Identification and analysis of low molecular weight dissolved organic carbon in subglacial basal ice ecosystems by ion chromatography

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Abstract

Determining the concentration and composition of dissolved organic carbon (DOC) in glacial ecosystems is important for assessments of in situ microbial activity and contributions to wider biogeochemical cycles. Nonetheless, there is limited knowledge of the abundance and character of DOC in basal ice and the subglacial environment and a lack of quantitative data on low molecular weight (LMW) DOC components which are believed to be highly bioavailable to microorganisms. We investigated the abundance and composition of DOC in basal ice via a molecular level DOC analysis. Spectrofluorometry and a novel ion chromatographic method, which has been little utilised in glacial science for LMW-DOC determinations, were employed to identify and quantify the major LMW fractions (free

amino acids, carbohydrates and carboxylic acids) in basal ice from four glaciers, each with a different type of overridden material (i.e. the pre-entrainment sedimentary type such as lacustrine material or paleosols). Basal ice from Joyce Glacier (Antarctica) was unique in that 98% of the LMW-DOC was derived from the extremely diverse free amino acids (FAA) pool, comprising 14 FAAs. LMW-DOC concentrations in basal ice were dependent on the bioavailability of the overridden organic carbon (OC), which in turn, was influenced by the type of overridden material. Mean LMW-DOC concentrations in basal ice from Russell Glacier (Greenland), Finsterwalderbreen (Svalbard) and Engabreen (Norway) were low (0 - 417 nM C), attributed to the relatively refractory nature of the OC in the overridden paleosols and bedrock. In contrast, mean LMW-DOC concentrations were an order of magnitude higher (4430 nM C) in basal ice from Joyce Glacier, a reflection of the high bioavailability of the overridden lacustrine material (>17% of the sediment OC comprised extractable carbohydrates, a proxy for bioavailable OC). We find that the overridden material may act as a direct (via abiotic leaching) and indirect (via microbial cycling) source of DOC to the subglacial environment and provides a range of LMW-DOC compounds that may stimulate microbial activity in wet subglacial sediments.

1. Introduction

Basal ice forms part of the subglacial environment, which also includes subglacial sediments and subglacial waters (Hodson et al., 2008). It hosts viable microbial communities that may play a significant role in the organic carbon (OC) turnover in glaciated regions (Sharp et al., 1999; Skidmore et al., 2000; Foght et al., 2004). Basal ice is typically defined as ice that has acquired distinctive physical and/or chemical characteristics due to processes operating at or near to the bed of an ice mass (Hubbard et al., 2009). Basal ice layers may comprise ice and debris entrained from beneath the glacier and meteoric ice derived from the surface and diagenetically modified by hydraulic, thermal and strain conditions at the glacier bed (Knight et al., 1997). A range of processes can form basal ice, which we highlight briefly. For instance, new basal ice may form from basal accretion of supercooled subglacial water, a freeze-on (or adfreezing) mechanism (Lawson et al., 1998), or by regelation, the localised melting and refreezing of ice at the glacier bed, e.g. around a bedrock obstacle,which represents an important mechanism to entrain subglacial debris into the basal ice (Iverson and Semmens, 1995). Sediment may also be incorporated into basal ice by folding (Hubbard and Sharp, 1989), cavity/crevasse infilling, structural deformation, thrusting, traction/shearing

and metamorphism of existing ice at the glacier bed (Knight et al., 1997). Metamorphosis of meteoric glacier ice can thicken basal ice layers (Sharp et al., 1994) and post-formational tectonic deformation of basal ice can cause intermixing of glacier and basal ice (Waller et al., 2000).

The chemical composition of basal ice reflects characteristics of the parent water prior to being frozen (Knight, 1997). In temperate and polythermal glaciers, this may include supraglacial inputs, whereas in cold-based glaciers where there is little surface meltwater penetration, the majority of meltwater at the glacier bed likely derives from basal ice melting. This water may flow at the base of the glacier, be held in porewaters in overridden water-saturated sediment or represent refrozen water from pressure melting during the regelation process. The parent water has potential to acquire dissolved compounds (including DOC and LMW-DOC) via biogeochemical interactions with the overridden subglacial material. To date, there has been only limited examination of the potential for different bedrock types and overridden organic matter, such as paleosols and lacustrine material (Wadham et al., 2008; Stibal et al., 2012), to act as a source of bioavailable DOC to basal ice, subglacial meltwaters and runoff, either directly (via abiotic leaching or in situ abiotic processes such as dissolution in water films around basal debris and in liquid water veins (Mader et al, 2006)) or indirectly (via microbial cycling). Further knowledge is needed to determine the abundance and composition of potentially bioavailable LMW-DOC in basal ice at the base of glaciers and ice sheets and the implications this may have on subglacial DOC cycling.

OC cycling in the subglacial environment can be investigated by incubation experiments that monitor DOC decline and/or biogenic gas (CO_2 and CH_4) production (Montross et al., 2012; Stibal et al., 2012) and provide a direct measure of bioavailability. Analysis of marker compounds in the DOC, such as free amino acids (FAAs) (Pautler et al., 2011), may provide an indirect assessment of bioavailability. These analyses may be complemented by fluorescence spectroscopy, where fluorescing components (fluorophores) are identified and associated with particular DOC compounds, e.g. protein-like and humic-like components. The protein-like compounds are more easily utilized by aquatic heterotrophs when compared with the more aromatic humic-like components (Fellman et al., 2008) and are indicative of recent microbial activity (Barker et al., 2006, 2010). More recently, glacial DOC has been

characterised at the molecular level by electrospray ionization (ESI) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) (Grannas et al., 2006; Bhatia et al., 2010; Lawson et al., 2014a), and by solution-state ^{1}H nuclear magnetic resonance (NMR) spectroscopy (Pautler et al., 2011; 2012). Both methods have provided unprecedented high resolution mass spectral information on DOC, but are not fully quantitative. Ion chromatography has been used to quantify a much smaller range of common LMW-DOC compounds, including carboxylic acids in ice cores and snow from Greenland, Antarctica, and alpine glaciers (Saigne et al., 1987; Maupetit and Delmas 1994; Tison et al., 1998). These LMW-DOC compounds typically represent small fractions of the bulk DOC (Borch and Kirchmann, 1997), yet are believed to be highly bioavailable to microorganisms owing to their rapid turnover and uptake rates (Rich et al., 1997; Skoog and Benner, 1997). Ion chromatography has yet to be widely employed to determine the molecular structure of glacial LMW-DOC due to the trace analyte concentrations (Lawson et al., 2014b). Recent advances in ion chromatography instrumentation and system optimisation (e.g. greater column sensitivities, low flow rates, multiple eluents and gradient elution) enabled this study to identify and quantify numerous LMW-DOC compounds at low (<70 nM C) concentrations, and demonstrates a novel methodological approach to glacial LMW-DOC analysis.

