Figure 3: Example time series plots of major ions in sample resolutions matching those of the new organic analyses, for major ions ammonium and calcium in the Belukha ice core. Note the decreasing sample resolution back in time. Dashed grey lines represent mid-year (summer) time points, and shaded grey boxes represent breaks in time.

3.2 Back trajectory analyses

Before further statistical investigation of the data, back trajectories are presented to guide an idea of the sources of the compounds in the ice samples, and in particular the variations in concentrations between seasons. Two main features appear from these results (Figure 4), firstly that there is seasonal variation in transport, and that this variation is consistent between these years. It is worth noting here that these output plots only indicate the source regions of the air masses arriving at the sampling site, not source strength of the organic compounds analysed here, and therefore the scales are not quantitative assessments of compound concentrations in

the aerosol reaching Belukha. Furthermore, these back trajectories ran for 5-day periods to account for lifetimes of the organic compounds only and therefore do to necessarily represent the transport of inorganic major ions and their sources, which may be much further away.

However, these plots agree with the idea that transport has a seasonal variation that likely contributes to differences in concentrations observed in the compound time-series: in summer, air masses appear to reach from greater distances from the ice core location within the lifetimes of the organic compounds, in particular further northwards and westwards. In winter back trajectories have a smaller range. This is in line with the mechanism of greater convective strength in summer described by Olivier et al. (2006). Figure 5 overlays an example summer back trajectory on a summer satellite image of the region, showing how the greater reach of back trajectories to the North in particular covers a larger area of forested region. The two closest major cities, Astana and Almaty, the capital and the largest cities of Kazakhstan respectively, are outside the boundary of the back trajectory transport for organic compounds but may feasibly be the source of anthropogenically produced inorganic compounds, which may have greater potential transport distances.

Since back trajectories are consistent between these example years and in the variation between seasons, it is reasonable to now go on to try to interpret the environmental differences in the source region behind any variations in compound concentrations.
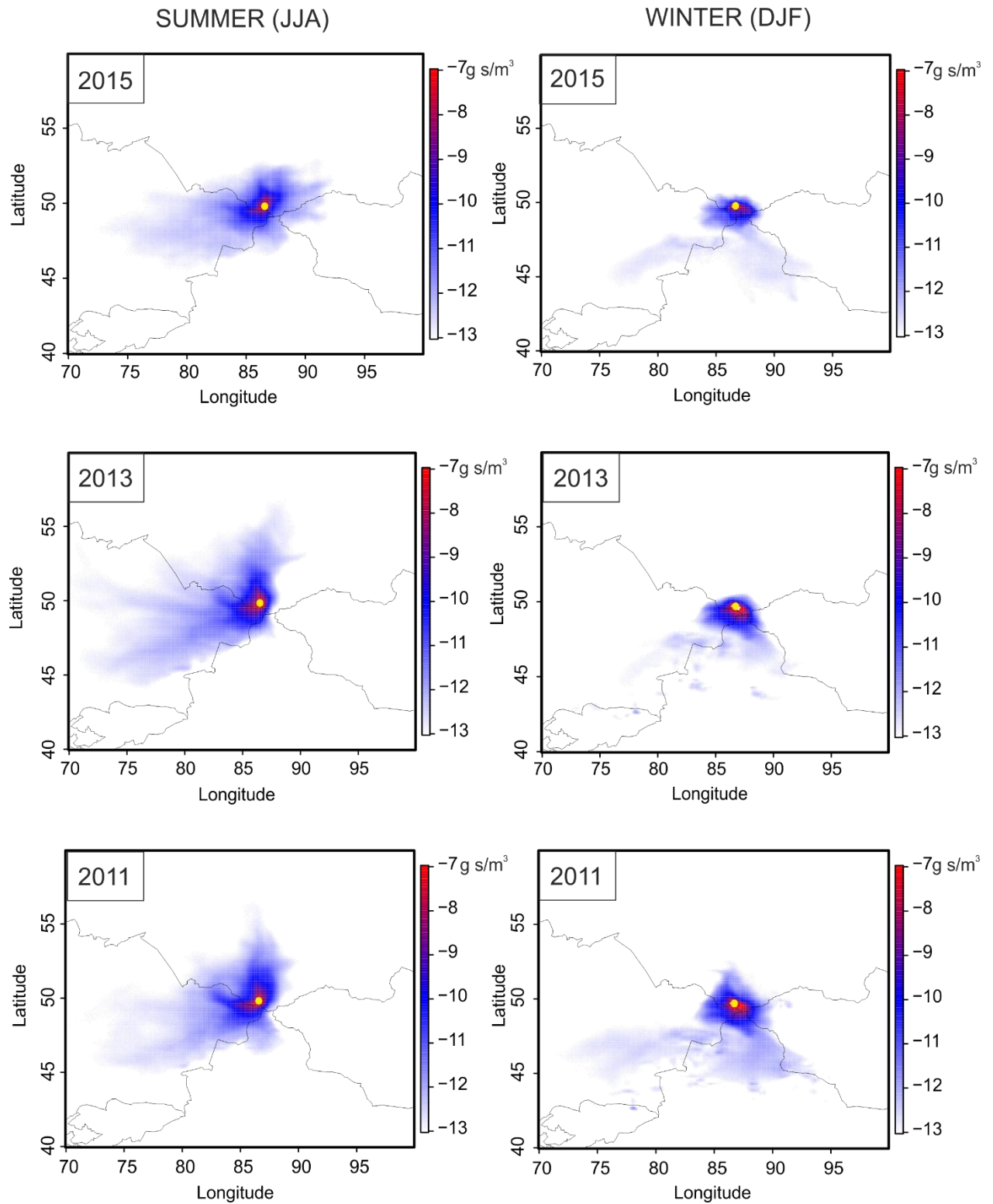
Figure 4: 3-month averages of 5-day back trajectory plots for each of the summer and winter seasons in example years of 2015, 2013 and 2011. Scales are log values.
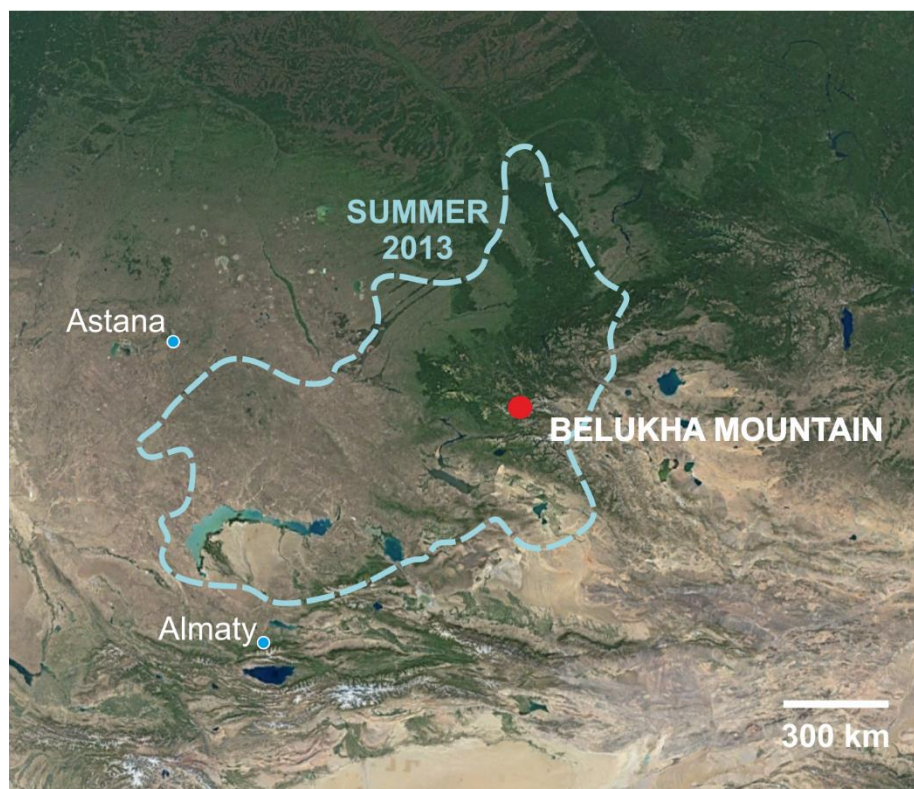
Figure 5: Example back trajectory outline (from Summer 2013, as in Figure 4) overlying a Landsat (NASA) satellite image (source: Google Earth) from summer 2018.

