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Carbon Sources to Microbes and Cryoconite on Alaskan Alpine Glacier Surfaces

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CARBON SOURCES TO MICROBES AND CRYOCONITE ON ALASKAN ALPINE GLACIER SURFACES

by

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ABSTRACT

Cryoconite are depressions in the ice surface filled with diverse microorganisms and dark debris, which are reducing albedo and increasing glacier melt. In order to understand cryoconite carbon composition and carbon sources to microorganisms living on glacier surfaces, bulk organic carbon and microbial lipids from supraglacial cryoconite sediment within the ablation zones of Spencer, Matanuska, and Mendenhall glaciers in southern Alaska have been coupled with radiocarbon (^{14}C) analyses. The microbial lipids analyzed in these studies, phospholipid fatty acids (PLFA), are components of microbial membranes, quickly degrade after cell death, and give a snapshot of the viable microbial community. PLFA structure distributions indicated autotrophic and heterotrophic microorganisms in the supraglacial environment, in an abundance similar to surrounding Alaskan soils. The ^{14}C content of PLFA indicated that microbes were incorporating carbon that was recently in equilibrium with the atmosphere, which suggests autotrophic predominance or the use of modern carbon by heterotrophs. The ^{14}C content of bulk cryoconite organic carbon on Matanuska and Spencer glaciers was depleted relative to the modern atmosphere, where it was modern and in some cases enriched on Mendenhall glacier. This difference was hypothesized to originate from surrounding geology. These results reveal two distinct carbon pools: a highly abundant microbial community, which uses young carbon as a carbon source, living within and minimally interacting with a larger, sometimes old carbon pool. Ultimately, this study highlights the carbon isotopic heterogeneity of cryoconite material.

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CHAPTER 1

INTRODUCTION

Alpine glaciers currently represent less than one percent of global glacier ice volume, yet their rapid mass loss accounts for nearly one third of the current observed sea level rise (Mengel et al., 2016). While continental glaciers contain most of the world's ice and cover landmasses such as Greenland and Antarctica, their contribution to global sea level rise is currently small, whereas alpine glaciers are found only on mountains at high elevations and are significantly contributing to sea level rise today (Gardner et al., 2013; Mengel et al 2016). On some regions of a glacier, the rate of ice loss exceeds the rate of accumulation. The ablation zone of a glacier is the total area between the terminal end, or toe, and the snowline, where glacier melt outpaces glacier growth (Benn and Evans, 2010). Ablation zone surfaces of both alpine and continental glaciers around the world are populated with dark material. This dark material promotes melting of the ice locally, creating depressions in the ice surface filled with water and dark material, referred to as cryoconite. Overall, this dark cryoconite material is reducing glacier albedo, or reflectivity, and increasing glacier melt within the ablation zone (Dumont et al., 2014; Benning et al., 2014). Cryoconite only populate the bare ice of the glacier ablation zone and as it expected for glacier ablation zones to grow at higher elevations in a warming climate, it is likely that cryoconite abundance will increase as well.

The overall paradigm of the makeup of cryoconite is a combination of dust, soot, and biology—rock dust blown from surrounding areas, soot from the combustion of fossil fuels and forest fires, and microorganisms present in high abundance—however, the ultimate composition of these supraglacial impurities, including when the material was deposited, remains unknown. To date, there is no direct evidence on the relative proportion of dust, soot and biology in cryoconite carbon. Some studies point to indirect evidence of soot being an important component. Ice cores from Greenland have been found to contain combustion derived material (Kehrwald, 2012), from the burning of biomass. Dissolved organic carbon (DOC) within export of glacial watersheds around the Gulf of Alaska has been found to be thousands of years old (Hood et al., 2009; Stubbins et al., 2012; Fellman et al., 2015), yet the export has also been found to be microbial in nature (Stubbins et al., 2012). Many theories have been put forward about the source of this old DOC, which include old microbial sources or the deposition of combusted fossil carbon on glacier surfaces (Hood et al., 2009; Stubbins et al., 2012), however, DOC export is a reflection of both supra- and sub-glacial processes, and therefore does not directly relate to the age of the organic carbon within cryoconite on the surface of glacier ablation zones. Therefore, this work aims to understand the age and sources of organic carbon within cryoconite on glacier surfaces and not within glacier export.

Carbon can accumulate on a glacier through either external inputs or in situ biological production, however at this time it is not known which process dominates (Hotaling et al., 2017). Aerosols can be an important mechanism to transport carbon from long distance locations. The deposition of aerosol-delivered carbon could happen at the time of snow deposition or at any point later and can originate from several sources,

including the ocean, the forests, surrounding geology, or combusted carbon from burning biomass or fossil fuels. Other possible external carbon sources to glacier surfaces could be from local and surrounding vegetation, soil, or rocks. Another mechanism for carbon accumulation on glaciers is through biological production, such as microbes, which photosynthesize carbon. It is suspected that microbial carbon is a major component of cryoconite on ice sheets such as Greenland and Antarctica (Smith et al., 2017; Musilova et al., 2017), but less so on alpine glaciers, due to other potential carbon inputs (Xu et al., 2009).

