Organic Geochemistry 118 (2018) 63–72

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Supraglacial microbes use young carbon and not aged cryoconite carbon

Drake O. McCrimmon, Michael Bizimis, Alexandra Holland [1], Lori A. Ziolkowski *

University of South Carolina, School of the Earth, Ocean and Environment, 701 Sumter St., EWS 617, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 24 July 2017
Received in revised form 22 November 2017
Accepted 8 December 2017
Available online 15 December 2017

Keywords:
Cryosphere
Lipids
Radiocarbon
Cryoconite
Microbial
Phospholipid fatty acids
Trace elements

ABSTRACT

Cryoconite holes are depressions in glacial ice surfaces filled with dark debris that reduce albedo. The relative contributions of combustion and microbial carbon to cryoconite carbon are currently not known. To constrain cryoconite organic carbon composition and carbon sources to microorganisms living on glacier surfaces, measurements of bulk organic carbon and microbial phospholipid fatty acids (PLFAs) from supraglacial cryoconite sediment within the ablation zones of Spencer and Matanuska glaciers in southern Alaska were coupled with radiocarbon (^{14}C) analyses. The ^{14}C content of bulk cryoconite organic carbon on both glaciers was depleted relative to the modern atmosphere, while the PLFAs contained carbon that was recently in equilibrium with the atmosphere. Because the bulk cryoconite material is isotopically distinct from the PLFAs, these results indicate that cryoconite organic carbon is not bioavailable to the microbes. Instead, modern carbon in the microbes suggests that carbon is being quickly cycled by them within the cryoconite. Biomarker and stable isotope analyses of cryoconite organic carbon points to fresh carbon inputs to cryoconite and indicates that combusted fossil carbon is a not a major component of cryoconite organic carbon. Trace element analyses of the bulk cryoconite show crustal inputs and no evidence for excess metals associated with recent combustions sources, indicating that the aged bulk cryoconite organic carbon is likely of rock origin (e.g., shale). This study highlights that microbes in cryoconite holes on these glaciers are fixing atmospheric carbon and not using the aged carbon surrounding them.

1. Introduction

Alpine glaciers currently represent < 1% of global glacier ice volume, yet their rapid mass loss accounts for nearly one third of the currently observed sea level rise (Mengel et al., 2016). The region of the glacier where the rate of ice loss outpaces the rate of accumulation, the ablation zone, has been shown to contain impurities that lower the albedo, or reflectivity, relative to other parts of the glacier (Bøggild et al., 2010; Box et al., 2012; Dumont et al., 2014). To date, analyses of the composition of these impurities have primarily focused on characterizing the glacial dissolved organic carbon and microbial communities. For example, in the Gulf of Alaska, glaciers export aged organic matter (Hood et al., 2009) that is consumed by organisms downstream (Fellman et al., 2015). The source of this aged organic matter has been suggested to be of anthropogenic origin (Stubbins et al., 2012) delivered to the glaciers by aerosol inputs. However, supraglacial microbial communities have been found to be abundant and diverse (Stibal et al., 2012; Lutz et al., 2016). Additionally, microbes in supraglacial streams have been shown to preferentially consume the most labile carbon (Musilova et al., 2017; Smith et al., 2017). While these studies have provided powerful insights into impurities that effect aqueous glacial carbon, much remains unknown about carbon in the sediment on glacier surfaces.

Cryoconite, the dark sediment that accumulates in depressions of the ablation zone (Hodson et al., 2008), is thought to originate from a mixture of aerosol inputs from afar and locally produced microbial carbon (Cook et al., 2015; Hotaling et al., 2017). To date, there is no direct evidence on the relative proportions of dust, soot, and microbial carbon in cryoconite carbon. Some studies point to indirect evidence of aerosol-derived soot and humic material being important components, as these components can be found in ice cores (Grannas et al., 2006; Kehrwald et al., 2012). Additionally, microbial communities have also been found to be an important component of cryoconite. Genetic sequencing (16S/18S) and microbial lipid analyses on supraglacial microbes have shown that glacier surfaces are dominated by gram positive and gram negative bacteria, as well as cyanobacteria (Christner et al., 2003; Xu et al.,

* Corresponding author.
E-mail addresses: drakemccrimmon@gmail.com (D.O. McCrimmon), mbizimis@geol.sc.edu (M. Bizimis), ah16063@bristol.ac.uk (A. Holland), lziolkowski@geol.sc.edu (L.A. Ziolkowski).
[1] Current address: Bristol University, School of Geographical Sciences, 12 Berkeley Square, Bristol BS8 1SS, England, United Kingdom.

https://doi.org/10.1016/j.orggeochem.2017.12.002
0146-6380/

2010). The microorganisms present within supraglacial material represent both heterotrophic and autotrophic communities, with autotrophy suspected to dominate (Anesio et al., 2009; Stibal et al., 2012; Lutz et al., 2016). However, the specific carbon sources available to microbes in the supraglacial environment is unknown. While molecular approaches to characterizing the microbes can provide powerful information about what microbes are living on the surface of the glacier, these approaches cannot answer the question of the source(s) of the carbon to the microorganisms or to the glacier in the first place.

One way to identify the relative contributions of organic carbon from different carbon sources is by determining the age, or radiocarbon (^{14}C) content, of the cryoconite total organic carbon. In general, $\Delta^{14}C$ ranges from −1000‰ for radiocarbon-free ancient organic carbon to ~+20‰ for the modern atmosphere (Levin et al., 2013). Overall, if the cryoconite organic carbon contains depleted ^{14}C, it contains carbon that was last in equilibrium with the atmosphere between 100 and 50,000 years ago. On the other hand, if the carbon was recently in equilibrium with the atmosphere, it would be abundant in ^{14}C and would be modern ($\Delta^{14}C$ > 0‰) or contain radiocarbon that originated from nuclear weapons testing during the 1950s. In the supraglacial environment, possible old carbon sources could be recently deposited soot from the combustion of fossil fuels (e.g., Stubbins et al., 2012), organic carbon that was deposited long ago and is now exposed due to glacier melt (e.g., aerosols co-deposited with the snow), or carbon of rock origin (e.g., shales) deposited by erosion of proximal rock formations. Modern carbon sources could include soot from forest fires, modern photosynthesizing microorganisms, and terrestrial plant material from nearby forests. The radiocarbon content of bulk cryoconite organic carbon would therefore represent the complicated mixing of all these possible isotopically distinct end members and by itself be difficult to interpret. Therefore, additional evidence beyond bulk radiocarbon measurements of cryoconite organic carbon, such as molecular biomarker analyses, is needed to help identify specific carbon sources to the cryoconite.

Radiocarbon measurements can be coupled with biomarker analyses to elucidate carbon sources to cryoconite as well as the carbon sources to microorganisms residing within cryoconite. Microbial lipids quantified in this study, phospholipid fatty acids (PLFAs) are components of microbial membranes that quickly break down after cell death and are therefore biomarkers of the viable microbial community (White et al., 1979). By measuring the radiocarbon content of the microbial lipids themselves, we can determine isotopic composition of the carbon taken up by the microorganisms. This technique has been used to elucidate carbon sources to microbes in other environments, including the degradation of oil by microbes (Slater et al., 2006; Mahmoudi et al., 2013) and the use of modern carbon to microbes in rocks (Ziolkowski et al., 2013a, 2013b). However, to date there have been no studies assessing the age of carbon sources to microbes living on glacier surfaces or carbon sources specific to alpine glacier environments.

In this study, we investigated the variability of carbon usage by microbes and organic carbon sources to cryoconite from the lower ablation zones of Matanuska and Spencer glaciers in southern Alaska. These two glaciers were studied because they were likely to receive aerosol inputs from nearby forest fires as well as possible anthropogenic inputs (e.g., Stubbins et al., 2012). We coupled compound-specific radiocarbon measurements of PLFAs with total organic carbon ^{14}C and analyses of alkane biomarkers and trace metals. This study provides, for the first time, compound-specific radiocarbon measurements on extracted microbial lipids from cryoconite in order to directly determine the age of carbon used by microbes on glacier surfaces. Additionally, ^{14}C analyses of the extractable and non-extractable organic carbon components of the total cryoconite organic carbon were compared to a readily available combusted carbon standard to determine the amount of combusted fossil carbon within cryoconite on the glacier surfaces. Our data indicate that the primary source of carbon to the cryoconite microbes is recent atmospheric carbon and not the aged geologic carbon in the cryoconite holes.