3.3 Principle Components Analysis

PCA for comparison of seasonal and annual resolution datasets, and pre- and post-industrial datasets, are presented in Tables 2 and 3 respectively. The results are presented as the significant components, and the respective loadings (or the extent to which each compound contributes to the variance explained by each component) of each compound, and discussed further in the following text.

Table 2: Results of the annual and seasonal PCA. Table (A) for an annual average record from all samples analysed (1598-1983), and table (B) for the ice core sections allowing e a seasonal record (1866 onwards). Compounds with the largest contribution (>0.5) to each component are emphasised in bold text.

A. ANNUAL PCA (1598-1983)	Component (variance explained)		
Compound	PC1 (40%)	PC2 (29%)	PC3 (11%)
D-malic acid	0.291	0.194	0.129
Pimelic acid	-0.240	**0.758**	
Terebic acid	**0.559**	0.196	0.453
Methyl tetrols	0.165	**0.852**	
Keto-pinic acid		**0.906**	-0.158
Cis-pinonic acid	-0.293	**0.836**	0.128
Chloride	**0.664**	-0.391	**0.576**
Nitrate	0.146		**0.875**
Sulfate	0.312		**0.923**
Oxalate	**0.618**	**-0.567**	-0.297
Sodium	**0.902**	-0.132	
Ammonium	0.234	**0.581**	**0.638**
Magnesium	**0.985**		0.334
Calcium	**0.885**		0.346

B. SEASONAL PCA (1866-1983)	Component (variance explained)		
Compound	PC1 (52%)	PC2 (23%)	PC3 (9%)
D-malic acid	0.229	0.476	**0.536**
Pimelic acid		**0.828**	0.393
Terebic acid	0.368	0.105	**0.776**
Methyl tetrols	0.158	**0.816**	0.185
Keto-pinic acid	-0.113	**0.912**	
Cis-pinonic acid	0.285	0.290	**0.830**
Chloride	**0.947**		0.186
Nitrate	**0.847**	-0.217	0.382
Sulfate	**0.652**	**-0.528**	0.486
Oxalate	**0.527**	**0.729**	-0.110
Sodium	**0.738**	0.367	0.230
Ammonium	**0.992**		0.228
Magnesium	**0.927**		0.281
Calcium	**0.924**	0.104	0.192

Table 3: Results of the pre- and post-industrial PCA, as the significant components and respective loadings (or compound contribution the component variance). Table (A) for the pre-industrial record representing 1598-1869 and Table (B) for the post-industrial, 1981-1983. Compounds with the largest contribution (>0.5) to each component are emphasised in bold text.

A. PRE-INDUSTRIAL PCA (1598-1869)	Component (variance explained)		
Compound	PC1 (40%)	PC2 (28%)	PC3 (10%)
D-malic acid	0.279	0.366	**0.677**
Pimelic acid	-0.188	**0.883**	
Terebic acid	**0.612**	0.482	0.284
Methyl tetrols	0.185	**0.902**	
Keto-pinic acid		**0.790**	-0.279
Cis-pinonic acid	-0.165	**0.855**	0.469
Chloride	**0.679**	-0.393	**0.924**
Nitrate			**0.694**
Sulfate	**0.543**		**0.555**
Oxalate	**0.596**	-0.390	0.201
Sodium	**0.812**		
Ammonium	0.500	**0.518**	0.292
Magnesium	**0.971**		0.117
Calcium	**0.947**	-0.123	

B. POST-INDUSTRIAL PCA (1981-1983)	Component (variance explained)	
Compound	PC1 (66%)	PC2 (19%)
D-malic acid	0.222	**0.797**
Pimelic acid		**0.935**
Terebic acid	0.432	**0.607**
Methyl tetrols	**0.650**	**0.533**
Keto-pinic acid	0.106	**0.882**
Cis-pinonic acid	0.247	**0.910**
Chloride	**0.972**	
Nitrate	**0.978**	0.107
Sulfate	**0.918**	0.214
Oxalate	**0.777**	0.384
Sodium	**0.732**	**0.566**
Ammonium	**0.933**	0.268
Magnesium	**0.950**	0.258
Calcium	**0.964**	0.207

All PCA analyses find a large number of compounds explain high proportions of variance for the dominant component (PC1), especially for major ions and most significantly when considering the higher-resolution records of the seasonal and post-industrial PCAs. This component could therefore be indicating common variations of all components attributed to transport processes; this is the factor most likely to have at least some influence on the concentrations of all compounds in the ice core particularly in the case of inorganic dust particles such as calcium and magnesium. This will be referred to here-in as the transport dominated component. The transport dominated component shows that transport is a contributing factor in both seasonal records and longer-term annual records of compound concentrations.