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., 2009). The microorganisms present within supraglacial material represent both heterotrophic and autotrophic communities, with autotrophy suspected to dominate (Anesio et al., 2009; Stibal et al., 2012a). It has also been determined that microbes themselves can decrease glacier albedo (Benning et al., 2014; Musilova et al. 2016). While molecular approaches to characterizing the microbes can provide powerful information on 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 glacier in the first place. Additionally, microorganisms are only a component of the overall organic carbon pool within alpine glacier cryoconite and additional lines of evidence are needed in order to determine other sources of carbon to alpine glacier surfaces.

One way to identify the relative contribution of organic carbon from different carbon sources is by determining the age, or radiocarbon (^{14}C) content, of the cryoconite

organic carbon. $\Delta^{14}C$ ranges from -1000‰ for radiocarbon-free organic carbon to ~+20‰ for the modern atmosphere (Levin et al., 2013), with $\Delta^{14}C$ greater than +20‰ indicating bomb carbon that was introduced into the atmosphere due to nuclear weapons testing in the 1950s. 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. Further, if the cryoconite contains organic carbon enriched relative to the modern atmosphere, there would be bomb carbon influence and could therefore indicate the carbon cycled on decadal time scales (Sharpenseel et al., 1989). For this environment, possible carbon sources could be soot from the combustion of fossil fuels or organic carbon that was deposited long ago and is being exposed due to glacier melt, or a combination of the two types of old carbon. Modern carbon sources could include soot from forest fires, modern photosynthesizing microorganisms, and terrestrial plant material. The radiocarbon content of bulk cryoconite organic carbon represents the radiocarbon content of all organic carbon components to the bulk and if there are inputs of both modern and fossil inputs, the radiocarbon content will reflect the mixing of these isotopically distinct end members. Therefore, additional evidence beyond bulk radiocarbon measurements of organic carbon is needed in order to determine specific carbon sources to the cryoconite.

Biomarker analyses can also help to determine carbon sources to cryoconite material. Biomarkers are chemical fingerprints of specific biological processes and through analysis can indicate specific carbon sources to cryoconite organic carbon. Alkanes, which are components of leaf waxes on plants, are straight-chained

hydrocarbons, with variable amounts of carbon atoms depending on carbon source (Eglinton and Hamilton, 1967; Nott et al., 2000; Volkman et al., 1998). Terrestrial material from higher land plants typically produce alkanes that have longer chain lengths than aquatic microbes (Eglington and Hamilton, 1967). Therefore, by looking at the relative contribution of alkanes of different chain lengths, it can reveal info about the alkane carbon source. Additionally, the relative concentration of odd chain lengths to even chain lengths can indicate the state of alkane degradation. Fresh plant material typically biosynthesizes alkanes with odd chain lengths (Bush and McInerney, 2013), which are degraded over time to alkanes with a near equal number of even and odd chain lengths (Bray and Evans, 1961). For example, alkane biomarker analyses have been completed on Athabasca Glacier in Canada and were found to represent carbon inputs of mossy and vascular plant origin (Xu et al., 2010) with a preference of odd chain lengths, indicating fresh carbon sources to the system. In this study, alkane biomarkers are used to show the relative contribution of terrestrial, aquatic, and microbial carbon to the bulk cryoconite material.

A second set of biomarkers analyzed in this study are phospholipid fatty acids (PLFA). These microbial lipids are components of microbial membranes, quickly hydrolyze after cell death, and are therefore biomarkers of the viable microbial community (White et al., 1979). An alternative approach to determining what role microbes play in the source(s) and cycling of carbon in the supraglacial environment, besides genetic identification, is to measure the radiocarbon content, or age, of the microbes themselves. In glacial environments, this approach has been used to determine that heterotrophic microbes in glacial outflow streams are fixing ^{14}C depleted carbon

(Fellman et al., 2015). However, to date there have been no studies assessing the direct age of carbon sources to microbes living on glacier surfaces or carbon sources specific to alpine glacier environments.

In this study, we coupled the microbial lipids, PLFA, with ^{14}C to determine what carbon microbes in cryoconite were using. This study provides, for the first time, compound-specific radiocarbon (^{14}C) measurements on extracted microbial lipids from cryoconite, in order to directly determine the age of carbon used by microbes on glacier surfaces. 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 my microbes in rocks in the Atacama Desert (Ziolkowski et al., 2013).

In these studies, we couple compound-specific radiocarbon measurements of microbial lipids with bulk organic carbon ^{14}C and biomarker analyses on Matanuska, Spencer, and Mendenhall Glaciers in southern Alaska, in order to investigate the variability of carbon usage by microbes and organic carbon sources to cryoconite between glacier locations.

CHAPTER 2

METHODS AND MATERIALS

Study sties

Supraglacial cryoconite sediment samples were collected in August of 2015 from Matanuska (n=6) and Spencer (n=15) glaciers in southern Alaska (Figure 2.1, 2.2). Spencer glacier is ~50 km south of Anchorage and only accessible by foot or train. The surface of Spencer 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 Spencer and Matanuska were found in the Chugach Mountains in southern Alaska, which are made up of sedimentary rocks, which include shale. Overall, the cryoconite samples collected consisted of a fine gray sediment with the exception of one sample, 'MAT orange', which was unique in that the material in the cryoconite was predominately an orange biofilm like material.