Here, we investigate the abundance and composition of LMW-DOC compounds (free amino acids, carbohydrates and carboxylic acids) in debris-rich basal ice. We investigate four different glaciers with distinct temperature regimes, overridden substrates, and hence, contrasting sources of terrestrial organic matter. These glaciers were Joyce Glacier (Antarctica – lacustrine organic matter, cold-based), Russell Glacier (Greenland Ice Sheet, GrIS – paleosols, polythermal), Finsterwalderbreen (Svalbard – bedrock with high OC, polythermal), and Engabreen (Norway – bedrock with low OC, temperate). We investigate whether LMW-DOC abundance in basal ice is influenced by the magnitude and bioreactivity of the OC in the overridden material.

2. Sampling sites, basal ice description and sample collection

2.1. Joyce Glacier, Antarctica

Joyce Glacier (67°06′S, 50°09′W, 90 km^2) is situated in the Garwood Valley, Antarctica. A large proglacial lake, dammed by an ice sheet grounded in the McMurdo Sound >23,000 ^{14}C yr BP (Péwé, 1960; Hendy, 2000), is thought to have previously occupied the valley (Hendy, 2000). Joyce Glacier is cold-based, meaning that it is completely frozen to the underlying substrate. The bedrock lithology includes dolomite, granite and metamorphic rocks. Joyce Glacier recently advanced over lake sediment (Stuiver et al., 1981) and hence, the basal material is thought to contain labile OC and algal-derived organic matter of Holocene age.

2.2. Russell Glacier, GrIS

Russell Glacier (67°03′N, 50°10′W, >600 km^2), situated on the west margin of the GrIS, is polythermal-based. Warm ice, with a temperature at the pressure melting point, in the interior is surrounded by a frozen layer beneath the thinner ice of the margins. Surface melting delivers supraglacial meltwaters to the subglacial system from the onset of the spring thaw. The bedrock is predominantly Archaean gneiss (Escher and Watt, 1976). Basal debris contains overridden Quaternary deposits (including paleosols), and relatively fresh organic matter (Knight et al., 2002), which was buried during the Holocene (Simpson et al., 2009).

2.3. Finsterwalderbreen, Svalbard

Finsterwalderbreen (77º28′N, 15º18′E, 44 km^2) is located on the southern side of Van Keulenfjorden, south Svalbard, and is a polythermal surge-type glacier. Finsterwalderbreen last surged between 1898 and 1920 (Liestøl, 1969) which may have influenced the formation of the basal ice as has been shown, for example, at Variegated Glacier (Sharp et al., 1994). The glacier is currently retreating at a rate of 10-45 m a^{-1} (Wadham et al., 2007). The bedrock consists of Precambrian carbonates, sandstones, limestones and shales (Dallmann et al., 1990). Shales exposed to water may provide a steady source of DOC (Schillawski and Petsch, 2008). The shale beneath Finsterwalderbreen contains up to 2.3 % OC (Wadham et al., 2004).

2.4. Engabreen, Norway

Engabreen (66°41′N, 13°46′E, 40 km^2) is temperate and part of the western Svartisen ice cap, northern Norway. Engabreen bedrock consists mostly of schist and gneiss, with calcite filled cracks (Jansson et al., 1996), and contains relatively little OC. A combination of in-washed material from the glacier surface and overridden soils of Holocene age may be the principal OC sources (Stibal et al., 2012).

2.5 Basal ice description and sample collection

Joyce Glacier basal ice samples were collected in the austral summer of 2010 from recently exposed, upthrust bands of debris-rich basal ice at the margin on the southern flank of the glacier. Basal ice was sampled where the facies were composed of frozen debris and only weakly exhibited layers that were >1 mm thick but < 1m thick, classified as solid banded basal ice (Hubbard et al., 2009). We assume that the basal ice was formed under cold-based conditions.

Debris-rich basal ice blocks from the Russell Glacier margin were collected in spring 2008. Samples were collected from the southern corner of the glacier where it has previous advanced into a dune and from within 1.5 m of the ice-bed contact. The basal ice samples contained subglacial sediment that had been extruded up from the glacier bed via fissures near the terminus. This comprised banded basal ice where the debris was generally restricted to narrow sediment layers and large vein networks were clearly evident. As Russell Glacier is polythermal, we assume that the basal ice was formed by a combination of regelation and cold-based processes such as basal adfreezing onto the glacier sole.

Finsterwalderbreen was sampled in autumn 2008. Basal ice blocks were collected from the terminus on the northern flank of the glacier from sections of dispersed banded basal ice (referred to as DB basal ice), within 1.5 m of the ice-bed contact, and from surface outcrops of frozen subglacial material, or thrust bands, with distinct debris layers (referred to as solid banded (SB) basal ice). It is probable that the thrust bands were formed during the most recent surge during two phases of thrusting; primary thrusting during the early surge phase in the subglacial zone between temperate ice and cold ice, and secondary thrusting during the surge termination due to ice flow compression, as envisaged for the similar polythermal surge-type Kuannersuit Glacier (Larsen et al., 2010). Finsterwalderbreen DB and SB basal ice are reported separately due to the very different mean debris concentrations (by mass); 20 ± 27% (DB basal ice) and 86 ± 6% (SB basal ice, where the debris component was much higher). As Finsterwalderbreen is polythermal, we assume that the basal ice was formed by a combination of regelation and cold-based processes. Basal ice samples from Finsterwalderbreen and Russell Glacier were collected from the same sites as the samples that were analysed in Stibal et al., (2012) and hence, we use their ^{14}C ages (Table 2).

Debris-rich basal ice samples from Engabreen were collected in autumn 2009 from an underground tunnel system excavated through bedrock beneath 210 m of sliding ice (Cohen, 2000). The basal ice stratigraphy comprises sediment-rich ice layers overlain by clean sediment-free and bubble-free ice (Jansson et al., 1996). We collected samples from sections of banded basal cryofacies from within 1.5 m of the ice-bed contact. Hot-water drilling was first implemented to create a basal cavity and the ice subsequently extracted by chain-sawing (described below). As Engabreen is temperate, we assume that the basal ice was formed primarily by regelation (Jansson et al., 1996).

Basal ice blocks (~40 cm^3) were collected by chain-sawing in all sample locations. The outermost ~0.5 m of the ice surface was removed before the blocks were cut. The blocks were wrapped in pre-combusted foil and stored at ≤-20 °C, before being transported frozen to the University of Bristol and subsequently stored at ≤-20 °C.