2. Materials and methods

2.1. Study sties

Supraglacial cryoconite sediment samples were collected in August of 2015 from Matanuska (n = 7) and Spencer (n = 10) glaciers in southern Alaska (Fig. 1, Table 1). Spencer glacier is ~50 km south of Anchorage and accessible only by foot or train. The surface of this glacier was a friable ice that was highly weathered and had channels of running water that contained cryoconite material. Matanuska glacier is ~50 km northeast of Anchorage and easily accessible by road. The surface of Matanuska glacier was hard clean ice, and samples of traditional cryoconite holes, as well as sediment accumulated in cracks in the ice, were collected.

Both Matanuska and Spencer glaciers are found on the Chugach mountains. The Chugach mountains contain the plutonic core and extrusive portions of a Jurassic age intra-oceanic island arc juxtaposed with faulted subduction complex rocks, principally of Cretaceous age, to the south (Burns et al., 1991). The Matanuska glacier crosses over an area of the Chugach mountain dominated by Jurassic age volcanic rocks of the Talkeetna arc, as well as younger sedimentary formations and Eocene age felsic intrusives (Burns et al., 1991). Based on the geologic maps of the area from the Burns et al. (1991) study, the western side of the glaciers contacts the younger Chichaloon formation of fluvial origin that also contains thin coal beds. The Spencer glacier crosses the Cretaceous flysch subterrain (Valdez group) of the Chugach terrane (deep water turbidites associated with a subduction zone). In general, both glaciers cross typical upper continental crustal rock formations that may have imprinted a geochemical signal to the cryoconite. Because of their close proximity, this study is a comparison of cryoconite carbon composition and microbial carbon usage across two glaciers in a similar geologic and climatic setting. Overall, the cryoconite samples collected consisted of fine gray sediment with the exception of one sample – the 'MAT orange' that was unique in that the material in the cryoconite was predominately an orange biofilm-like material.

Solid impurities were collected using disposable pipettes into Falcon tubes and Whirlpak bags. Samples were kept frozen until they could be returned to the lab at the University of South Carolina and freeze dried. All glassware used was baked at 450 °C for 4 h prior to use.

2.2. Bulk organic carbon and nitrogen measurements

The bulk organic carbon and nitrogen content of the cryoconite sediment was determined via Elemental Analysis (EA) at the University of Georgia. EA analysis was completed by placing dry cryoconite sediment that was pre-treated with 5% HCl to remove inorganic carbon into small tin capsules, then placing the tin capsules into the instrument where the sediment was oxidized and the resulting CO_2, H_2O, and N_2 gases were purified and quantified.

2.3. Alkane extraction

Analysis of plant leaf wax *n*-alkane biomarkers was performed to determine the relative contributions of aquatic biomarkers to

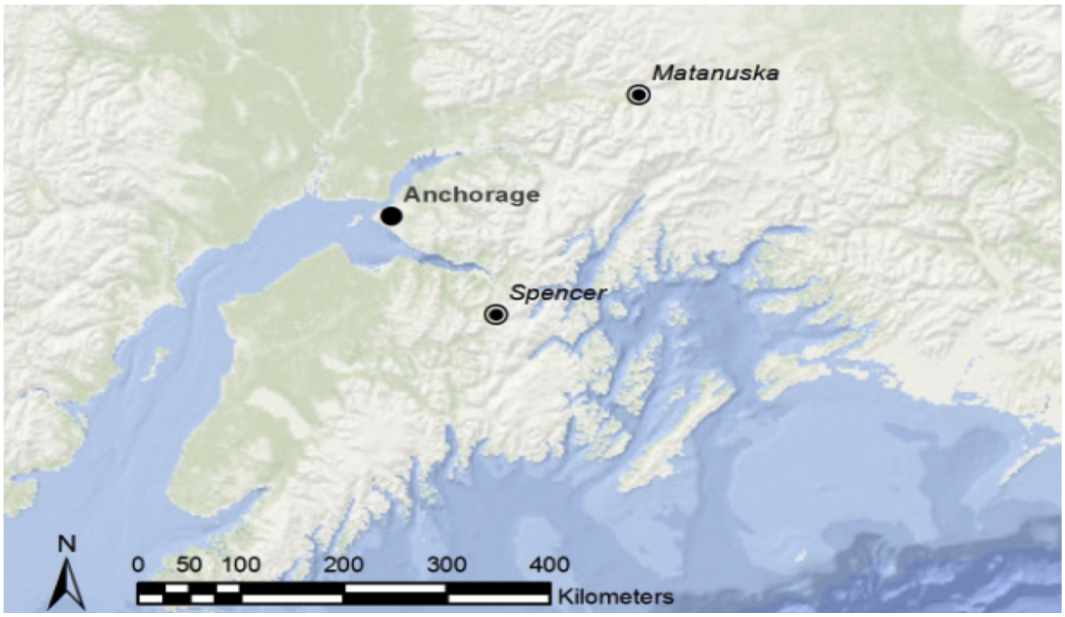

Fig. 1. Map of glacier locations in southern Alaska. Samples locations (Table 1) were collected within a 4 km range of each other near the foot of the ablation zone on each glacier.

Table 1
Cryoconite sample locations, %C, %N, C/N, PLFA concentrations from Matanuska (MAT) and Spencer glaciers collected in August 2015. Dashes indicate no data available.

Sample name	Location	%C	%N	C/N	μg PLFA/g
MAT 1	N 61°46.474′ W 147°44.987′	–	–	–	83.5
MAT 2	N 61°46.423′ W 147°44.784	0.64	0.06	10.6	50.1
MAT 3	N 61°46.396′ W 147°44.757′	1.28	–	–	38.1
MAT Orange	N 61°46.387′ W 147°44.779′	1.47	–	–	98.4
MAT 4	N 61°46.345′ W 147°44.685′	0.54	–	–	170.8
MAT 5	N 61°46.329′ W 147°44.680′	0.64	0.05	12.8	56.3
MAT 6	N 61°46.313′ W 147°44.658′	0.98	–	–	55.8
Spencer 15	N 60°40.769′ W 149°00.424′	0.82	0.06	13.6	25.8
Spencer 14	N 60°40.812′ W 149°00.346′	1.25	0.12	10.4	84.6
Spencer 13	N 60°40.823′ W 149°00.316′	–	–	–	28.5
Spencer 12	–	–	–	–	170.5
Spencer 10	N 60°40.762′ W 148°59.799′	0.56	–	–	139.9
Spencer 5.1	N 60°40.786′ W 148°59.766′	1.30	–	–	294.7
Spencer 6	N 60°40.761′ W 148°59.669′	–	–	–	41.1
Spencer 7	–	–	–	–	207.1
Spencer 8	N 60°40.827′ W 148°59.654′	0.25	–	–	107.9
Spencer 9	N 60°40.770′ W 148°59.525′	0.95	0.10	9.5	110.1

terrestrial leaf waxes in the cryoconite material. Around 2 g of freeze-dried cryoconite sediment was sonicated in 50 mL of a DCM:MeOH (9:1, v:v) solution for 30 min and filtered three times, using fresh 50 mL DCM:MeOH (9:1, v:v) each time. The samples were subsequently blown down to a small volume under a stream of high purity (UHP) N_2 and placed on 4 g of activated silica gel in a glass chromatography column. The extract was then chromatographically separated into polarity fractions using 40 mL each of hexane and DCM. Alkanes were contained in the hexane fraction that was blown down to 1 mL before analysis via gas chromatography–mass spectrometry (GC–MS).

Alkanes were injected (1 μL, splitless mode) and separated using an Agilent 7890B/5977A GC–MS equipped with an HP-5MS column. He was the carrier gas, and a temperature program that

began at 100 °C, ramped up 8 °C/min to 300 °C, and then held isothermal for 23 min. Selected ion monitoring (SIM) of *m*/*z* of 57 was used for the identification of *n*-alkanes. Quantification was performed using external standards (*n*-alkane standards C_{20}, C_{24}, C_{26}, and C_{30}). Laboratory blanks were analyzed with each sample set of six samples.