All PCA analyses show there is a significant second component (PC2) of variance. In the annual PCA a range of organics as well as ammonium are highly loaded, while many inorganic compounds are weakly or not loaded. In the seasonal PCA the second component is also loaded with several organic compounds, this time without ammonium but including oxalate, and inorganics are again more weakly loaded. The pre-industrial PCA shows a very similar result to that of the annual PCA, the same organics highly loaded alongside ammonium, while the comparative post-industrial PCA shows a change, loading all the new organic compounds highly and, with the exception of sodium, all major ions more weakly or not loaded. On a broad scale, we can interpret that PC2 of all PCAs may be indicative of biogenically produced organic compounds, and it will be referred to here-in as a biogenic-dominated component. More specifically, PC2 of the pre- and post-industrial PCAs could be interpreted to show the effect of anthropogenic emissions on compound concentrations; after the industrial era, those compounds with only terrestrial biogenic emissions are more clearly separated from those which also have anthropogenic emissions (i.e. oxalate and ammonium) than in the pre-industrial, and from inorganic major ions. We can tentatively attribute PC2 to an actual emissions signal, rather than transport. This biogenic-dominated component is present throughout all PCAs and therefore indicates that source emissions contribute to both seasonal records and longer-term records of compound concentrations.

A third component, PC3, is identified as significant for the annual, seasonal and pre-industrial PCAs. Whilst significant, the interpretation of the outcome of each is not as clear as for the transport and biogenic emission components. In particular it is difficult to infer much at all from the pre-industrial PC3 since the comparative post-industrial PC3 was not statistically significant, and therefore no further discussion is given here. PC3 of the annual PCA is loaded

highly by a number of major ions, but no organics. Since transport is already accounted for in PC1, this is likely to indicate different sources for these inorganic compounds. These variances are over longer term scales, since these major ions are weakly loaded in the comparative PC3 of the seasonal PCA. Instead, PC3 of the seasonal PCA is highly loaded by two organic compounds. Again, with transport already accounted for in the signal this may indicate a second emission component, differing to that of the biogenic emissions of PC2, but this can only be hypothesized until a larger dataset is analysed.

4. Conclusions

Multiple organic compounds were detected in the Belukha Glacier ice core dating back to 1598. All compounds detected show sub-annual cycles, where core resolution allows, with peaks in concentrations that appear to occur in summer. Compounds also show variations in concentrations over annual scales and between decades, which vary for each individual compound.

Variations in compound concentrations on both annual and seasonal timescales are explained by both transport and source emissions, for both major ions and organic compounds. Principle components analyses particularly highlight a source signal of biogenic emissions. This is interesting because it can be challenging to differentiate source and transport signals from ice core records, and pin-pointing emission concentrations of organics may be incredibly useful in understanding Earth's past atmosphere. For example, organic compounds are important in tropospheric ozone formation and atmospheric radical cycles (Pitts & Stephens 1978). They are also important in cloud forming processes, and hence the Earth's radiation budget, which has given them increasing precedence in model studies reported by the intergovernmental panel on climate change (IPCC) (Boucher et al. 2013). Many of these processes are involved in assessing health effects of such aerosol in the atmosphere. There is however still much room for improvement in these aerosol budgets. This study indicates that a number of substantial components in organic aerosol, isoprene and monoterpene SOA, are quantifiable in ice core records and, with further study to build on these findings, may describe source emission quantities to use as input date for more accurate atmospheric models. This study shows that investigation of these organic compounds will be possible over at least several hundred years.

This is an exciting prospect which justifies further investigation over longer, continuous, ice core sections.

Principle components analyses also show an influence of the industrial era on compound records of the Belukha Glacier; in post-industrial samples there is clear segregation between records of those compounds only with terrestrial biogenic emission sources from those that also or only have anthropogenic sources.

This pilot study highlights the potential of terrestrially sourced biogenic compounds in ice cores as environmental records, both as a stand-alone suite of compounds and in combination with traditional ice core proxies. Further longer-term records will be useful in progressing the understanding of their use as biomarkers.

5. References

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Chapter 6: Summary and conclusions

This PhD thesis aimed to investigate the potential for analysing a suite of organic compounds in ice cores, exploring their application to palaeoclimate reconstruction. The main driver of the project was that the majority of ice core studies focus on the inorganic component of atmospheric aerosol trapped in ice cores, and yet organic compounds can account for half or more of total aerosol composition. Furthermore, a few organic compounds have shown to be clearly detectable in ice core samples over timescales of a few hundred years, and shown links to environmental factors ranging from the robust (such as the sea ice marker MSA) to the tentative (such as secondary oxidation products of terpenes being linked to Northern Hemisphere temperatures).

The first objective of the study was to identify a long-list of organic compounds which show potential for preservation and subsequent detection in ice core samples, which may be related in concentrations to an environmental parameter at the time of initial compound emission. Following a review of the literature in Chapter 1, it was decided to target two organic compound groups, fatty acids and secondary oxidation aerosol (SOA) of terpenes. Chapter 1 discussed the potential of these compounds for ice core palaeoclimate studies and finalised a list of target compounds as those showing most promise. This is the longest list of organic compounds yet targeted by a single analytical method, but following other published exploratory work for these, and similar, organic compounds it is considered timely to produce such a unifying method of analysis to allow maximum output per-sample.

Chapter 2 dealt with contamination, with the objective to declare whether detected concentrations in the samples are a valid environmental signal. This is an important preliminary consideration when analysing new compounds in ice core samples, investigating and quantifying contamination potential of target compounds throughout the drilling, storage, processing and analyses steps. Some aspects, such as laboratory processing suited to organics, are easily adapted from inorganic procedures and recommendations are summarised in the conclusions of the chapter, for example baking of glassware. Other aspects such as the drilling process are intrinsically unfavourable for organic compounds. Investigation found that drilling fluids, layflat storage bags and laboratory cleaning solutions all contain substantial numbers of organic compounds, both in general and within the target groups. Using firstly MS and then MS-MS analysis, those exactly matching the target compounds were reduced to only a very few. Different batches of the same drill fluids showed variability, suggesting testing would be required on a core-by-core basis in the future.

With the contamination potential of ice core drilling and storage media in mind, a core diffusion test aimed at defining the risk of contaminants to different ice portions found there to be very little diffusion beyond the outer few millimetres of a proxy solid ice core over three months. Therefore, using inner portions of ice or, if not available, removing the outer few millimetres of the ice section, should mitigate contamination of organic compounds from drilling and storage stages. Future work should certainly investigate this risk in both firn cores and fractured sections, in which diffusion may be substantially greater. However, for the purposes of this study, we were able to conclude that the threat of contamination to the target compounds is within those bounds which may be managed and allow reliable quantification of organic compound concentrations in ice samples. Of course, many of the suggested methods in this chapter would not be required with the development of specialist facilities such as clean rooms suited to organics, something which would be worth investing in if the potential of organics analyses in ice cores came to fruition in the future.