3. Methodology

3.1. Basal icemelt and sediment sample preparation

Subsamples of the basal ice were prepared for analysis by chipping ~15 cm^3 chunks from the main block using a flame sterilised chisel. The outer ~10-30 mm of the chips was removed by rinsing with ultrapure (≥18.2 MΩcm) deionized water (DI) (Millipore), and the remaining ice was transferred into a pre-combusted glass beaker covered with foil. The ice was allowed to melt inside a laminar flow cabinet (Telstar Mini-H) under ambient laboratory conditions, which allowed any sediment to settle out of suspension. The icemelt was then decanted into smaller pre-combusted beakers. Icemelt was filtered through Whatman polypropylene Puradisc™ 0.45 μm syringe filters. Water samples for subsequent OC analysis were stored in clean pre-combusted borosilicate glass bottles (thrice rinsed with the sample before storage). Five samples of filtered icemelt were taken from the ~15 cm^3 chunks cut out of the Joyce Glacier basal ice block, the Finsterwalderbreen DB ice block, and the Finsterwalderbreen SB ice block. Slightly larger volumes of icemelt permitted six samples of filtered icemelt to be collected from the Engabreen basal ice chunk, and seven from the Russell Glacier chunk. DI procedural blanks (n = 5) were subject to identical processing as the samples from the filtration stage onwards to monitor for possible contamination during processing and storage. Sample concentrations were subsequently blank corrected (see Sect. 3.3.4).

Sub-samples from each ice block were also collected for free carboxylic acid (FCA) determination. Ice was melted in an inert gas (O_2-free-N_2, OFN) atmosphere to limit potential contamination during the melting process (Saigne et al., 1987). The OFN gas first travelled through a hydrocarbon trap (HT200-4, Agilent) to remove any volatile OC compounds. Icemelt was filtered through Whatman polypropylene Puradisc™ 0.45 μm syringe filters into 1.5 mL vials with PTFE caps (Chromacol). Samples were analysed within 24 hours of melting to minimise losses due to the volatile nature of the FCA compounds. Procedural blanks were collected in concert.

The subglacial sediment OC content was derived from analysis of the settled particles, which were transferred from the beakers with clean, ethanol-rinsed metal spatulas and stored in sterile 0.5 L Whirl-pak bags (Nasco). Every effort was made to collect as much of the finer sediment as possible from the bottom and sides of the beakers. However, some fine sediment may have remained in the beaker and were thus excluded from the OC determinations. We were also unable to collect the fine particles that remained in suspension owing to the use of syringe filters to filter the icemelt. The total mass of this finer sediment was small compared to the mass of the settled sediment; therefore OC determinations were not unduly compromised. Sediment and filtered samples were stored in the dark at ≤-20ºC until analytical processing.

3.1.1 Basal ice debris concentration

Basal ice debris concentrations (% by mass) were determined by mass subtraction. Basal ice debris typically comprised sediment particles predominantly <2 mm plus some small gravel in the Finsterwalderbreen SB samples. First, the melted basal ice samples (sediment + icemelt) were weighed and the sediment extracted according to the procedure described above. The sediment was dried in a hot air oven (105 °C) for a minimum of 12 hours and weighed. The basal ice debris concentration was expressed as a percentage of a mass (of sediment) to mass (total mass of ice and sediment) basis.

3.2. Basal sediment analysis

3.2.1. Elemental analysis

The subglacial sediments were first dried in a hot air oven (105 °C, 12 hours) and then manually homogenized by grinding. Total carbon (TC) was measured on an EA1108 Elemental Analyser (EuroVector). Inorganic carbon (InC) was determined by a modified Coulomat 702 Analyser (Strohlein Instruments). Total OC was calculated as the difference between TC and InC. The precision of determinations was <5%. Samples were calibrated using external reference standards at a detection limit of 0.1 mg g^{-1} (or 0.01%).

3.2.2. Carbohydrate sediment extractions

Previous studies have estimated sediment OC bioavailability based on the concentration of extractable carbohydrates (Biersmith and Benner, 1998; Pusceddu et al., 2009). We employed this method to provide a conservative estimate and acknowledge that this is not a comprehensive assessment of bioavailable OC in the subglacial material, as other compounds, such as enzymatically hydrolysable amino acids, were not quantified. Operationally-defined minimum estimates of extractable carbohydrate concentrations in basal sediment were quantified by ion chromatography following an acid-extraction protocol to convert any polysaccharides and sugar derivatives to lower molecular weight components (Jensen et al., 2005). We followed the protocol described in (Stibal et al., 2010) and conducted each extraction procedure in triplicate. Monosaccharide losses occurred during hydrolysis, including the total loss of fructose, and were not compensated for (Borch and Kirchmann, 1997; Jensen et al., 2005). This methodological limitation means that some of the variability between samples will be due to procedural effects, rather than a true disparity between sediment carbohydrate concentrations.

3.2.3. Cell counts

Cell counts were conducted to quantify the microbial abundance in basal sediment and determine whether there is potential for subglacial microbial activity. We followed the protocol described in (Stibal et al., 2012). For Joyce Glacier samples, the method followed that of (Porter and Feig, 1980) (detailed in the Supplementary Methods).

3.3 Analysis of basal icemelt

3.3.1. Bulk DOC

DOC was determined by high temperature combustion (680ºC) using a Shimadzu TOC-V_{CSN}/TNM-1 Analyzer equipped with a high sensitivity catalyst. Precision and accuracy of standard solutions (5-170 µM C) of potassium hydrogen phthalate ($C_8H_5KO_4$) (Merck) were < ± 6%, and the limit of detection (LOD) was 5 µM C.

3.3.2. Fluorescence spectroscopy

Fluorescence spectra were determined on a HORIBA Jobin Yvon Fluorolog-3 spectrofluorometer equipped with excitation and emission monochromators, a Xenon lamp (excitation source) and FluorEssence software. Synchronous scans were performed at 1 nm increments with a 0.1 s integration period, 10 nm bandwidth and an 18 nm offset between excitation and emission monochromators (Barker et al., 2006). The accuracy of the monochromators was ± 0.5 nm. Synchronous scans of DI were run under identical scanning conditions and subtracted from all sample spectra to correct for Raman scattering. All scans were dark corrected and internally corrected for inner filter effects and variations in lamp performance. Post-scan data correction followed the protocol described by (Barker et al., 2006). Fluorophore recognition was based on values reported in the literature (Miano and Senesi, 1992; Ferrari and Mingazzini, 1995; Coble, 1996; Yamashita and Tanoue, 2003) and all spectra were normalized to the sample fluorescence peak spectral maximum.

3.3.3. OC compound determination by ion chromatography

Free amino acid (FAA), carbohydrate (FCHO) and carboxylic acid (FCA) determinations were performed by an ICS-3000 dual-analysis reagent-free ion chromatography system, employing electrolytic NaOH eluent generation (Dionex™, part of Thermo Fisher Scientific). Precision and accuracy were monitored by periodically running certified external standards (Dionex™), and internal standards during each sample run, at concentrations within the range of sample concentrations (10 - 2000 nM C). The limit of quantification (LOQ) was defined as the concentration of the lowest standard that could be significantly differentiated from the next highest. The 28 basal ice samples and five DI blanks were analysed in small batches. To limit any potential change in analyte abundance or composition over the course of the batch analysis due to inorganic or organic activity within the sample vial, we typically ran 8-10 samples (plus standards and DI to flush the system) during each run. Prior to running the samples we assessed the level of drift (which may account for instrumental drift plus changes

in LMW-DOC compounds) in a low level standard (50 µg L^{-1}) and found that *c.* 21 samples could be run before significant drift (exceeding the precision of the instrument) was noted. Due to the scarcity of sample volume we were unable to explore whether LMW-DOC concentrations in each sample changed over the course of the sample run.