2.4. PLFA extraction

To determine the amounts and types of bacteria and eukaryotes present, PLFAs were extracted from the supraglacial material. Using a modified Bligh and Dyer (White et al., 1979) method, the PLFAs were purified from the total solvent extractable lipids. Briefly, about 3 g of freeze-dried supraglacial material was sonicated in duplicate for two min and extracted overnight in a DCM:MeOH:phosphate buffer (1:1:0.8, v:v:v) solution and then filtered through pre-rinsed GF/F filters into separatory funnels. After bringing the solvent ratio to 1:1:0.9 DCM:MeOH:water, samples were allowed to equilibrate overnight into organic (total lipid extract, or TLE) and aqueous phases. The TLE was then separated from the aqueous phase, evaporated to a small volume under a gentle stream of UHP N_2. A quantitative portion of the TLE was saved for future analyses. The remaining TLE was placed onto 4 g of activated silica gel in a glass column and then separated by column chromatography into polarity fractions with 40 mL each of DCM, acetone, and MeOH. The MeOH fraction, which contained the PLFAs, was blown down to dryness under UHP N_2 and mild alkaline methanolysis was performed to transesterify the PLFAs into fatty acid methyl esters (FAMEs) (Guckert et al., 1985). Free fatty acids are not esterified under base catalyzed transesterification conditions. The FAMEs solution was then chromatographically separated into polarity fractions with 4 mL each of hexane:DCM (4:1, v:v), DCM, and MeOH. FAMEs were present in the DCM fraction, which was blown down to 1 mL before analysis via GC–MS.

An Agilent 7890B/5977A GC–MS was used for separation, identification, and quantification of FAMEs. For quantification, 1 μL of FAMEs was injected using splitless mode into an HP-5MS column with He as a carrier gas and a temperature program that began at 50 °C, ramped up 20 °C/min to 130 °C, then 4 °C/min and held isothermal at 160 °C (5 min), ramped up 8 °C/min to 300 °C (held isothermal 5 min). The total ion current (TIC) was used for quantification of FAMEs. Identification of FAMEs was completed using mass fragmentation patterns and retention times relative to commercially available standards. Quantification was completed using external standards (FAME standards $C_{8:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{18:2}$, Sigma-Aldrich). Laboratory blanks were regularly analyzed to assess contamination. The amount of PLFAs extracted was converted to a cell abundance using the conservative conversion 2×10^4 cells/pmol PLFA (Green and Scow, 2000). Nomenclature for PLFAs is based on the number of carbons followed by the number of double bonds. For example, 18:2 indicates a PLFA with 18 carbons and 2 double bonds.

2.5. Carbon isotope measurements

The ^{14}C contents of bulk cryoconite material, extracted PLFAs, and the total lipid extract were measured to determine the age of cryoconite and its solvent extractable components. Bulk cryoconite sediment was treated with 5% HCl at 80 °C for 1 h to remove carbonate carbon and then was washed with deionized water and dried at 60 °C. Dried carbonate-free cryoconite was then placed in pre-combusted quartz tubes containing cupric oxide and silver wire. Quantitative portions of previously extracted TLE and total PLFAs were separately placed in pre-combusted quartz tubes and dried under UHP N_2 before adding cupric oxide and silver wire. All samples were evacuated under vacuum before being flame sealed and combusted at 900 °C for four hours. Evolved CO_2 was purified, quantified manometrically and sealed into Pyrex tubes before being sent to the University of Georgia for ^{14}C analysis using accelerator mass spectrometry (AMS). An aliquot of CO_2 was analyzed for ^{13}C using isotope ratio mass spectroscopy on some samples. $\Delta^{14}C$ values were corrected to $\delta^{13}C$ values of −25‰ to correct for isotope fractionation and reported in the per mil (‰) notation relative to the standard Oxalic Acid I (NBS SRM 4990) (Stuiver and Polach, 1977).

The transesterification of PLFAs to FAMEs cleaves the polar head group of the molecule and adds a methyl group that was assumed to be radiocarbon-free ($\Delta^{14}C_{MeOH} = -1000‰$). To correct for the isotopic value of the added carbon the following formula was used:

$$\Delta^{14}C_{PLFA} = [(\Delta^{14}C_{measured} \times n) + (\Delta^{14}C_{MeOH} \times 1)]/(n+1) \quad (1)$$

where n is the average chain length of the PLFAs in the same sample.

Uncertainties for $\Delta^{14}C$ were assumed to be ± 5‰ for bulk cryoconite organic carbon, ± 10‰ for the total lipid extract, and ± 20‰ for PLFAs. To regularly quantify any extraneous carbon added to samples, laboratory blanks were regularly combusted and found to have < 5 μg C. Samples submitted for ^{14}C analysis were > 100 μg C. Because the ^{14}C ages were not calibrated, we report the age of the material as "^{14}C years before present" (BP). Uncertainties for $\delta^{13}C$ were ± 0.5‰ for bulk cryoconite organic carbon.

2.6. Statistical analyses of PLFAs

A standard *t*-test in Microsoft Excel© was run to determine if the amounts of PLFAs on the two glaciers were significantly different. The test was run between two sample sets, assuming unequal variances. Therefore, we also ran a one-way ANOVA to determine if the sample sets were significantly different from each other. Additionally, cluster analysis, a descriptive statistical technique, was used to assess differences between the relative distributions of PLFAs in sampling locations and to investigate if PLFA distributions influence the ^{14}C of extracted lipids. Hierarchical cluster analysis using statistical software *R* compared the mole percentage (mol %) of 37 distinct PLFAs observed in 17 samples. The average space between clusters was measured using the average Euclidean distance between samples. On the cluster dendrogram, which is computed by the cluster analysis, an average distance (height) of 100 represents complete dissimilarity between samples, while clusters with an average distance of 0 represents no dissimilarity, or identical samples.

2.7. Trace element analysis

A suite of trace and major element (Al, Rb, Sr, Y, Zr, Nb, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Pb, Th, U, Li, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga) concentrations were determined on the bulk cryoconite material on a Thermo ELEMENT 2 High Resolution ICP–MS at the Center for Elemental Mass Spectrometry, University of South Carolina using standard methods (e.g., Das et al., 2013; Frisby et al., 2016). Briefly, 50 mg of freeze-dried cryoconite was digested in Teflon vessels with sub-boiled distilled concentrated HF:HNO_3 (3:1, v:v). Samples were subsequently dried down several times with concentrated HNO_3 and finally picked up at 250 ppm total dissolved solid dilution and spiked with In as internal standard. Quantification was against the BHVO-2 USGS rock standard using the preferred concentrations from Jochum et al. (2016) and a 10 ppb in-house mixed elemental solution. Precision is typically better than 5% for all reported elements.

3. Results

3.1. Bulk organic carbon properties

Overall, the bulk organic carbon concentration of the supraglacial cryoconite material on the Matanuska and Spencer glaciers was low. The amount of organic carbon was 0.92% ± 0.38% and 0.86% ± 0.40% for Matanuska (n = 6) and Spencer (n = 6) glaciers, respectively (Table 1). Because the concentrations were similar and within the standard deviation of the six samples from each of the two glaciers and their systematic variation in carbon concentrations based on sample location were also similar (Table 1), we pooled the data. When the two glaciers were treated as a single dataset, the organic carbon content was 0.89% ± 0.38% (n = 12, Table 1). The nitrogen concentration of the cryoconite was measured on only five samples and ranged from 0.05% to 0.12%, which yielded C/N weight/weight ratios that ranged from 9.5 to 13.6 (Table 1).

3.2. Solvent extractable carbon and composition

The carbon in the samples that was solvent extractable was represented by the TLE. The TLEs of two samples, MAT 6 and Spencer 15, were examined to determine the amount of extractable carbon and radiocarbon content of the extractable carbon. In both samples, the amount of extractable carbon was low (7.6% and 1.3% of the total organic carbon for MAT 6 and Spencer 15, respectively).

n-Alkanes from C_{15} to C_{31} were observed on both glaciers, but the chain length with the highest relative abundance differed between the two glaciers (Fig. 2). Spencer had a shorter maximum chain length (C_{23}) than that of the Matanuska *n*-alkanes (C_{27}). Additionally, when the relative abundance of odd to even chain lengths was examined, both glaciers had a similar odd to even (O/E) carbon preferences (O/E of 5 and 3, for Spencer and Matanuska glaciers respectively).