Chapter 3 developed and optimised a method of HPLC-MS analysis with preceding rotary evaporation preconcentration. The objective was to find the 'best' overall method, the optimal of which would be that which performs best on-average for all compounds on the target list. Considering the challenging aim of a method targeting a long list of chemically different compounds, the success of the final method was surprising; average recoveries of 80% were achieved, alongside reproducibility of 9% RSD. This met the aim of this study which was to target a wide range of organic compounds, giving the best overview for the potential of organics analysis of ice. However, there was substantial variability on the success of preconcentration methods on a compound-to-compound basis suggesting that the development of more compound-specific analyses would benefit results in the future. For example, use of alternative preconcentration methods for the smallest SOA compounds for which rotary evaporation recoveries were extremely low would be beneficial.

Following the preconcentration HPLC-MS method, the project tested the possible application of direct injection analyses (by eliminating the preconcentration step). This used a more sensitive mass spectrometer than that used previously to allow lower detection limits. This method would be beneficial in the future extension of organics analysis to older ice layers, where sample volumes would need to be small to retain high resolution in the time series records. It would also reduce handling and processing of the sample preceding analysis, and therefore substantially reduce contamination particularly for fatty acids which are common in all environments and showed high background contamination throughout this study. This

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method was successful for SOA compounds, including those which showed low recoveries during rotary evaporation, but less successful for fatty acids which showed high contamination from the instrument used. Again, the conclusion here is that more targeted methods could be applied in the future, optimising the analyses of each compound or compound class to that which is most appropriate based on these findings. Alternatively, a non-target mass spectrometry method could be useful in the future; this method would not be limited to the identified target list but could detect any organic compound present in the sample, whether initially known or not. This would open the method to an array of new compounds not quantified in this study.

The preconcentration HPLC-MS method was carried forward to analyses of ice core samples. The objective was to apply the developed method to samples of two distinct ice cores, one of a dominantly terrestrial aerosol input (Belukha Glacier, Russian Altai Mountains) and one of a dominantly marine aerosol input (Bouvet Island, Sub-Antarctic). A subsequent objective was to investigate the potential environmental signals shown by any detected time-series records of compounds in the samples, by various methods of comparison to better-known ice core compounds, statistical analyses, and back-trajectory modelling. Chapter 4 shows the results from the sub-Antarctic Bouvet Island ice core, alongside records of commonly measured major ions, oxalate, formate, acetate and methanesulfonic acid (MSA). This core, dominated by marine aerosol input and representing a modern record back to 2001, displayed one new, continuous, record for the fatty acid compound oleic acid. Statistical investigation showed this compound may be related to the sea ice marker MSA, and indeed further investigation showed both compounds to have positive correlations to sea ice concentration during the period July – September each year. This is related to the source of both compounds, marine algae, for which blooms are greater in spring seasons when amounts of sea ice have been greater in the preceding winter. The correlation region extents geographically west from Bouvet Island along the sea ice margin, where blooms occur, consistent with transport of emitted aerosol along the margin by westerly winds. These dominant westerly winds are persistent throughout seasons and years, providing a consistent source region to the Bouvet ice core. Other compounds showed summer time correlations to sea ice but their sources require further investigation.

The results from the Bouvet ice core show exciting potential for developing a suite of organic sea ice markers which would help to better resolve past sea ice records. To test this idea further in the future, it would be valuable to test other sub-Antarctic cores, of which there is a series available, to see if these findings persist in similar locations. These records may be a signal

from a much more specific region than previous records from the Antarctic continent, which represent transport and subsequent compound input from a wider geographical area. If found to be a more widely applicable sea ice proxy, records of fatty acids and other organics could then be tested in ice cores from regions such as coastal Greenland and coastal Antarctica, where sea ice would be expected to have an influence on compound records.

Chapter 5 conducted a pilot study for ice core samples of a very contrasting core to that of Chapter 4; the Belukha Glacier core is from a terrestrial aerosol dominated site in the Russian Altai Mountains, and allowed testing of organic compounds much further back in time to the 1590s. This is a clear comparison between those compounds in marine aerosol and those in terrestrial aerosol, at least for these specific locations. The chapter also used previously analysed inorganic major ion data. Analyses of this core obtained a series of records of biogenic SOA compound in clearly detectable concentrations throughout all samples tested. The organic records show seasonal signals, with summer peaks in concentrations, as do major ions. Throughout statistical and back-trajectory modelling, these signals are attributed to both transport and emission processes, but strikingly the organic compounds show a clearly distinct emission signal. The potential of terrestrial biogenic emission signals in ice cores is certainly of great interest; aerosol concentrations of SOA compounds are important in modelling of Earth's radiation budgets, interacting in ozone reactions, cloud condensation processes, and signalling the state of the terrestrial biosphere. If budgets of organic aerosol were better known, modelling of both past and future climates would be much more accurate. Future work on this idea would require analysis of a longer, more continuous core section of both Belukha and other cores of terrestrial-dominated input.

The final findings of this study show clear and exciting potential for marine and terrestrial biosphere-sourced organic compound analyses of ice core samples. While this study forms a starting platform for this area of investigation, it is hoped that a number of offshoots are now available for future work.

The developed method should be applied in future to further ice core analyses, but a refined method for more specific target analysis will begin to generate even more successful output. The method should also be carefully considered to improve the detection limits achieved throughout this study before future application to other ice samples. For example, the reduction of background contamination of fatty acids is required to allow improved detection of these compounds. High background contamination certainly contributes to the fact that only one fatty

acid record is achieved throughout the Bouvet core and none at all in the Belukha samples. Ideally, limits of detection in the range of ppt should be the target to clearly identify compound records, whereas this current method gives background levels of contamination for fatty acids in the range of ppb. SOA compounds also require these lower limits of detection; the more sensitive instrument used in the inter-lab comparison of this study allowed detection of SOA compounds at ppt levels, therefore detecting the additional compound MBTCA in the Belukha ice samples. This compound was below detection limits on the primary, less sensitive, instrument used for sample analyses.

It is recommended that a pilot study for a particular core be tested with this studies preconcentration HPLC-MS method and, with the present compounds then in mind, a refined method developed based on the extensive optimisation work here presented to suggest which methods would be best for each compound group. Analyses would also require the use of an instrument with suitable sensitivity, one which could be thoroughly cleaned to limit the background contamination sources of these compounds in conjunction with the steps used to limit contamination in the preceding sample preparation. With a shorter target list also comes the capability of a more thorough investigation of contamination potential for each specific compound. Of course, the development of specialist clean facilities such as a clean room for organic compounds, in which all sample preparation could be carried out and the instrument could be operated, would be the most ideal scenario.

With these future method developments in mind, the use of organic compounds as sea ice proxies and indicators of emissions of the terrestrial biosphere is worth further investigation by applying organics analysis to other, similar cores as a start, and then pushing the investigation to more diverse, and older, ice core samples. This project is a hopeful starting point to a wealth of new information from ice core analyses.

Appendix 1

Table A1.1: Annual resolution concentration (ppb) record of major ions.