FAA: were separated via gradient anion exchange on an AminoPac PA10 column (2x250 mm) after passing through an AminoPac PA10 guard column (2x50 mm). Pulsed electrochemical detection with an Au electrode was employed. A gradient mix of 0.25 M NaOH, 1.0 M Na-acetate (NaOAC) and DI was used to elute 14 FAAs (lysine, alanine, threonine, glycine, valine, serine/proline, isoleucine, leucine, methionine, phenylalanine, cysteine, aspartic acid, glutamic acid and tyrosine) at a flow rate of 0.25 mL min^{-1}. Serine and proline were reported together due to co-elution. Precision was typically c. ± 5% for lysine, alanine, threonine, glycine, valine, serine/proline, isoleucine, leucine, methionine, and cysteine, and c. ± 10% for phenylalanine, aspartic acid, glutamic acid and tyrosine. Accuracy was < ± 7% for all analytes (certified external standard, Fluka Analytical). The LOQ ranged from 10-60 nM C.

FCHO: fucose, rhamnose, arabinose, galactose, glucose, xylose/mannose, fructose/sucrose, ribose and lactose were separated isocratically at a flow rate of 0.35 mL min^{-1} on a CarboPac PA20 column (3x150 mm) after passing through a CarboPac PA20 guard column (3x30 mm). Xylose and mannose, and fructose and sucrose, were reported together due to co-elution. Precision for fucose, rhamnose, arabinose, glucose and xylose/mannose was generally c. ± 5%, and c. ± 10% for galactose, fructose/sucrose, ribose and lactose. Accuracy of a certified external standard (Dionex™) was < ± 7% for all analytes. The LOQ ranged from 10-80 nM C.

FCA: acetate, formate, propionate and butyrate were separated via gradient anion exchange on an IonPac Hydroxide-Selective Anion Exchange AS11-HC column (2x250 mm) with an AS11-HC guard column (2x50 mm) and Anion Self-Regenerating Suppressor (ASRS). Electrolytic eluent generation was employed to allow analyte separation along a NaOH gradient during the 30 minute run at a flow rate of 0.5 mL min^{-1}. Precision and accuracy of the four FCAs in a certified reference standard (Supelco Analytics) was 5-8% (precision) and 3-4% (accuracy). The LOQ ranged from 90-130 nM C.

3.3.4. Blank corrections

Preparation of DI blanks is described in Sect. 3.1. Blank corrections were not required for FAAs due to the negligible blank concentrations. Minimal corrections were required for FCHOs (1.3 nM C), but larger corrections were required for DOC (5.85 µM C) and FCAs (23.06 nM C).

4. Results

4.1. Basal sediment characteristics

Basal ice debris concentrations (by mass) differed between glaciers. Finsterwalderbreen solid banded (FSB) and Russell Glacier basal ice contained the highest concentration of debris (86 ± 7% and 55 ± 25%, Table 1), which are similar to percentages in GrIS banded ice (46-57%), solid ice (61%) (Yde et al., 2010) and debris bands (71%) (Sugden et al., 1987). Debris concentrations in basal ice from Engabreen (37 ± 21%), Joyce Glacier (21 ± 6%), and Finsterwalderbreen dispersed banded (FDB) (20 ± 27%) were lower than percentages in GrIS and FSB ice.

We investigated possible correlations between DOC (and LMW-DOC) and the debris content of the basal ice, which may provide information on DOC provenance and the potential for DOC to leach from sediments into the basal ice. We acknowledge that if DOC is leached from sediments the controlling variable will be the surface area, rather than the debris concentration. However, a detailed investigation into the particle size distribution was beyond the scope of this study. We thus conducted a preliminary analysis to determine if the relationship with debris concentration differed for DOC and LMW-DOC. Significant positive associations between debris concentration and DOC were only evident in Joyce Glacier (R^2 = 0.71, $p < 0.05$) and Russell Glacier (R^2 = 0.72, $p < 0.05$) basal ice (Figure 1a). No significant associations between LMW-DOC and debris concentrations were observed (Figure 1b, R^2 < 0.1, $p < 0.05$).

The sediment OC content was low (<0.6%) in all basal ice samples (Table 2). Minor fractions of extractable carbohydrate (<0.5% of the sediment OC) were measured in Engabreen, Russell Glacier and Finsterwalderbreen sediments. A higher carbohydrate fraction (17% of the sediment OC) was measured at Joyce Glacier (Table 2). We use this as a proxy for lability

(Biersmith and Benner, 1998; Pusceddu et al., 2009) and thus make the assumption that Joyce Glacier sediment is bioavailable. Microbial cell abundance was comparable in all samples (1 - 7 x 10^5 cells g^{-1}, Table 2).

4.2. Subglacial DOC quantity and complexity

DOC abundance and composition varied between the four glaciers. The highest mean DOC concentrations were observed in basal ice from Joyce Glacier (272 ± 99 µM C) and Engabreen (114 ± 106 µM C), with lower concentrations in Russell Glacier basal ice (53 ± 29 µM C), FDB (15 ± 10 µM C) and FSB (33 ± 33 µM C) (Table 1). The relatively large standard deviations show that subglacial DOC concentrations are highly variable, even in basal ice from the same glacier. Between 5 and 7 replicate samples were taken from each of the ~15 cm^3 chunks cut out of the main ice blocks from each glacier (detailed in Sect. 3.1). The variability in the DOC concentrations suggests that there is significant spatial heterogeneity even at the level of the ~15 cm^3 basal ice chunks analysed from each glacier.

The composition of the subglacial DOC was investigated by spectrofluorescence and ion chromatography. The synchronous fluorescence spectra of all basal ice samples illustrated the dominance of three key fluorophores of a marine humic-like/fulvic acid type, at c. 340, 385 and 440 nm (excitation wavelengths, Figure 2, Table 3), and several unresolved fluorophores at longer excitation wavelengths. Protein-like peaks (~279 nm excitation wavelength), indicative of tyrosine-like compounds (Ferrari and Mingazzini, 1995; Yamashita and Tanoue, 2003), were only evident in Joyce Glacier and FSB basal ice (Table 3). Ion chromatographic analyses provided a greater level of detail on the molecular composition of the DOC. LMW-DOC compounds, with concentrations > LOQ, accounted for <3% of the DOC in all basal ice samples. Mean LMW-DOC concentrations in Engabreen, Finsterwalderbreen and Russell Glacier basal ice were <420 nM C (Table 1). Mean LMW-DOC concentrations were an order of magnitude higher (4430 nM C) in Joyce Glacier basal ice. As with DOC concentrations, the variability in the LMW-DOC compound concentrations suggests high spatial heterogeneity within the basal ice.