The concentration of PLFAs (Table 1) was 75.6 ± 48.9 μg/g sample for Matanuska Glacier (n = 7) and 121.0 ± 85.6 μg/g sample for Spencer Glacier (n = 10). It was determined that the abundance of microbes on the two glaciers was significantly similar to each other (*t*-test, p = 0.059, one way ANOVA, p = 0.2564). When the PLFA concentrations were converted to cellular abundance, using a conservative conversion factor (Green and Scow, 2000), the abundance of microbial cells was $6.8 \times 10^9 \pm 4.8 \times 10^9$ cells/g cryoconite.

The distribution of PLFA structures can indicate, broadly, the type of microorganisms present in the cryoconite environment (Supplementary Table S1). The most abundant fatty acids in these samples were $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and terminally branched i15:0 and a15:0 (Supplementary Table S2). To determine if there were any systematic differences in the PLFA distributions between sample sites, a cluster analysis was run on the PLFA structure distributions (37 PLFA structures across 17 samples). The resulting cluster dendrogram (Supplementary Fig. S1) showed that the vast majority of samples clustered very close to zero, indicating a high degree of similarity between structure variations and microbial communities across most sample locations. Those PLFA samples that clustered away from the rest of the samples (MAT 1 and Spencer 15) shared similar structure distributions with the rest of the samples. It should be noted that MAT 1 and Spencer 15 were samples closest to the terminus of each glacier.

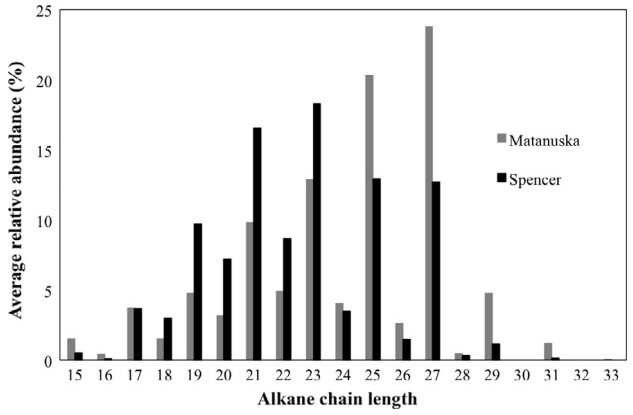

Fig. 2. Distribution of *n*-alkane chain lengths on Matanuska and Spencer glaciers, represented as average relative abundance (%).

3.3. Isotopic composition of cryoconite carbon

The average $\delta^{13}C$ values of bulk organic carbon for Matanuska (n = 6) were −26.2‰ ± 0.9‰ and −26.6‰ ± 1.0‰ for Spencer (n = 6). For all supraglacial samples on Matanuska and Spencer, the average $\delta^{13}C$ value of bulk organic carbon was −26.3‰ ± 0.9‰ (n = 12, Table 2). The bulk organic carbon radiocarbon values (Table 2) were highly variable on both glaciers, ranging from almost modern ($\Delta^{14}C$ = −92‰ ± 5‰) to ancient ($\Delta^{14}C$ = −836‰ ± 5‰). Even though the values varied greatly, there was no systematic variation in the bulk ^{14}C of the cryoconite material based on the location on the glacier or the amount of carbon in the sample, and the average values for each glacier were similar (Matanuska $\Delta^{14}C$ = −335‰ ± 189‰, n = 6, Spencer, $\Delta^{14}C$ = −277‰ ± 282‰, n = 6).

The average $\Delta^{14}C$ value for the extracted PLFAs was −25‰ ± 116‰ and 17‰ ± 55‰ for Matanuska (n = 5) and Spencer (n = 4), respectively. When both glaciers were treated as one data set, the $\Delta^{14}C_{PLFA}$ was −6‰ ± 91‰ (n = 9, Table 2). Based on a mass balance calculation, most of the extracted PLFA samples contained carbon that was recently in equilibrium with the atmosphere (Table 2), with the except of the PLFAs from MAT 2 and Spencer 5.1 that were more depleted in ^{14}C relative to the rest of the samples as well as the recent atmosphere (−233‰ ± 20‰ and −65‰ ± 20‰, respectively). Comparing the ^{14}C content of PLFAs to the ^{14}C content of the cryoconite bulk organic carbon from which the PLFAs were extracted (Fig. 3) showed that PLFAs were significantly more modern than bulk organic carbon ($\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ = 288‰ ± 128‰). Even though the PLFAs from MAT 2 and Spencer 5.1 were far more depleted in ^{14}C than the other PLFA samples, the $\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ for these two samples were similar to the other samples ($\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ = 377‰ ± 15‰ and 71‰ ± 15‰ for MAT 2 and Spencer 5.1, respectively). There was a strong relationship between the ^{13}C content of the bulk cryoconite and the ^{14}C of the bulk cryoconite (R^2 = 0.85, Supplementary Fig. S2).

Different compounds are present within the solvent extractable and non-solvent extractable organic carbon components of the bulk cryoconite organic carbon. To assess whether the solvent-extractable or non-extractable carbon was responsible for the old age of the bulk carbon, we investigated the radiocarbon content of the total extractable lipids. $\Delta^{14}C$ values of the solvent extracted carbon were −30‰ ± 10‰ and −162‰ ± 10‰ for MAT 6 and Spencer 15, respectively, which was more modern than the bulk cryoconite organic carbon (bulk $\Delta^{14}C$: MAT 6 = −310‰ ± 5‰, Spencer 15 = −839‰ ± 5‰, Table 2). For Mat 6, the $\Delta^{14}C$ value of the TLE was within the analytical uncertainty of the microbial lipids ($\Delta^{14}C_{PLFA}$: MAT 6 = 34‰ ± 20‰). Because of sample size limitations for ^{14}C analysis, no PLFA ^{14}C was determined for Spencer 15.

Based on these results, it appears that the modern PLFAs are only a small portion of the total lipid extract. The TLE can contain a wide range of extractable compounds such as alkanes, levoglucosan, free fatty acids, and some black carbon compounds, among others. Using mass balance equations (Eqs. 2 and 3), it was possible

Table 2
Isotopic composition of bulk cryoconite sediment and extracted PLFAs in per mil (‰) notation or ^{14}C years BP. $\Delta\Delta^{14}C$ is the difference between the bulk cryoconite organic carbon and the PLFAs. TLE is the total lipid extract. %C from the atm is the percentage of carbon from the atmosphere incorporated into the PLFAs using an isotopic mass balance between the atmosphere (+20‰) and the $\Delta^{14}C_{bulk}$. Uncertainties for the respective measurements are given in parentheses. Dashes indicate no data available.

Sample name	$\Delta^{14}C$ Bulk (± 5‰)	^{14}C age BP	$\delta^{13}C$ Bulk (± 0.5‰)	$\Delta^{14}C$ PLFA (± 20‰)	$\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ (± 25‰)	$\Delta^{14}C$ TLE (± 10‰)	%C from atm
MAT 2	−611	7580 ± 30	−25.0	−234	377	–	60
MAT 3	−207	1860 ± 25	−27.2	27	234	–	103
MAT Orange	−96	810 ± 20	−26.3	–	–	–	
MAT 4	−289	2740 ± 25	−26.4	25	314	–	102
MAT 5	−500	5560 ± 30	−25.2	22	522	–	100
MAT 6	−311	2990 ± 30	−27.0	34	345	−30	104
Spencer 15	−839	14,680 ± 40	−24.8	–	–	−162	
Spencer 14	−92	770 ± 25	−27.5	–	–	–	
Spencer 10	−175	1540 ± 25	−26.3	47	222	–	114
Spencer 5.1	−136	1170 ± 25	−27.3	−65	71	–	46
Spencer 8	−284	2680 ± 30	−26.5	39	323	–	106
Spencer 9	−141	1220 ± 25	−27.1	48	189	–	117