	Compound							
Year	Ammonium	Nitrate	Sulfate	Sodium	Chloride	Potassium	Magnesium	Calcium
2016	26.0	46.4	194.7	1001.3	1659.8	48.8	80.7	91.0
2015	20.3	53.0	65.0	754.3	1187.8	38.4	38.8	62.0
2014	20.5	147.9	79.5	749.8	1168.7	33.3	52.5	42.2
2013	31.1	28.7	211.0	1221.6	1989.4	102.2	75.9	61.1
2012	38.9	54.0	173.6	1040.0	1695.7	60.8	71.3	101.5
2011	32.1	53.2	42.3	268.5	465.2	19.8	27.7	65.3
2010	29.2	46.0	124.0	533.9	998.9	44.8	47.3	68.5
2009	25.7	104.0	111.5	498.5	927.0	40.1	56.4	55.9
2008	35.0	47.0	65.2	300.8	561.1	27.4	52.2	84.8
2007	30.3	35.0	128.9	506.0	889.1	50.3	54.7	76.3
2006	20.2	22.9	86.6	342.9	601.4	27.0	36.7	39.2
2005	21.1	52.2	61.3	262.1	445.8	28.0	21.2	58.1
2004	24.7	39.3	79.3	295.5	547.5	33.1	26.7	35.8
2003	12.7	37.5	36.7	125.5	219.3	17.8	10.1	34.5
2002	15.2	39.6	34.9	140.7	257.3	18.1	15.3	31.4
2001	25.7	29.7	123.3	431.7	812.9	43.7	37.6	37.2

Table A1.2: Annual resolution concentration (ppb) record of major ions cntd. and oleic acid.

	Compound					
Year	Oxalate	Formate	Acetate	MSA	Bromide	Oleic acid
2016	10.5	93.9	123.0	9.2	6.5	7.6
2015	14.6	110.0	165.7	3.4	4.6	3.7
2014	7.3	47.9	40.1	1.6	5.0	3.6
2013	8.5	42.0	32.4	1.6	8.6	6.3
2012	15.8	123.9	200.8	1.3	7.2	4.0
2011	15.7	138.6	187.4	2.0	2.0	2.6
2010	12.7	121.6	199.2	1.4	3.6	3.1
2009	9.2	71.2	93.4	0.9	4.0	3.6
2008	23.3	286.7	412.8	3.3	2.1	4.3
2007	12.9	109.6	57.0	1.7	3.4	4.0
2006	2.8	35.9	37.1	1.1	2.0	3.5
2005	4.5	36.8	50.4	0.8	1.5	3.8
2004	8.6	54.8	70.8	1.2	1.6	2.4
2003	1.1	22.0	13.3	0.5	0.8	2.5
2002	4.6	50.6	54.8	0.7	0.9	2.5
2001	6.7	47.0	40.8	0.7	2.7	3.0

Table A1.3: Annual resolution concentrations (ppb) of non-continuous organic compound records

Year	Compound			
	D-malic acid	Pimelic acid	Meso-erythritol	β-nocaryophillonic acid
2016	0.2	0.2	0.7	1.2
2015			0.1	0.2
2014		0.2	0.4	0.9
2013	2.5	0.4	0.5	1.7
2012	0.2	0.2	0.5	0.9
2011	0.1	0.1		0.5
2010	0.2	0.0		0.1
2009	0.3		0.1	0.3
2008	0.3		0.1	0.3
2007	0.3		0.0	0.4
2006			0.1	0.2
2005	0.3	0.1	0.2	0.9
2004	0.6			0.2
2003	1.0			
2002	0.1		0.4	
2001	0.5			

Appendix 2

Table A2.1a: Sample resolution dataset of all compounds detected (conc. ppb) in the Belukha ice core (continued in Table A2.1b)

Age	D-malic acid	Pimelic acid	Terebic acid	Methyl tetrols	Keto-pinic acid	Cis-pinonic acid	Chloride
1598.2	3.4	1.5	4.9	10.3	0.3	2.0	1.3
1599.3	10.0	0.8	7.1	10.7	0.1	0.2	1.6
1600.4	1.7	0.3	7.2	5.2	0.1	0.1	1.3
1601.5	0.6	0.1	2.4	1.2	0.1	0.1	2.0
1602.6	1.5	0.1	2.2	0.3	0.0	0.2	1.1
1826.2	0.6	1.2	0.7	6.9	0.3	0.0	0.7
1826.7	0.9	0.2	0.6	1.0	0.1	0.6	0.5
1826.9	1.4	0.8	1.1	3.6	0.2	0.8	0.3
1827.2	2.7	1.1	3.0	3.3	0.2	1.7	0.9
1827.5	2.0	0.9	3.3	8.0	0.1	2.0	0.5
1827.8	3.1	1.8	4.0	11.9	0.3	1.6	0.7
1828.0	2.9	1.3	2.7	9.4	0.1	1.5	0.6
1866.2	1.4	1.4	3.4	5.6	1.3	1.1	0.5
1866.5	1.4	1.6	6.9	42.7	2.5	3.5	0.8
1866.8	1.2	1.3	4.0	21.7	0.7	2.5	0.2
1867.0	0.8	1.7	5.1	33.8	0.8	3.2	0.2
1867.2	2.1	2.0	4.5	88.4	1.1	2.6	0.3
1867.5	3.0	5.3	7.2	151.7	2.4	5.9	1.2
1867.9	0.9	1.3	3.2	27.7	0.3	2.0	0.5
1868.3	1.3	2.6	2.1	30.7	0.9	3.1	0.3
1868.7	6.1	2.0	4.9	100.1	1.1	5.8	0.8
1868.9	2.5	1.3	2.2	13.4	0.5	1.4	1.3
1869.1	1.6	1.3	1.8	3.4	0.3	1.2	0.3
1981.1	2.7	1.9	4.0	4.5	0.5	4.7	0.1
1981.2	2.0	1.5	5.2	5.9	0.4	4.8	1.4
1981.3	4.5	1.9	6.2	29.1	0.4	4.3	2.1
1981.5	4.2	2.3	6.6	18.1	0.5	6.7	3.8
1981.7	0.9	1.6	6.0	15.1	0.3	5.6	1.6
1981.9	0.6	1.0	2.4	13.3	0.1	2.9	0.7
1982.3	1.3	0.7	4.6	12.5	0.1	3.0	1.5
1982.4	6.3	2.0	11.1	20.6	0.3	5.9	2.1
1982.6	1.8	1.2	4.7	11.3	0.3	3.2	1.0
1982.7	1.2	0.9	3.8	3.8	0.2	2.6	0.6
1982.9	0.7	0.2	3.4	3.0	0.1	1.7	0.8
1983.1	2.3	1.2	7.0	6.1	0.1	2.4	0.6
1983.2	1.0	0.2	4.3	4.3	0.0	1.5	0.7
1983.4	0.8	0.8	4.1	6.2	0.2	2.8	1.7