LMW-DOC was typically dominated by FCAs (Table 1), except in Joyce Glacier samples which are subsequently discussed. Overall, acetate was the most common analyte (Figure 3), being present in 60% of the samples that contained FCAs at concentrations > LOQ. Basal ice

FCHO concentrations were typically < LOQ (<4% of the LMW-DOC, Table 1) and only detected in Joyce Glacier samples, comprising glucose (16 - 49 nM C) and ribose (16 - 19 nM C, data not shown). Joyce Glacier basal ice DOC was unique in that most (98%) of the LMW-DOC was derived from the extremely diverse FAA pool (Figure 4). Mean FAA concentration in Joyce Glacier basal ice (4353 ± 2643 nM C) was an order of magnitude higher than mean FAA concentrations in Engabreen, Finsterwalderbreen and Russell Glacier basal ice (0 – 51 nM C, Table 1). Some 14 FAAs were detected in Joyce Glacier basal ice, including methionine, glutamic acid, aspartic acid and cysteine, which were not observed in the other basal ice samples. Serine/proline, alanine and valine dominated the Joyce Glacier FAA pool. FAAs accounted for 59% of the LMW-DOC in Russell Glacier basal and FSB ice, primarily in the form of alanine and valine, respectively.

5. Discussion

The application of a novel methodological approach (within the field of glacial science) using ion chromatography has allowed the identification and quantification of a range of LMW-DOC compounds in debris-rich basal ice, including FCAs, FCHOs and FAAs, at unprecedented low concentrations (<70 nM C). This represents, to our knowledge, the first study to quantify LMW-DOC in basal ice from a range of glaciers and ice sheets. We demonstrate that ion chromatographic systems that have been optimised for the detection of trace level LMW-DOC concentrations, e.g. by using multiple eluents, low flow rates and gradient elution, can be utilised as an additional quantitative technique to supplement characterisations of glacial LMW-DOC by ESI FT-ICR MS (Grannas et al., 2006; Bhatia et al., 2010; Lawson et al., 2014a) and solution-state ^{1}H NMR spectroscopy (Pautler et al., 2011, 2012).

5.1. The influence of debris type on sediment OC and basal ice DOC concentrations

We find little evidence that the type of overridden material (i.e. the pre-entrainment sedimentary type such as lacustrine material or paleosols) and the mean sediment OC content has a significant influence on the DOC content in basal ice. Indeed, the mean basal ice DOC concentrations (Table 1) and mean sediment OC content (Table 2) were relatively similar in all basal ice samples despite the differences in the types of overridden material. Furthermore, the fact that the highest mean DOC concentration was observed in Joyce Glacier basal ice

(272 µM C) yet the corresponding sediment OC% was the lowest (0.01%) of all four sites demonstrates the lack of a relationship between sediment OC% and basal ice DOC. This may be due to the particular section of basal sediment that was sampled as, in the case of Joyce Glacier, higher OC content has previously been observed in other Antarctic lacustrine samples, such as subglacial sediment beneath Lower Wright Glacier (0.7% OC) (Stibal et al., 2012), and Antarctic Dry Valley lacustrine sediments containing microbial mats (~9% OC) (Squyres et al., 1991). This suggests a more diverse basal sediment matrix comprising algal mats and organic lacustrine material that mixed with sand and/or other low-OC, mineral-based material during basal ice formation beneath Joyce Glacier. However, we acknowledge that some of the difference in sediment OC (and extractable carbohydrate concentrations) may be due to the different analytical methods employed in this and previous studies. The concentrations that we present may also be conservative as our methodological approach meant that fine sediment fractions, which may be OC-rich, remained in suspension and were not included in the OC determinations.

Key differences were, however, observed in the proportions of extractable carbohydrates (a proxy for bioavailable compounds in the basal sediment) and LMW-DOC concentrations in basal ice from the four sites. The LMW-DOC concentrations in Joyce Glacier basal ice, which were an order of magnitude higher than LMW-DOC concentrations in samples from the other three sites and predominantly due to high FAA concentrations, may have derived from the relatively large pool of potentially-bioreactive extractable carbohydrates in Joyce Glacier basal sediment (17% of the sediment OC, compared with <0.5 % of the sediment OC in samples from Russell Glacier, Engabreen and Finsterwalderbreen). The bioreactive OC pool in Joyce Glacier basal sediment may have been enhanced by the assimilation of proglacial algal mats into overridden material during glacial advance, which likely enriched the basal ice with lacustrine material and associated algal necromass (Pautler et al., 2012), which may include autochthonous material produced by microorganisms prior to basal ice formation. Indeed, lacustrine material is generally acknowledged as a source of reactive OC to microorganisms (Meyers and Ishiwatari, 1993). The lower extractable carbohydrate concentrations in basal sediment from Russell Glacier, Engabreen and Finsterwalderbreen (compared with Joyce Glacier) are thought to reflect the more refractory nature of the overridden material. OC in subglacial material beneath this sampled section of Russell Glacier is thought to derive from a soil origin, based on relatively high concentrations of n-alkanoic acids, steroids, and other soil-derived functional compounds that have been

identified in basal ice samples (Stibal et al. 2012). Due to this, and the relatively young age of Russell Glacier sediment OC (<1900 ^{14}C yrs BP), we expected the total and bioreactive OC concentrations to be higher than 0.44% and 0.47% of the OC, respectively. For instance, OC content in Greenland soils range from 0.1 - 44.8% in C horizons and peat soils (Horwath Burnham and Slettern, 2010). The low OC and extractable carbohydrate concentrations in Russell Glacier basal ice may reflect a heterogeneous sediment matrix that incorporates a lower proportion of paleosols mixed with other low-OC, mineral-based material. However, as discussed earlier, these differences in sediment OC concentrations may be due to the conservative nature of our methodological approach that may have excluded the potentially OC-rich fine sediment fractions. The low extractable carbohydrate concentration (0.04% of the OC) in Finsterwalderbreen basal sediment is likely influenced by the predominance of OC from kerogen in the overridden shale bedrock (Wadham et al., 2004) that has been incorporated into the basal ice matrix. Kerogen is ancient carbon comprising stable carbon macromolecules (Petsch et al., 2001) and has limited bioreactivity. Similarly, low bioreactive OC in Engabreen basal sediment (0.17% of the OC comprised extractable carbohydrates) is influenced by the subglacial substrate comprising overridden continental shield rock depleted in reactive OC, the limited opportunity for material from supraglacial environments to be in-washed, and the limited input of overridden paleosols (Stibal et al., 2012). A lack of organic biomarkers (derived from algal and higher plant inputs) in Engabreen basal ice further suggests that incorporation of organic material is probably limited (Stibal et al., 2012). Alternatively, the lack of organic biomarkers may be due to debris entrainment by regelation rather than freezing-on (adfreezing).

In summary, our data suggest that where glaciers and ice sheets override lacustrine sediments, there is an injection of particulate and dissolved bioavailable compounds into the basal ice at the glacier bed, which is less evident where the glacier overrode paleosols or bedrock. This has implications for subglacial LMW-DOC cycling as this abiotic input of LMW-DOC (via leaching) has the potential to stimulate microbial activity in wet sediments in the subglacial environment. We go on to investigate the DOC and LMW-DOC signatures in basal ice from these contrasting subglacial environments.