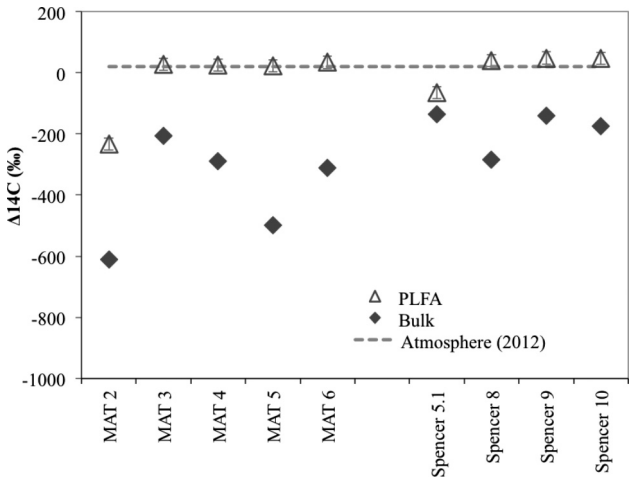

Fig. 3. The radiocarbon (^{14}C) content of bulk cryoconite organic carbon and extracted phospholipid fatty acids (PLFAs) in the per mil (‰) notation. The error associated is ± 5‰ for $\Delta^{14}C_{bulk}$ and ± 20‰ for $\Delta^{14}C_{PLFA}$. The error bars for bulk ^{14}C are smaller than the symbols. The ^{14}C content of the 2012 atmosphere (+20‰, dashed line) is included for reference (Levin et al., 2013).

to determine the age of the organic carbon present in cryoconite that was not solvent-extractable (extracted residue).

$$M_{bulk} = M_{extractableC} + M_{non\text{-}extractableC} \quad (2)$$

$$\Delta^{14}C_{bulk} \times (M_{bulk}) = \Delta^{14}C_{extractableC} \times (M_{extractableC}) + \Delta^{14}C_{non\text{-}extractableC} \times (M_{non\text{-}extractableC}) \quad (3)$$

where the mass (M) of the bulk is composed of the mass fractions (M) of the extractable and non-extractable carbon. Using Eqs. 2 and 3, the $\Delta^{14}C$ values of the non-extractable carbon were determined to be −334‰ ± 15‰ and −848‰ ± 15‰ for MAT 6 and Spencer 15, respectively (Table 2). As expected because the modern lipid extract was only a small fraction (< 10%) of the total organic carbon in the samples, the non-extractable carbon was responsible for the old age of the bulk cryoconite organic carbon.

3.4. Trace metal composition of cryoconite

Based on their low MgO (< 2.8 wt%) and CaO (< 3.2 wt%) contents, moderately high Al_2O_3 (~15 wt%) and inferred (by weight difference) high SiO_2 contents (~70 wt%), the cryoconite material resembles in composition felsic volcanic rocks from the Matanuska Valley (Greene et al., 2006) or upper continental crust (UCC; Rudnick and Gao, 2003). The trace element systematics of cryoconite are plotted on a conventional primitive mantle – normalized extended trace element diagram (Fig. 4a; normalization values from McDonough and Sun, 1995) and show an enrichment in light over heavy rare earth elements (REE), Ti depletion, positive Pb anomaly and high Ba, Th and U concentrations. These are characteristic patterns of UCC suggesting that the inorganic material in these cryoconite are sourced from typical crustal rocks. There is no obvious difference between the Matanuska and Spencer samples, although we acknowledge that the sample size is small. One exception is sample MAT Orange with the orange hue. This has much higher FeO content (31.6 wt%) than the other samples (6, 6.5 wt %), consistent with the presence of Fe-oxides, which also explains the orange color. The trace element pattern of MAT-Orange is similar to the other samples analyzed, but at lower overall concentrations. The Pb enrichment is at the levels of the other samples, but because of the lower REE concentrations, the Pb enrichment appears to be higher.

These REE cryoconite concentrations are comparable to data from Greenland cryoconites (Wientjes et al., 2011). The two data sets agree remarkably well and are similar to the average UCC. Consistent with the inferences of Wientjes et al. (2011), we infer that the bulk inorganic material deposited in the cryoconite holes at both Matanuska and Spencer glaciers is of crustal origin. In detail, there are subtle differences between the patterns of the UCC, Alaskan, and Greenland cryoconites, exemplified in the UCC-normalized REE patterns (Fig. 4b). The Alaskan samples have slightly lower light REE and slightly higher HREE than the average crust and show crossing patterns with the Greenland cryoconites. Proximal felsic rocks from the Matanuska valley have lower light REE and higher heavy REE than the UCC (Fig. 4b; sample 1719A7 from Greene et al., 2006) and may have contributed to the slight REE pattern differences. Overall however, the bulk trace element patterns of the cryoconite material are dominated by upper crustal sources, as was also determined for the Greenland case (Wientjes et al., 2011).

4. Discussion

4.1. Principal source of carbon used by supraglacial microbes

The microbial PLFAs quantified in this study predominately contained carbon that was recently in equilibrium with the atmosphere. Using the concept of 'you are what you eat', the isotopic content of microbial lipids can indicate the isotopic content of the carbon source to the microorganism from which the microbial lipids were extracted (Boschker and Middelburg, 2002). Because PLFAs degrade quickly after cell death, these biomarkers represent only the viable, living microbial community (White et al., 1979). Therefore, the modern radiocarbon content of the microbial lipids

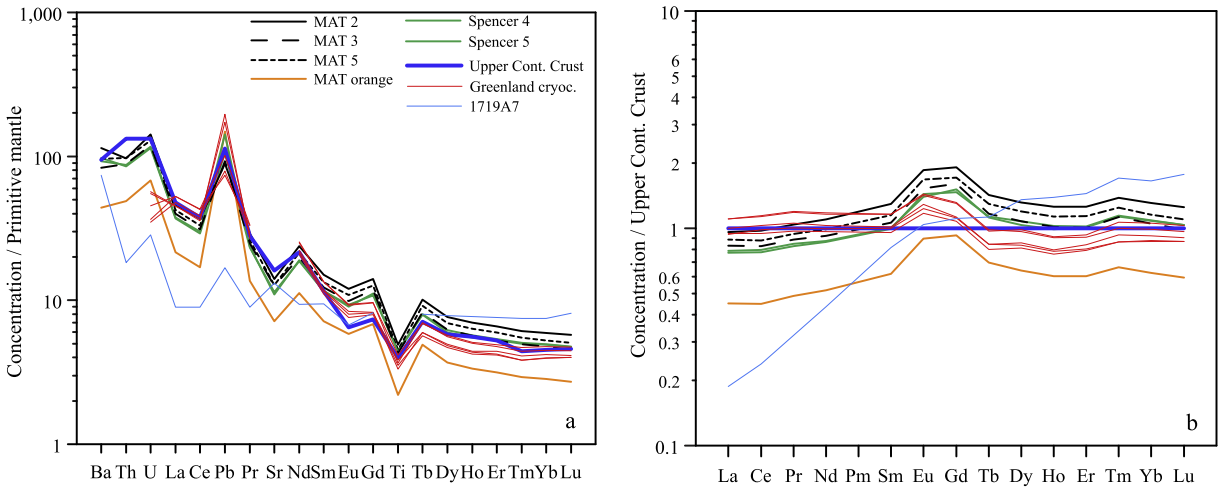

Fig. 4. Trace element systematics of the Spencer and Matanuska glacier cryoconite (this study), compared to Greenland cryoconite material (Wientjes et al., 2011), estimates of the upper continental crust (Rudnick and Gao, 2003), and a felsic plutonic rock proximal to the Matanuska glacier (sample 1719A7; Greene et al., 2006). (a) Primitive mantle normalized extended trace element diagram; (b) upper continental crust normalized rare earth element diagram. Note the close similarity of the Alaskan cryoconite to average upper crustal estimates and to Greenland samples. The slightly lower light to heavy rare earth element pattern of the Alaskan cryoconite compared to the Greenland ones might indicate contribution from locally sourced felsic plutonic rocks exemplified by sample 1719A7 (see text for further discussion).

on Spencer and Matanuska glaciers indicated the use of modern carbon as the primary carbon source to the microbes in the supraglacial environment. The modern radiocarbon content of the lipids is also likely reflected in their DNA, as previously it has been shown that DNA and PLFA have similar ^{14}C contents (Mailloux et al., 2012). Modern carbon sources to these microbes include directly fixing atmospheric CO_2 ($\Delta^{14}C_{atmosphere}$ = +20‰, Levin et al., 2013) through photosynthesis (e.g., autotrophy) or by consuming modern carbon that was recently fixed (e.g., heterotrophy).