Table A2.1b: Sample resolution dataset of all compounds detected (conc. ppb) in the Belukha ice core (continued from Table A2.1a)

Age	Nitrate	Sulfate	Oxalate	Sodium	Ammonium	Potassium	Magnesium	Calcium
1598.2	3.4	4.0	2.9	2.6	6.6	0.8	2.1	13.4
1599.3	6.8	7.2	6.9	2.9	11.5	1.5	3.1	24.3
1600.4	4.6	6.2	2.9	4.3	6.1	0.8	1.4	9.1
1601.5	3.9	4.7	3.3	1.9	5.3	0.7	1.3	15.1
1602.6	2.0	2.2	2.4	0.8	5.6	0.6	1.3	13.6
1826.2	2.0	1.3	0.9	0.4	6.2	0.2	0.2	2.3
1826.7	2.0	1.1	0.9	0.1	6.6	0.3	0.4	2.6
1826.9	7.0	3.2	0.9	0.6	3.8	0.2	0.1	0.5
1827.2	5.0	2.0	0.6	0.2	6.4	0.5	0.3	2.0
1827.5	2.9	1.4	1.0	0.2	9.8	0.3	0.3	1.2
1827.8	9.9	7.5	1.6	0.8	7.3	0.2	0.3	2.3
1828.0	1.4	1.4	2.2	1.5	8.5	0.4	0.8	3.1
1866.2	1.7	1.2	0.6	0.7	4.4	0.2	0.7	7.9
1866.5	2.5	7.2	1.0	2.6	13.6	0.5	2.5	10.8
1866.8	1.5	1.8	0.7	0.8	8.6	0.1	0.7	4.3
1867.0	2.1	1.3	0.1	0.8	5.2	0.1	0.4	2.0
1867.2	2.4	1.0	0.7	0.4	7.6	0.1	0.5	3.2
1867.5	5.7	4.6	1.8	1.4	15.6	0.7	1.2	6.9
1867.9	3.5	1.8	0.8	0.9	11.7	0.5	0.6	5.5
1868.3	1.8	1.6	0.4	0.9	4.9	0.4	0.4	2.6
1868.7	4.0	2.8	1.7	0.9	13.9	0.4	1.5	9.8
1868.9	3.1	3.4	1.3	2.0	12.8	0.4	1.0	5.4
1869.1	1.6	1.1	0.4	0.4	5.0	0.2	0.3	1.4
1981.1	1.1	5.6	0.0	0.5	3.6	0.2	0.2	0.8
1981.2	6.8	19.7	0.3	1.5	15.5	0.6	1.5	10.2
1981.3	8.8	27.3	0.7	3.2	25.3	1.0	3.4	21.5
1981.5	12.6	27.3	1.0	3.8	36.5	0.8	3.8	28.2
1981.7	6.1	15.8	0.2	1.1	19.0	0.5	1.6	9.3
1981.9	4.2	12.5	0.1	0.5	14.2	0.4	0.9	5.2
1982.3	6.7	18.1	0.7	1.4	15.6	0.5	1.9	9.7
1982.4	11.6	31.7	1.6	2.2	24.1	0.7	2.9	22.8
1982.6	6.4	18.7	0.7	1.1	13.8	0.4	1.5	13.1
1982.7	3.2	10.4	0.0	0.7	6.8	0.2	0.9	4.3
1982.9	4.8	20.4	0.0	0.8	9.7	0.3	0.9	6.9
1983.1	4.4	11.1	0.2	0.4	8.1	0.3	0.6	3.3
1983.2	4.7	8.2	0.2	0.4	8.3	0.2	0.6	3.3
1983.4	5.5	17.5	0.6	0.8	13.7	0.3	1.4	8.1

Appendix 3

This thesis presents results of all sample analysis in concentrations (ppb). However, it is also common in ice core studies to use flux ($\mu g/m^2/yr$) as a measure. Flux is calculated using the concentration, multiplied by the snow accumulation rate. The decision to use concentration is taken from the discussion in:

Wolff, E.W. (2012). Chemical signals of past climate and environment from polar ice cores and firn air. Chem.Soc.Rev. 41, pp 6247-6258'(see page 6249),

which states that the use of concentrations versus fluxes depends on whether the ice core is retrieved from an area of high or low snow accumulation. The paper explains that concentration is a more appropriate measure in high accumulation sites due to the dominance of wet over dry deposition, as opposed to low accumulation sites where dry deposition dominates and flux is more appropriate. Examples of the former include Greenland and coastal Antarctica, and the latter would be the central Antarctic continent.

Based on the definitions in this paper, both Belukha and Bouvet ice cores are from high accumulation areas and concentration is best applied.

However, aerosol deposition may be varied and complex depending on the compound, and on a number of varying climatic factors in a region which may affect dry versus wet aerosol deposition. A comparison of concentration and flux measurements for some example compounds from both ice cores is presented here, to test for significant change in results from using one versus the other.

At Bouvet Island, average yearly snow accumulation is considered high at 0.58 m weq for the period 2002-2016. This, along with concentration and flux of oleic acid and MSA, is presented in Table A3.1. Plotting these values as time series allows a visual comparison (Figure A3.1). For both oleic acid and MSA, the year-to-year variation in values seems somewhat dampened by use of flux, however overall year-to-year trends appear consistent. There are strong correlations coefficients when plotting concentration versus flux for both compounds.

Considering whether these small differences may carry over into further analysis, the flux of oleic acid is used in a repeat spatial correlation with NSIDC sea ice concentration and compared to the previous output (as presented in Chapter 4) for concentration (Figure A3.2). The output shows that there are slight changes in the significance of the correlation in some regions of the

plot, but that in general positive correlations are observed over the same regions using flux as in the regions calculated using concentrations.