5.2. Basal ice LMW-DOC signatures and provenance

503 The presence of LMW-DOC compounds and the similarities in the types of compounds
504 detected in basal ice samples from the four sites may reflect common sources and pathways
505 of transformation of DOC in subglacial environments beneath glaciers and ice sheets. The
506 potential for interactions between basal sediment and subglacial icemelt suggest that inputs
507 from the overridden subglacial material may represent a key contribution to basal ice DOC.
508 The chemical composition of basal ice, including DOC compounds, should reflect
509 characteristics of the parent water prior to being frozen (Knight, 1997), where this water
510 might be either flowing at the base of the glacier, held in porewaters in overridden water-
511 saturated sediment, or refrozen water from pressure melting during the regelation process.
512 These water sources have extensive contact with the subglacial material and so have the
513 potential to acquire dissolved compounds via biogeochemical interactions. However, these
514 processes are highly site specific and where there are well-developed quick-flow components
515 and scoured bedrock channels, for instance, there will be less scope for fast-flowing waters to
516 acquire dissolved compounds from biogeochemical interactions with the overridden material.
517 DOC and LMW-DOC components in basal ice may also be acquired by in situ abiotic
518 processes, e.g. by reactions, such as dissolution, in water films around debris and in liquid
519 water veins (Mader et al, 2006). It is likely that certain organic compounds will remain
520 associated with the debris and others will dissociate to become DOC. To fully assess whether
521 DOC is largely terrestrially-derived and leached from sediments, we would need data on the
522 surface area of the debris and information on particle size distribution. As this was beyond
523 the scope of this study we instead used debris concentrations for a preliminary investigation.
524 We find that for sites where there is a bioavailable OC source in sediments (Joyce Glacier)
525 there is a significant relationship between DOC and debris concentration (Figure 1a). This
526 suggests that subglacial meltwater contact with subglacial sediment beneath Joyce Glacier,
527 which is cold-based and so has little supraglacial meltwater penetration to the glacier bed, is a
528 major control on DOC acquisition. We find several additional lines of evidence to support the
529 leaching of DOC from subglacial sediments, including the presence of fulvic acids that have
530 previously been associated with terrestrial material (> 440 nm fluorescence wavelengths) in
531 all basal ice samples (McKnight et al., 2001). The basal ice LMW-DOC compounds may also
532 be a leached relic of the overridden material that has been preserved in the ice when frozen.
533 However, the lack of significant association between LMW-DOC and debris concentration
534 (Figure 1b) is reflective of additional sources and sinks of these compounds in the basal ice
535 layer and/or in the parent water body from which basal ice formed. The LMW-DOC
536 signature in basal ice may also be influenced by in situ microbial production and

consumption, as illustrated in earlier work that has proposed a range of microbial processes to be active in the subglacial environment, including in situ chemoautotrophic production (Bhatia et al., 2006, 2013), chemoheterotrophic oxidation of OC substrates to protein-like LMW-DOC compounds (Bhatia et al., 2010) and release of LMW-DOC from decaying cells. It is probable that subglacial microbial activity cycles LMW-DOC both before and after the formation of basal ice. For instance, microorganisms in subglacial sediment porewaters and basal meltwaters flowing at the rock:water interface may actively utilise OC substrates and energy sources derived from the overridden material. Via this activity, they may also go on to produce simple LMW-DOC compounds which may subsequently be incorporated into basal ice. The protein-like peaks that were observed in the spectrofluorescence spectra in Joyce Glacier and FSB ice (Table 3) tentatively suggests that some of the LMW-DOC is of a microbial provenance. Protein-like fluorescence is linked with recent biological activity (De Souza Sierra et al., 1994) and is associated with active FAA production during microbial metabolism (Yamashita and Tanoue, 2003). The finding that FSB samples contained larger protein-like peaks and had higher mean FCA and FAA concentrations when compared with FDB samples may be explained by the different basal ice formation processes at Finsterwalderbreen. FSB debris, sampled from surface outcrops of frozen subglacial material, or thrust bands, is expected to derive from further upglacier than FDB debris and likely formed during the most recent surge *c.* 1898-1920 (Liestøl, 1969). This suggests that FSB debris may have been glacier-covered for a much longer period than FDB debris. These conditions may have led to enhanced leaching of LMW-DOC from the subglacial material and/or greater production (vs. comsumption) of LMW-DOC by in situ microorganisms. It is also possible that LMW-DOC in basal ice from the polythermal and warm-based glaciers sampled in this study (Finsterwalderbreen, Russell Glacier and Engabreen) could derive from supraglacial inputs as glacially-overridden material is not the sole source of DOC in basal ice.

In this study, we were not able to categorically separate LMW-DOC derived from biotic and abiotic processes as, at a molecular level, many LMW-DOC compounds are non-specific biomarkers due to their pervasive occurrence in plants and microorganisms (Biersmith and Benner, 1998). For example, valine, a common FAA in most basal ice samples, can be synthesized in plants via several steps starting from pyruvic acid (e.g. described in Singh, 1999). Valine can also be microbially-synthesized from pyruvate (Blombach et al., 2007) and produced by aerobic gram-positive microbes (Valle et al., 2008). Similarly, glucose can be produced by photosynthesis (Kirchman et al., 2001) and chemoautotrophic bacterial activity

(Jansen et al., 1982). The key point is that the presence of numerous LMW-DOC compounds in basal ice from all four glacial sites provides evidence that viable substrates for microbial growth, whether derived from a terrestrial or microbial source, are available in subglacial environments. These LMW-DOC compounds may help support microbial communities within the present-day basal ice, e.g. beneath Russell Glacier, where recent work has shown that the basal ice may be microbially-active in the current frozen state (Yde et al., 2010). The microbial cell counts observed in all basal ice samples in this study (10^5 cells g^{-1}, Table 2) are comparable to microbial populations (10^5 - 10^8 cells g^{-1}) reported in other subglacial sediments that have been proven to be microbially-active (Sharp et al., 1999; Foght et al., 2004; Kastovska et al., 2007; Yde et al., 2010; Montross et al., 2012).