Overall, the microbes contained ^{14}C contents that was more similar to atmospheric CO_2 than the bulk cryoconite carbon (Fig. 3). Microbial lipids were significantly more modern than bulk organic carbon ($\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ = 288‰ ± 128‰) for all samples, which means that the microbes contained carbon that is thousands of years younger than the carbon that surrounds them. This difference in radiocarbon content between bulk organic carbon and microbial lipids, regardless of sample location, illustrates that supraglacial microbes were using predominately young carbon instead of aged cryoconite organic carbon. An isotopic mass balance between the atmosphere and the bulk cryoconite carbon illustrated that seven of the nine samples were consistent with the radiocarbon content of the modern atmosphere (Table 2). The preferential use of young carbon over the aged cryoconite carbon suggested that most of the cryoconite carbon is not bioavailable as a carbon source to the microbes within this environment.

There were two microbial lipid samples that were more depleted in ^{14}C relative to the other samples analyzed. These two samples, from near the toe of Matanuska Glacier (MAT 2) and near the medial moraine on Spencer Glacier (Spencer 5.1), were from geographically different parts of the glacier so their isotopic composition does not appear to be related to their sampling location. Apart from the ^{14}C content of both the PLFAs and the bulk organic carbon, there were no measured parameters that indicated these two samples were different in their carbon quality. The ^{14}C-depleted PLFA values indicate that the microbes in these samples contained older carbon. This older carbon could be in the microbial lipids because the aged bulk cryoconite carbon is more accessible to the heterotrophs in these samples or because the microbes cycle carbon more slowly. However, the ^{14}C age of the bulk cryoconite organic carbon is still much older relative to the microbes in these samples, suggesting that microbes did not solely use aged cryoconite organic carbon and must also be using some modern carbon sources.

Previous work has suggested that supraglacial microbial communities are either primarily autotrophic carbon sinks (Anesio et al., 2009) or act as both carbon sinks and sources, depending on the relative abundance of autotrophy and heterotrophy on the glacier surfaces (Stibal et al., 2012). Autotrophic microorganisms use atmospheric CO_2 as a carbon source when cryoconite is open to the atmosphere in the summer and can use dissolved CO_2 when cryoconite is frozen in the winter, whereas heterotrophic microorganisms use previously fixed organic carbon present in their surroundings, such as the organic carbon that autotrophs fix into the cryoconite environment. In order to determine the types of microbial metabolisms present, the distribution of microbial lipid compounds was analyzed. Different microorganisms make different microbial lipid structures, and thus the distribution of lipids within a sample can identify, broadly, the type of microorganism(s) present. In these cryoconite samples, the distributions of microbial lipid (PLFA) (Supplementary Table S2) structures observed indicated, broadly, the presence of a mixture of autotrophic and heterotrophic metabolisms within the supraglacial environment. In these samples, the observed PLFA structures are typical of microbial communities that are composed of cyanobacteria (autotrophs), gram positive and gram negative bacteria (heterotrophs), and algae (autotrophs) (Zelles, 1999; Green and Scow, 2000; Boschker et al., 2005; Dijkman et al., 2010). Additionally, cluster analysis of the distribution of microbial lipids in these samples illustrated that there were no systematic differences in PLFA distributions between sample locations on either glacier. Similar microbial lipid distributions, and therefore the same attributed microbial communities, have also been found in cryoconite samples from Greenland, Canada, and Antarctica (Xu et al., 2010; Pautler et al., 2013). Genetic identification (16S/18S) of microorganisms on glacier surfaces has identified similar microbial communities present on other glaciers (Christner et al., 2003; Anesio et al., 2009; Stibal et al., 2012). Although the amount of PLFAs within a microbial membrane can vary depending on factors such as temperature and stress, a greater PLFA concentration may not be directly comparable between different environments (Frostegård et al., 2011). Therefore, the PLFA distributions indicated that the microbes on Matanuska and Spencer glaciers were present in high abundance and the community consisted of a mixture of autotrophic and heterotrophic organisms.

Taken together, the overall modern radiocarbon content of the microbial lipids and the distribution of the microbial lipids suggested that carbon is cycling quickly within the microbial community. Because the data presented here are the $\Delta^{14}C$ values of all the microbial lipids in the samples and not lipids specific to autotrophic and/or heterotrophic organisms, these data represent the community as a whole. Measurements of the ^{14}C content of autotrophic and heterotrophic groups of compounds, rather than all of the microbial lipids as a whole, may have been able to distinguish more specifically what carbon source heterotrophs vs autotrophs were consuming. However, due to sample size limitations and the high degree of uncertainty associated with ^{14}C measurements of specific microbial lipid compounds, we determined only the radiocarbon content of the pooled microbial lipids. It is also worth noting that the most abundant PLFA ($C_{16:0}$), which was between 10% and 25% of the total PLFAs in all samples, is produced by both autotrophic and heterotrophic organisms. Therefore $C_{16:0}$ specific ^{14}C would not be helpful for distinguishing autotrophic and heterotrophic carbon usage.

Modern carbon usage by microbes on the surface of Spencer and Matanuska glaciers leads to two hypotheses: (1) autotrophy dominates in the supraglacial environment, and (2) heterotrophy is as abundant as autotrophy, but heterotrophic microorganisms in cryoconite predominately use the modern carbon fixed by autotrophic microorganisms and not the aged organic carbon that surrounds them. If only autotrophy dominated, then we would expect to find an accumulation of microbial carbon in cryoconite that would age over time, but this accumulation of aged microbial carbon was not observed. Therefore, it is most likely that the heterotrophs are consuming the modern carbon fixed by the autotrophs. While this study examined the carbon dynamics within cryoconite sediments, our findings are consistent with the work of Musilova et al. (2017) who found that microbes living on the surface of the Greenland ice sheet were responsible for the production and recycling of labile dissolved organic carbon. The ^{14}C data presented here indicated the presence of at least two distinct carbon pools on the surface of Spencer and Matanuska glaciers: a modern carbon source that is used by a large microbial community that is living within, but interacting minimally with a larger aged carbon pool. The data presented here are consistent with a study that showed heterotrophic microbes on a glacier in Antarctica were using only the carbon fixed by autotrophic microorganisms (Smith et al., 2017), rather than other sources of organic carbon. Furthermore, these findings suggest that the microbes are self-contained carbon cyclers, living within a carbon pool that they are not consuming. Thus, this highlights the question: Is the composition of the aged cryoconite organic carbon an important factor in why the microbes are consuming principally modern carbon?

4.2. Origin of aged cryoconite organic carbon

To determine the source of the cryoconite carbon, we studied the composition of not only the total organic carbon, but also of both the solvent-extractable and non-solvent-extractable carbon components. The total organic carbon present within cryoconite was carbon that remained after the removal of inorganic carbon with dilute acid, whereas the organic carbon that was solvent extractable was present in the TLE. Using two samples that met sample size requirements needed for additional extraction, we quantified how much carbon was solvent extractable. Both of these samples (MAT 6, Spencer 15) had low amounts of total solvent-extractable carbon (< 10%), meaning > 90% of the total organic carbon was not solvent-extractable. The radiocarbon content of these two TLE samples was also far more modern in ^{14}C than the bulk organic carbon, suggesting that the solvent-extractable organic carbon contains more carbon that was recently in equilibrium with the atmosphere than the total organic carbon samples. Additionally, the alkane biomarkers extracted from these samples indicated the presence of fresh land plant and microbial carbon entering the supraglacial environment, which is consistent with the TLE containing mostly modern carbon. It is assumed that the fresh land plant organic matter is blown by the wind onto the ablation zone of the glacier. Based on the low TOC concentration, alkane biomarker distribution, and isotopic composition of the TLE, it appears that solvent-extractable carbon contains young carbon and is only a small portion of the cryoconite carbon. Therefore, most of the carbon in cryoconite sediment is old and not solvent-extractable, which means that it is not accessible via traditional organic geochemical techniques.