Table A3.1: Snow accumulation of the Bouvet Island ice core, alongside original concentrations and calculated fluxes of both oleic acid and MSA.

|	Concentration (ppb)		Snow accumulation (m weq)	Flux ($\mu g/m^2/yr$)	
	Oleic	MSA		Oleic	MSA
2016	7.56	9.18	0.51	3.82	4.64
2015	3.72	3.35	0.68	2.52	2.27
2014	3.62	1.61	0.58	2.10	0.93
2013	6.30	1.63	0.52	3.28	0.85
2012	3.99	1.28	0.72	2.86	0.91
2011	2.64	2.03	0.68	1.79	1.38
2010	3.06	1.41	0.34	1.05	0.49
2009	3.62	0.93	0.61	2.20	0.56
2008	4.30	3.30	0.49	2.12	1.62
2007	4.03	1.65	0.58	2.35	0.96
2006	3.49	1.07	0.61	2.15	0.66
2005	3.79	0.83	0.57	2.16	0.47
2004	2.38	1.23	0.59	1.41	0.73
2003	2.46	0.51	0.58	1.43	0.30
2002	2.55	0.72	0.69	1.76	0.50
2001*	2.97	0.72			

*not a full year in the core
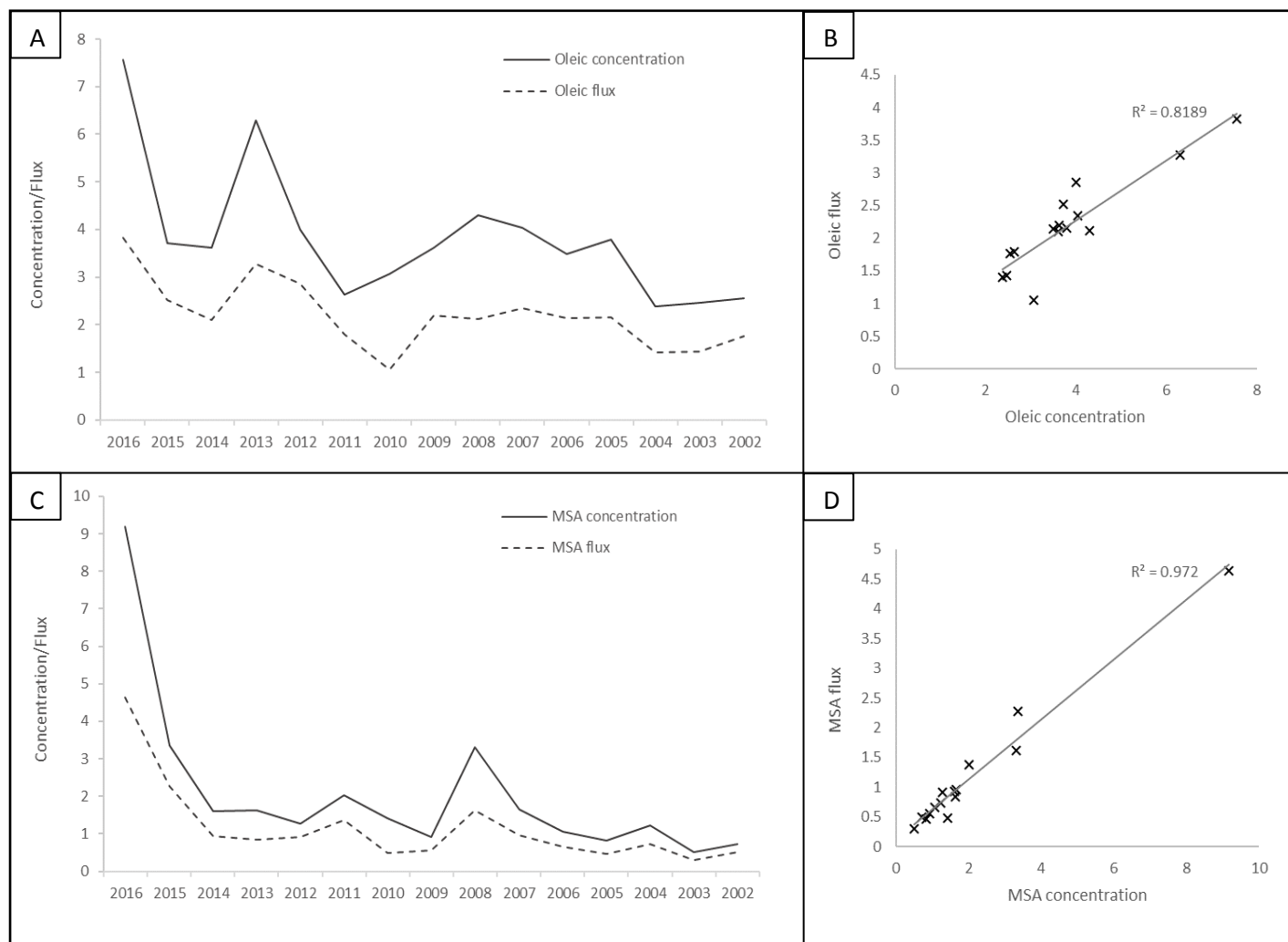
Figure A3.1: Plots showing timeseries comparisons of concentrations (ppb) and flux ($\mu g/m^2/yr$) of oleic acid (A) and MSA (C), alongside their respective correlation coefficients (B) and (D).
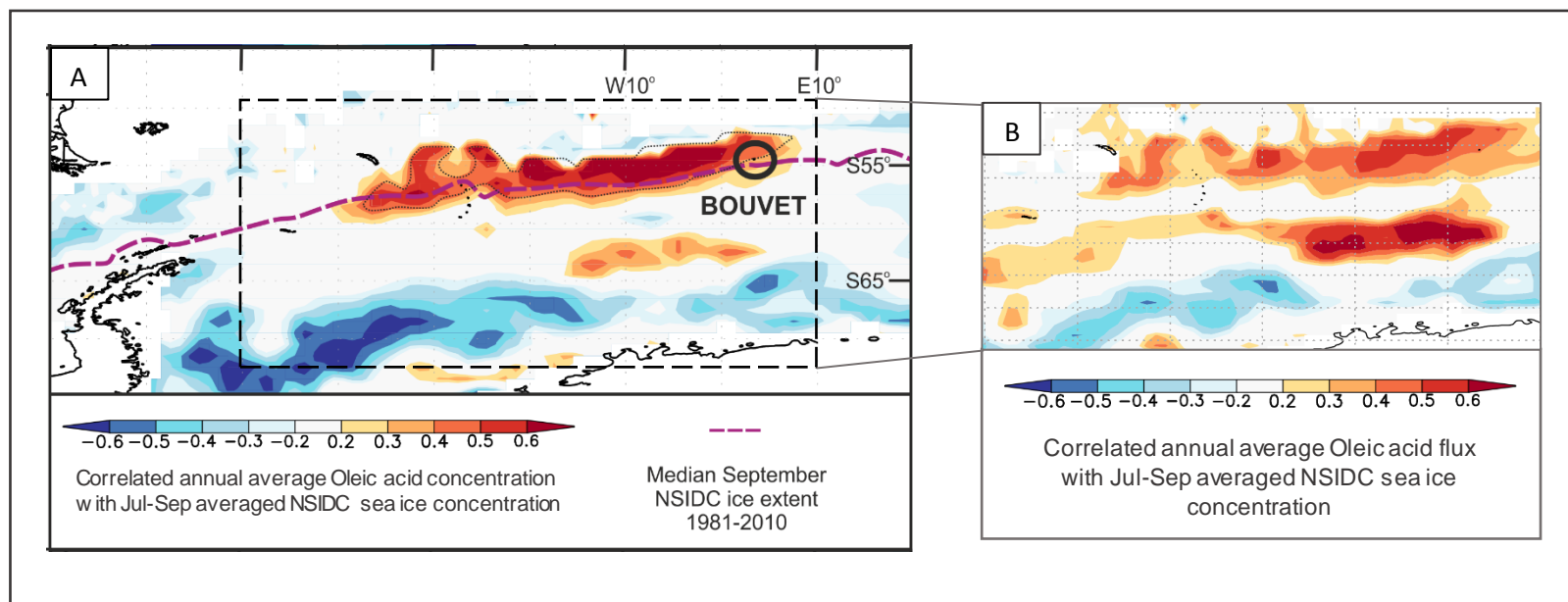
Figure A3.2: Spatial correlation plots between oleic acid concentration and NSIDC sea ice concentration (A) and oleic acid flux and NSIDC sea ice concentration (B).