5.3. Implications for LMW-DOC cycling beneath glaciers with bioreactive subglacial sediment

The margin of Joyce Glacier rests upon ancient lake sediments and hence, represents a case where a very labile organic matter source is overridden. This situation may have been common in past periods of glaciation, when, for example, the Pleistocene ice sheets advanced over regions with a high density of lakes, such as in northern Canada and Scandinavia (Wadham et al., 2008). Hence, the potential for LMW-DOC incorporation in Joyce Glacier basal ice and sediment may be applicable to these other types of lacustrine-based subglacial ecosystems. In addition, the abundance of LMW-DOC in Joyce Glacier suggests that overridden lacustrine material can be sequestered even if the glacier is cold-based. Contrary to traditional assumptions that drainage in cold-based glaciers is entirely supraglacial, it is possible that discrete subglacial channels exist where water is in contact with the substrate, e.g. at Longyearbreen (Yde et al., 2008). This mechanism may enable the release of DOC to downstream ecosystems. If the glacier was warm-based then the DOC could be flushed out during the summer melt seasons and contribute to the net export of bioavailable DOC to downstream environments. DOC in glacial runoff may derive from multiple sources; terrestrial DOC derived from overridden material at the bed (Hood et al., 2009); anthropogenic aerosol deposition on the glacier surface (Stubbins et al., 2012), and; biological activity in both supraglacial (Anesio et al., 2009) and subglacial (Bhatia et al., 2013) environments. The contribution from basal ice may be more significant in cold-based glacier systems, e.g. in the Antarctic Dry Valleys, where daily radiation melting of the steep ice cliffs may release solute from the debris-rich basal ice that is exposed on the cliffs. The

603 distributed drainage system beneath temperate and polythermal glaciers may also include a
604 constant source of water from basal ice melt and groundwater in contact with glacial till
605 (Paterson, 1999).
606
607 The dramatic difference in the DOC composition beneath glaciers resting on different OC
608 substrates that our data have highlighted may have implications for the rate and degree to
609 which this overridden OC can be cycled to biogenic gases in current subglacial environments,
610 which in turn, has relevance for the global carbon cycle (Wadham et al., 2008; Stibal et al.,
611 2012). While the DOC and LMW-DOC signatures of basal ice may arise from several
612 confounding factors which are difficult to disentangle, identifying the abundance and
613 composition of DOC in basal ice is an important first step to understanding LMW-DOC
614 cycling in subglacial environments.
615

616 ## 6. Conclusion

617 We employ a combined spectrofluorometric and ion chromatographic methodological
618 approach to produce the first identification and quantification, at trace level concentrations, of
619 major LMW-DOC fractions (free amino acids, carbohydrates and carboxylic acids) in debris-
620 rich basal ice. We demonstrate that ion chromatographic systems that are optimised for trace
621 level LMW-DOC analyte detection can supplement traditional methods of LMW-DOC
622 characterisation as a quantitative technique. Our work adds to the growing body of research
623 addressing sources and reactivity of DOC in subglacial ecosystems and provides a
624 characterisation of LMW-DOC in basal ice from four different glacial environments with
625 distinctive basal debris types including lacustrine material (Joyce Glacier), overridden soils
626 and tundra (Russell Glacier), kerogen in bedrock (Finsterwalderbreen) and bedrock/soils
627 (Engabreen). We infer that terrestrial inputs from the overridden subglacial material represent
628 a key contribution to basal ice DOC. Our data show that LMW-DOC concentrations in basal
629 ice are dependent on the bioavailability of the overridden OC, which in turn, is influenced by
630 the type of overridden material. We find that where glaciers and ice sheets override lakes,
631 such as at Joyce Glacier, there is an injection of particulate and dissolved bioavailable
632 compounds into the basal ice at the glacier bed, which is less evident where glaciers overrode
633 paleosols or bedrock. There is also potential for the overridden substrate to act as an indirect
634 (via microbial cycling) source of DOC, as the leached LMW-DOC compounds may stimulate
635 microbial activity in wet sediments in the subglacial environment. This has implications for

636 the cycling of overridden OC to biogenic gases in subglacial environments and concurs with
637 recent findings that accelerated melting of glaciers and ice sheets could constitute a
638 significant source of DOC and other, potentially-bioavailable dissolved organic matter, to
639 glacially-fed ecosystems. The abundance of LMW-DOC in Joyce Glacier basal ice suggests
640 that overridden material may be sequestered even if the glacier is cold-based. Identifying the
641 abundance and composition of DOC in basal ice is an important first step to understanding
642 LMW-DOC cycling in subglacial environments, which has relevance for local carbon cycling
643 and wider ecosystem processes.

Author Contributions

JLW and MT conceived the project. ECL, JLW, GPL, MS and SF collected field data. ECL, GPL, AEP, and MS undertook the lab analysis. PD, GPL and ECL assisted with the Dionex™ ICS-3000 ion chromatography system optimisation and method development. ECL, JLW and MT wrote the paper with additional comments from the co-authors.

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871 Gunnlaugsson, H.P., Nielsen, O.B. Basal ice microbiology at the margin of the Greenland ice
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873 Table 1 Biogeochemical data for basal ice from Engabreen (E), Finsterwalderbreen (F),
874 Russell Glacier (R) and Joyce Glacier (J). DB = dispersed banded and SB = solid banded.
875 Values given are the mean concentrations for each analyte and the standard deviation is given
876 in parentheses. LMW-DOC = free carbohydrates (FCHOs) + free amino acids (FAAs) + free
877 carboxylic acids (FCAs). Only values > LOQ have been included.

878

Sample	**% debris (by mass)**	**DOC (µM C)**	**LMW DOC (nM C)**	**FCHOs (nM C)**	**FAAs (nM C)**	**FCAs (nM C)**
E (n = 6)	36.83 (20.96)	113.56 (106.60)	417.70 (213.07)	0.00	22.41 (24.24)	442.19 (164.00)
FDB (n = 5)	20.22 (26.74)	14.85 (9.91)	169.67 (183.90)	0.00	0.00	169.67 (183.90)
FSB (n = 5)	86.47 (6.58)	33.38 (33.30)	312.67 (502.64)	0.00	46.47 (48.99)	274.91 (549.83)
R (n = 7)	54.89 (24.51)	53.31 (28.89)	343.72 (689.83)	0.00	50.59 (62.97)	365.62 (817.56)
J (n = 5)	21.22 (6.41)	272.09 (99.38)	4429.83 (2625.95)	28.29 (15.83)	4353.30 (2643.59)	0.00

879 Table 2 Mean sediment characteristics. [§]sediment OC age from Stibal et al., (2012), method
880 described in the online supporting information. ND = not determined. E = Engabreen, F =
881 Finsterwalderbreen, R = Russell Glacier, J = Joyce Glacier. Standard deviation is given in
882 parentheses.

Sample	**^{14}C age (years, BP)[§]**	**%OC**	**%InC**	**Extractable carbohydrates (µg/g)**	**Carbohydrate fraction (% of OC)[*]**	**Cell abundance (cell g^{-1})**
E (n = 5)	ND	0.19 (0.08)	0.24 (0.18)	3.26	0.17	6.80 x 10^5
F (n = 5)	3750 (150)	0.57 (0.12)	1.80 (0.25)	2.34	0.04	1.68 x 10^5
R (n=5)	1830 (50)	0.44 (0.09)	0.01 (0.00)	20.83	0.47	2.26 x 10^5
J (n= 5)	ND	0.01 (0.02)	0.28 (0.05)	23.95	17.11	1.16 x 10^5

883 Table 3 Summary of the dominant fluorophores in basal ice from four contrasting glacial
884 environments. The dominant fluorophores (denoted by *) have been identified according to
885 previous characterisation of spectral compounds (see Barker et al., 2009 and references
886 therein). DB = dispersed banded, SB = solid banded.