$\delta^{13}C$ values and C/N ratios of the bulk organic carbon were also utilized to help identify carbon sources to the supraglacial environment. The $\delta^{13}C$ values of bulk cryoconite organic carbon on Matanuska and Spencer glaciers ($-26.3‰ \pm 0.9‰$ for all samples) could originate from many sources, including fresh sources such as C_3 vegetation and microbes (Collister et al., 1994; Boschker and Middelburg, 2002; Peters et al., 2005) and fossil sources such as oil or organic carbon in sedimentary rocks (Peters et al., 2005). Due to the limited dynamic range of ^{13}C, relative to ^{14}C, and the similarity in $\delta^{13}C$ from different end members, it is difficult to determine the carbon sources of bulk organic matter using the $\delta^{13}C$ of bulk organic matter alone.

On the other hand, the observed C/N ratios could indicate microbial carbon and/or soil organic matter inputs to the supraglacial environment. The average C/N weight/weight value in cryoconite on Matanuska and Spencer glaciers was 11.4 ± 1.7. While C/N ratios between 5:1 and 15:1 eliminates land-plant organic matter as a primary carbon source, this range of C/N values is shared by algal, microbial and soil organic matter sources (Hedges and Oades, 1997). Thus, it is plausible that the measured C/N ratio indicates that a portion of the carbon content of cryoconite could have originated from the microbial life present on glacier surfaces. In contrast, the degradation of plants to form soil reduces the C/N to about the same range (Hedges and Oades, 1997), indicating that soil organic matter from surrounding areas could also be a component of the bulk cryoconite organic carbon. However, it is unlikely that surface soil organic carbon that was recently blown onto the glacier would be so depleted in ^{14}C, and therefore there must be other aged carbon sources to the cryoconite material.

One possible source of old carbon to dark impurities on glaciers has been hypothesized to be from fossil fuel combustion (e.g., Stubbins et al., 2012). We investigated the possibility that the old, non-extractable carbon from these glaciers could be due to fossil fuel derived carbon by comparing our data with the National Institute of Standards and Technology (NIST) standard reference material (SRM) 1649a. This SRM is commonly used to study the composition of black carbon from fossil fuel combustion; it is atmospheric particulate matter collected in a naval shipyard in the Washington, D.C. area in 1976–1977. The radiocarbon content of black carbon, the total lipid extract, and total organic carbon have all been measured on NIST 1649a. Radiocarbon analysis of black carbon in NIST 1649a was found to be the most depleted in ^{14}C ($\Delta^{14}C_{black\ carbon} \sim -900‰$ to $-1000‰$; Currie et al., 2002), which suggests that the highly aromatic material in NIST 1649a is from fossil fuel combustion. The solvent extractable component of NIST 1649a was also significantly more ^{14}C-depleted than the bulk organic carbon ($\Delta^{14}C_{bulk} \sim -400‰$, $\Delta^{14}C_{TLE} \sim -700‰$) (NIST SRM 1649a Certificate of Analysis). If there was a significant fraction of the cryoconite organic carbon from the glaciers in this study that could have originated from fossil fuel combustion, the $\Delta^{14}C_{TLE}$ from cryoconite should have been much more negative than the values we observed. Due to the near modern radiocarbon content

of the extractable carbon in the cryoconite samples, it is unlikely that the cryoconite material contains a significant amount of old carbon from fossil fuel combustion. In addition, certain small polycyclic black carbon compounds have also been found to be solvent extractable into the TLE (Weidemeier et al., 2015). Because the ^{14}C content of the extracted cryoconite carbon was modern, it is unlikely that fossil fuel derived black carbon or polycyclic aromatic hydrocarbons were a major part of the lipid extract and may not be present in these cryoconite samples. Therefore, by comparing our ^{14}C results of total organic carbon and total lipid extract to the well-studied NIST 1649a, we conclude that fossil fuel combustion did not contribute a significant amount of carbon to our samples.

If inputs from fossil fuels are unlikely to be responsible for the old age of the carbon, the aged carbon must originate from other carbon sources. One possibility could be that the non-extractable carbon was originally modern carbon that was deposited onto the glacier at some point in the past and that aged in situ. If the extracted carbon was therefore a signal of age of the glacier, one would expect to see the oldest extracted residue closest to the toe of the glacier. Because the samples collected on each glacier covered a limited geographic area of the ablation zone, further research should investigate if the age of cryoconite carbon varies between from the toe to the accumulation zone.

Another possible explanation for the old age of the non-extractable carbon could be an aged geologic carbon source. Shales and coal can contain organic carbon that is ^{14}C free ($\Delta^{14}C = -1000‰$), is not readily solvent extractable, and has a $\delta^{13}C$ value within the same range as terrigenous organic matter ($-25‰$ to $-30‰$). As discussed earlier, the trace element systematics of these cryoconite samples indicated an upper crustal origin with contribution from locally sourced rocks. The presence of coal beds within the Chichaloon Formation of the Chugach terrane, as well as shales and other marine type deposits of the Chugach flysch, are all reasonable proximal sources of aged carbon. Therefore, we hypothesize that the erosion of rocks within the geologic settings of these glaciers is a possible explanation for the old radiocarbon content of the cryoconite carbon.

5. Conclusions

Microorganisms are abundant on glacier surfaces and fixing modern carbon to the supraglacial ecosystem. Their modern carbon sources seem to be independent from the cryoconite organic carbon environment in which they live. This study highlights that there are at least two distinct carbon pools on these two Alaskan glaciers: carbon from a large microbial community that is living within a larger carbon pool of unreactive carbon, likely of geologic origin. This study highlights the radiocarbon heterogeneity of supraglacial organic carbon, especially when there are inputs of both aged rock-derived carbon and recently fixed microbial carbon. Because microbes were isotopically distinct from most of the cryoconite carbon, the microbes were consuming mostly modern carbon and only a small portion of the cryoconite carbon, suggesting that most of the carbon on the glacier will not be decomposed by the microbes. Finally, less than 10% of the cryoconite carbon was found to be extractable, with the remainder of the material being composed of inorganic material derived from rocks and not anthropogenic activities.

Acknowledgements

We thank Dr. Hongyu Li for helping collect these samples. This research was supported in part by University of South Carolina ASPIRE program. The authors also thank two anonymous reviewers and editors Philip Meyers and John Volkman for their time and constructive comments that helped improve this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orggeochem.2017.12.002.