Average accumulation at the Belukha glacier ice core site is also considered high, at an annual average of 0.54 m weq across all samples. An example comparision of concentrations versus flux is presented here for terebic acid, randomly chosen as a representative compound. Values of concentrations compared to flux (Table A3.2) show very similar timeseries (Figure A3.3) with excellent agreement on sample-to-sample changes and with a strong positive correlation between the two records.

Table A3.2: Concentrations, snow accumulation and calculated flux for samples from the Belukha glacier ice core.

Sample ID	Age	Terebic acid concentration (ppb)	Snow accumulation (m weq)	Terebic acid flux ($\mu g/m^2/yr$)
276-5	1598.15	4.87	0.46	2.24
276-4	1599.28	7.06	0.46	3.25
276-3	1600.41	7.18	0.46	3.30
276-2	1601.53	2.39	0.46	1.10
276-1	1602.64	2.20	0.46	1.01
165-7	1826.15	0.72	0.66	0.48
165-6	1826.67	0.57	0.66	0.37
165-5	1826.90	1.05	0.66	0.70
165-4	1827.17	2.98	0.66	1.97
165-3	1827.45	3.34	0.66	2.20
165-2	1827.80	4.04	0.66	2.67
165-1	1828.05	2.69	0.66	1.77
146-5	1866.15	3.41	0.56	1.91
146-4	1866.50	6.86	0.56	3.84
146-3	1866.75	4.03	0.56	2.26
146-2	1867.00	5.09	0.56	2.85
146-1	1867.20	4.45	0.56	2.49
145-7	1867.45	7.24	0.63	4.56
145-6	1867.87	3.16	0.63	1.99
145-5	1868.30	2.09	0.63	1.32
145-4	1868.67	4.92	0.63	3.10
145-3	1868.87	2.20	0.63	1.38
145-2	1869.10	1.84	0.63	1.16
68-7	1981.08	4.02	0.43	1.73
68-6	1981.23	5.15	0.43	2.22
68-5	1981.33	6.24	0.43	2.68

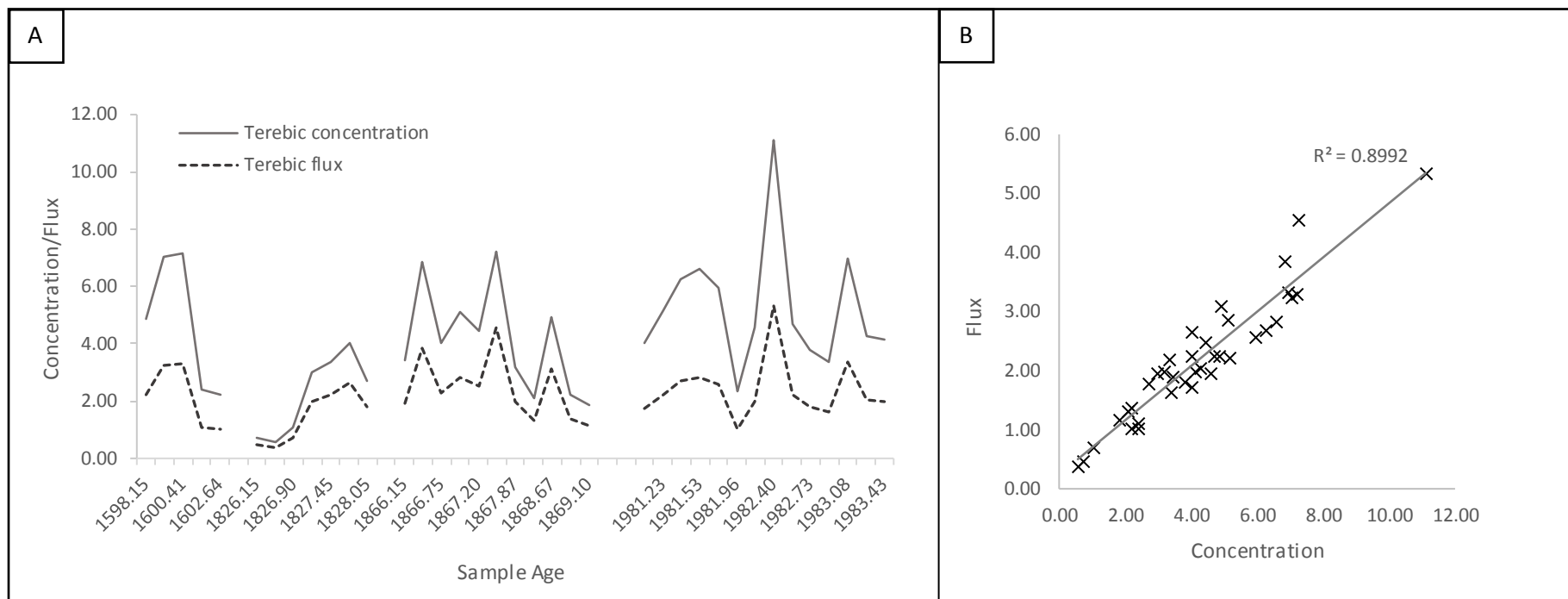| Sample ID	Age	Terebic acid concentration (ppb)	Snow accumulation (m weq)	Terebic acid flux ($\mu g/m^2/yr$)
68-4	1981.53	6.59	0.43	2.83
68-3	1981.71	5.95	0.43	2.56
68-2	1981.96	2.37	0.43	1.02
68-1	1982.27	4.57	0.43	1.96
67-7	1982.40	11.11	0.48	5.33
67-6	1982.57	4.69	0.48	2.25
67-5	1982.73	3.78	0.48	1.82
67-4	1982.88	3.39	0.48	1.63
67-3	1983.08	6.95	0.48	3.34
67-2	1983.23	4.27	0.48	2.05
67-1	1983.43	4.14	0.48	1.99

Figure A3.3: Timeseries plot of concentration (ppb) and flux ($\mu g/m^2/yr$) of terebic acid in the Belukha ice core samples (A), and a correlation coefficient for the two records (B).