Sample	Fluorophore (peak excitation wavelength, nm)	Dominant fluorophore identification	n
Engabreen	342, 386*, 440, 483	Fulvic acid, marine humic-like	6
Finsterwalderbreen DB	342, 389*, 440	Fulvic acid, marine humic-like	5
Finsterwalderbreen SB	276, 336, 389*, 440	Fulvic acid, marine humic-like	5
Russell Glacier	335*, 385, 440, 483	Protein-like/marine humic-like	7
Joyce Glacier	279, 342, 386*, 440, 460, 551	Fulvic acid, marine humic-like	5

887 **Figures**

888

889 Figure 1 Associations between a) DOC and debris concentration, and b) LMW-DOC and
890 debris concentration. Engabreen samples are given in black, Finsterwalderbreen in red (FDB
891 (dispersed banded ice) as triangles and FSB (solid banded ice) as circles), Russell Glacier in
892 blue and Joyce Glacier in green. $P < 0.05$ for all regression equations, only significant
893 correlations are shown.

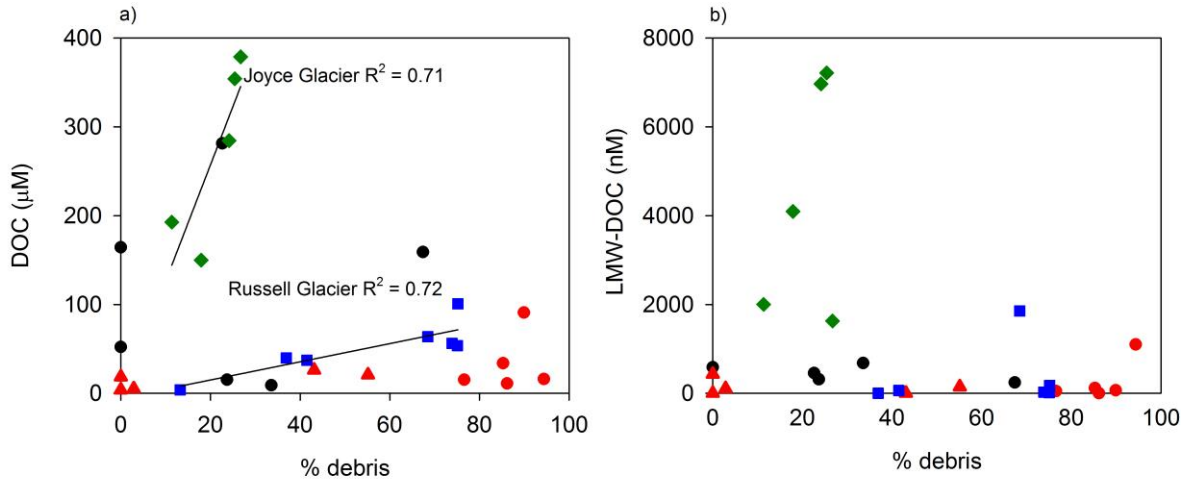

Figure 2 Mean normalized synchronous fluorescence spectra for basal ice samples. E = Engabreen, F = Finsterwalderbreen, R = Russell Glacier, J = Joyce Glacier, DB = dispersed banded, SB = solid banded.

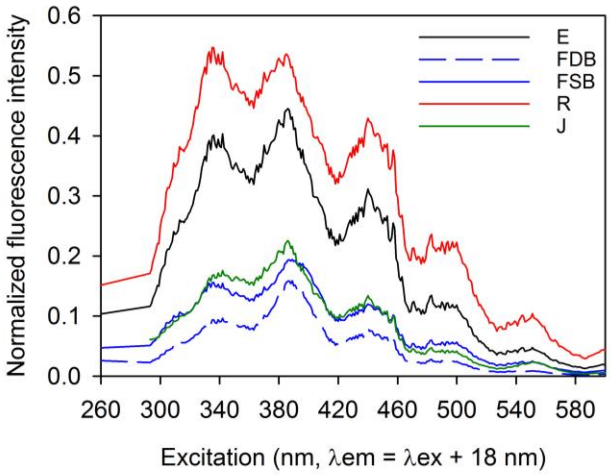

Figure 3 FCA compositions in basal ice samples. E = Engabreen, F = Finsterwalderbreen, R = Russell Glacier, DB = dispersed banded, SB = solid banded. Samples with zero concentrations have been excluded from the plot and only values > LOQ have been included.

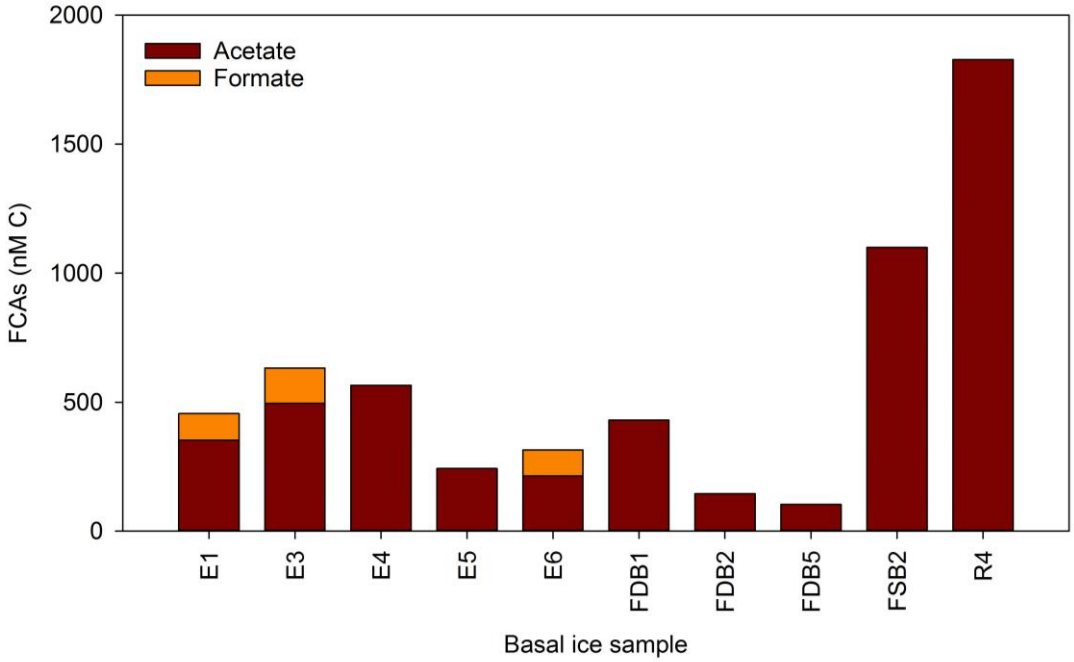

Figure 4 FAA composition in basal ice samples from a) Engabreen (E), Finsterwalderbreen (F), Russell Glacier (R) and Joyce Glacier (J), b) FAAs in Joyce Glacier basal ice, plotted separately due to an order of magnitude increase in concentrations. Samples with zero concentrations have been excluded from the plot and only values > LOQ have been included. S/P = serine and proline, reported together due to co-elution. SB = solid banded.