Associate Editor—**Philip Meyers**

References

Anesio, A.M., Hodson, A.J., Fritz, A., Psenner, R., Sattler, B., 2009. High microbial activity on glaciers: importance to the global carbon cycle. Global Change Biology 15, 955–960.
Bøggild, C.F., Brandt, R.E., Brown, K.J., Warren, S., 2010. The ablation zone in northeast Greenland: ice types, albedo and impurities. Journal of Glaciology 45, 101–113.
Boschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiology Ecology 40, 85–95.
Boschker, H.T.S., Kromkamp, J.C., Middelburg, J.J., 2005. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnology and Oceanography 50, 70–80.
Box, J.E., Fettweis, X., Stroeve, J.C., Tedesco, M., Hall, D.K., Steffan, K., 2012. Greenland ice sheet albedo feedback: thermodynamics and atmospheric drivers. The Cryosphere 6, 821–839.
Burns, L.E., Pessel, G.H., Little, T.A., Pavlis, T.L., Newberry, R.J., Winkler, G.R., Decker, J., 1991. Geology of the northern Chugach Mountains, Southcentral Alaska. Alaska Division of Geological and Geophysical Surveys Professional Report 94, 58 pp.
Christner, B.C., Kvitko, B.H., Reeve, J.N., 2003. Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7, 177–183.
Collister, J., Rieley, G., Stern, B., Fry, B., 1994. Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. Organic Geochemistry 21, 619–627.
Cook, J., Edwards, A., Takeuchi, N., Irvine-Flynn, T., 2015. Cryoconite: the dark biological secret of the cryosphere. Progress in Physical Geography 40, 66–111.
Currie, L.A., Benner, B.A., Kessler, J.D., Klinedinst, D.B., Klouda, G.A., Marlolf, J.V., Slater, J.F., Wise, S.A., Cachier, H., Cary, R., Chow, J.C., Watson, J., Druffel, E.R.M., Masiello, C.A., Eglinton, T.I., Pearson, A., Reddy, C.M., Gustafsson, O., Quinn, J.G., Hartmann, P.C., Hedges, J.I., Prentice, K.M., Kirchstetter, T.W., Novakow, T., Puxbaum, H., Schmid, H., 2002. A critical evaluation of interlaboratory data on total, elemental, and isotopic carbon in the carbonaceous particle reference material, NIST SRM 1649a. Journal of Research of National Institute of Standards and Technology 107, 279–298.
Das, R., Bizimis, M., Wilson, A.M., 2013. Tracing mercury seawater vs. atmospheric inputs in a pristine SE USA salt marsh system: mercury isotope evidence. Chemical Geology 336, 50–61.
Dijkman, N.A., Boschker, H.T.S., Stal, L.J., Kromkamp, J.C., 2010. Composition and heterogeneity of the microbial community in a coastal microbial mat as revealed by the analysis of pigments and phospholipid-derived fatty acids. Journal of Sea Research 63, 62–70.
Dumont, M., Brun, E., Picard, G., Michou, M., Libois, Q., Petit, J.R., Geyer, M., Morin, S., Josse, B., 2014. Contribution of light-absorbing impurities in snow to Greenland's darkening since 2009. Nature Geoscience 7, 509–512.
Fellman, J., Hood, E., Raymond, P.A., Hudson, J., Bozeman, M., Arimitsu, M., 2015. Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. Limnology and Oceanography 60, 1118–1129.
Frisby, C., Bizimis, M., Mallick, S., 2016. Seawater-derived rare earth element addition to abyssal peridotites during serpentinization. Lithos 248–251, 432–454.
Frostegård, A., Tunlid, A., Baath, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621–1625.
Grannas, A.M., Hockaday, W.C., Hatcher, P.G., Thompson, L.G., Mosley-Thompson, E., 2006. New revelations on the nature of organic matter in ice cores. Journal of Geophysical Research 111, D04304. https://doi.org/10.1029/2005JD0006251.
Green, C.T., Scow, K.M., 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal 8, 126–141.
Greene, A.R., DeBari, S.M., Kelemen, P.B., Blusztajn, J., Clift, P.D., 2006. A detailed geochemical study of island arc crust: the Talkeetna Arc section, south-central Alaska. Journal of Petrology 47, 1051–1093.
Guckert, J.B., Antworth, C.P., Nichols, P.D., White, D.C., 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Letters 31, 147–158.
Hedges, J.I., Oades, J.M., 1997. Comparative organic geochemistries of soils and marine sediments. Organic Geochemistry 27, 319–361.
Hodson, A., Anesio, A.M., Tranter, M., Fountain, A., Osborn, M., Priscu, J., Laybourn-Parry, J., Sattler, B., 2008. Glacial ecosystems. Ecological Monographs 78, 41–67.

Hood, E., Fellman, J., Spencer, R.G.M., Hernes, P.J., Edwards, R., D'Amore, D., Scott, D., 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. Nature 462. https://doi.org/10.1038/nature08580.

Hotaling, S., Hood, E., Hamilton, T.L., 2017. Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections, and implications of a warming climate. Environmental Microbiology 19, 2935–2948.

Jochum, K.P., Weis, U., Schwager, B., Stoll, B., Wilson, S.A., Haug, G.H., Andreae, M.O., Enzweiler, J., 2016. Reference values following ISO guidelines for frequently requested rock reference materials. Geostandards and Geoanalytical Research 403, 333–350.

Kehrwald, N., Zangarando, R., Gabrielli, P., Jaffrezo, J., Boutron, C., Barbante, C., Gambaro, A., 2012. Levoglucosan as a specific marker of fire events in Greenland Snow. Tellus B Chemical and Physical Meteorology 64, 18196. 10/3402/tellusb.v64i0.18196.

Levin, I., Kromer, B., Hammer, S., 2013. Atmospheric $\Delta^{14}CO_2$ trend in Western European background air from 2000 to 2012. Tellus B Chemical and Physical Meteorology 65, 20092. https://doi.org/10.3402/tellusb.v65io.20092.

Lutz, S., Anesio, A.M., Edwards, A., Benning, L.G., 2016. Linking microbial diversity and functionality of arctic glacial surface habitats. Environmental Microbiology 19, 551–565.

Mahmoudi, N., Porter, T.M., Zimmerman, A.R., Fulthorpe, R.R., Kasozi, G.N., Silliman, B.R., Slater, G.F., 2013. Rapid degradation of Deepwater Horizon spilled oil by indigenous microbial communities in Louisiana saltmarsh sediments. Environmental Science and Technology 47, 13303–13312.

Mailloux, B.J., Dochenetz, A., Bishop, M., Dong, H., Ziolkowski, L.A., Wommack, K.E., Sakowski, E.G., Onstott, T.C., Slater, G.F., 2012. A carbon free filter for collection of large volume samples of cellular biomass from oligotrophic waters. Journal of Microbiological Methods 90, 145–151.

McDonough, W.F., Sun, S.S., 1995. The composition of the earth. Chemical Geology 120, 223–253.

Mengel, M., Levermann, A., Frieler, K., Robinson, A., Marzeion, B., Winkelmann, R., 2016. Future sea level rise constrained by observations and long-term commitment. Proceedings of the National Academy of Sciences USA 113, 2597–2602.

Musilova, M., Tranter, M., Wadham, J., Telling, J., Tedston, A., Anesio, A.M., 2017. Microbially driven export of labile organic carbon from the Greenland ice sheet. Nature Geoscience 10, 360–366.

National Institute of Standards and Technology, 2007. Certificate of Analysis; Standard Reference Material (SRM) 1649 Urban Dust/Organics. National Institute of Standards and Technology, Gaithersburg, MD.

Pautler, B.G., Dubnick, A., Sharp, M.J., Simpson, A.J., Simpson, M.J., 2013. Comparison of cryoconite organic matter composition from Arctic and Antarctic glaciers at the molecular-level. Geochimica et Cosmochimica Acta 104, 1–18.

Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker Guide. Cambridge University Press, Cambridge.

Rudnick, R.L., Gao, S., 2003. The composition of the continental crust. In: Treatise on Geochemistry, vol. 3. Elsevier, Oxford, pp. 1–64.

Slater, G.F., Nelson, R.K., Kile, B.M., Reddy, C.M., 2006. Intrinsic bacterial biodegradation of petroleum contamination demonstrated in situ using natural abundance, molecular-level ^{14}C analysis. Organic Geochemistry 37, 981–989.

Smith, H.J., Foster, R.A., McKnight, D.M., Lisle, J.T., Littmann, S., Kuypers, M.M.M., Foreman, C.M., 2017. Microbial formation of labile organic carbon in Antarctic glacial environments. Nature Geoscience 10, 356–359.

Stibal, M., Sabacka, M., Zarsky, J., 2012. Biological processes on glacier and ice sheet surfaces. Nature Geoscience 5, 771–774.

Stubbins, A., Hood, E., Raymond, P.A., Aiken, G.R., Sleighter, R.L., Hernes, P.J., Butman, D., Hatcher, P.G., Striegl, R.G., Schuster, P., Abdulla, H.A.N., Vermilyea, A.W., Scott, D.T., Spencer, R.G.M., 2012. Anthropogenic aerosols as a source of ancient dissolved organic matter in glaciers. Nature Geoscience 5, 198–202.

Stuiver, M., Polach, H., 1977. Reporting of ^{14}C data. Radiocarbon 19, 355–363.

Weidemeier, D.B., Brodowski, S., Wiesenberg, G.L.B., 2015. Pyrogenic molecular markers: linking PAH with BPCA analysis. Chemosphere 119, 432–437.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51–62.

Wientjes, I.G.M., van de Wal, R.S.W., Reichart, G.-J., Sluijs, A., Oerlemans, J., 2011. Dust from the dark region in the western ablation zone of the Greenland ice sheet. The Cryosphere 5, 589–601.

Xu, Y., Simpson, A.J., Eyles, N., Simpson, M.J., 2010. Sources and molecular composition of cryoconite organic matter from the Athabasca Glacier, Canadian Rocky Mountains. Organic Geochemistry 41, 177–186.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111–129.

Ziolkowski, L.A., Wierzchos, J., Davila, A.F., Slater, G.F., 2013a. Radiocarbon evidence of active endolithic microbial communities in the hyperarid core of the Atacama Desert. Astrobiology 13, 607–616.

Ziolkowski, L.A., Myktczuk, N., Omelon, C.R., Whyte, L., Slater, G.F., 2013b. Isotopic and geochemical evidence of microbial activity of Arctic gypsum endoliths. Biogeosciences 10, 7661–7675.