Cryoconite sediment was collected along the length of the ablation zone in July of 2016 from Mendenhall glacier (n=19) in southeast Alaska (Figure 2.1, 2.3). Mendenhall glacier is located in southeast Alaska, within the city limits of the capital city of Juneau. The surface of Mendenhall was highly weathered, with both traditional cryoconite holes and sediment accumulation in cracks, as well as piles of supraglacial debris, all of which

were sampled. Samples on Mendenhall glacier were collected along a ~9km transect, starting at the toe of the glacier and ending at the snowline at the top of the glacier, moving up parallel to the medial moraine. While 19 samples were collected along the length of the ablation zone, four samples were analyzed in detail with the remaining samples being archived for future research.

Solid impurities were collected by scooping dark material out of cryoconite holes 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 hours prior to use.

Bulk organic carbon and nitrogen measurements

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

Alkane extraction

Alkane, or plant leaf wax, analysis was performed to determine the relative contribution of aquatic to terrestrial leaf waxes in the cyroconite material. Around 2 grams of freeze dried cryoconite sediment was sonicated in 50mL of a 9:1 DCM:MeOH solution for 30 minutes and filtered three separate times, using fresh 50mL 9:1

DCM:MeOH each time. The samples were subsequently blown down to a small volume under a stream of high purity (UHP) N_2 and placed on 4g of activated silica gel in a glass chromatography column. The extract was then chromatographically separated into polarity fractions using 40mL each of hexane and DCM. Alkanes were contained in the hexane fraction, were blown down to 1mL before analysis via GC/MS.

Alkanes were separated and quantified using an Agilent 7890B/5977A GC/MS, with an HP-5MS column, He as a carrier gas, and a temperature program that began at 100°C, ramped up 8°C/min to 300°C, then held isothermal for 23 min. Scanning ion monitoring (SIM), detecting ion m/z of 57, was used for the identification of n-alkanes. Quantification was completed using external standards (n-alkane standards C_{20}, C_{24}, C_{26}, and C_{30}). Laboratory blanks were analyzed with each sample set to assess contamination.

PLFA extraction

To determine the amount and type of microbial life present, PLFA were extracted from the supraglacial material. Using a modified Bligh and Dyer (1957) method, the PLFA were purified from the total solvent extractable lipids. Briefly, lipids from about 3 grams of freeze dried supraglacial material were sonicated in duplicate for two minutes and extracted overnight in a 1:1:0.8 DCM:MeOH:phosphate buffer solution and then were 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 drained, 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 grams of activated silica gel in a glass column and then chromatographically separated into polarity fractions with 40mL each of DCM, acetone, and MeOH. The MeOH fraction, which contained the PLFA, was blown down to dryness under UHP N_2 and mild alkaline methanolysis was performed to transesterify the PLFA into fatty acid methyl esters (FAME) (Guckert et al., 1985). Free fatty acids are not esterified under base catalyzed transesterification conditions. The FAME solution was then chromatographically separated into polarity fractions with 4mL each of 4:1 hexane:DCM, DCM, and MeOH. FAME were present in the DCM fraction, which was blown down to 1mL before analysis via GC/MS.

An Agilent 7890B/5977A GC/MS was used for separation, identification, and quantification of FAME. FAME were separated using 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). Identification of FAME 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 PLFA extracted was converted to a cell abundance using the conservative conversion $2x10^4$ cells/pmol PLFA (Green and Scow, 2000). Nomenclature for PLFA 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. The prefix cy- indicates a cyclic fatty acid structure and i- and a- indicate iso-branched and anteiso-branched PLFA. Limit of quantification was 1mg/L.

Carbon isotope measurements

The radiocarbon (^{14}C) content of bulk cryoconite material, extracted PLFA, and the total lipid extract (TLE) was measured in order to determine the age of cryoconite and its solvent extractable components. Bulk cryoconite sediment was treated with 5% HCl at 80°C for 1 hour to move inorganic carbon, then it was washed with deionized water and dried at 60°C. Dried bulk cryoconite was then placed in pre-combusted quartz tubes containing cupric oxide and silver wire. A quantitative portion of previously extracted TLE and total PLFA, separately, were 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 (IRMS) 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 PLFA to FAME cleaves the polar head group of the molecule and adds a methyl group, adding an additional carbon atom, which 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:

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

Uncertainties for $\Delta^{14}C$ were ±5‰ for bulk cryoconite organic carbon, ±10‰ for the total lipid extract, and ±20‰ for PLFA.

Statistical analyses of PLFA

A standard *t-test* in Microsoft Excel© was run in order to determine if the amount of PLFA was significantly different between glaciers. The test was run between two sample sets, assuming unequal variances. Additionally, cluster analysis, a descriptive statistical technique, was used to assess differences between the relative distributions of PLFA 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 PLFA 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.

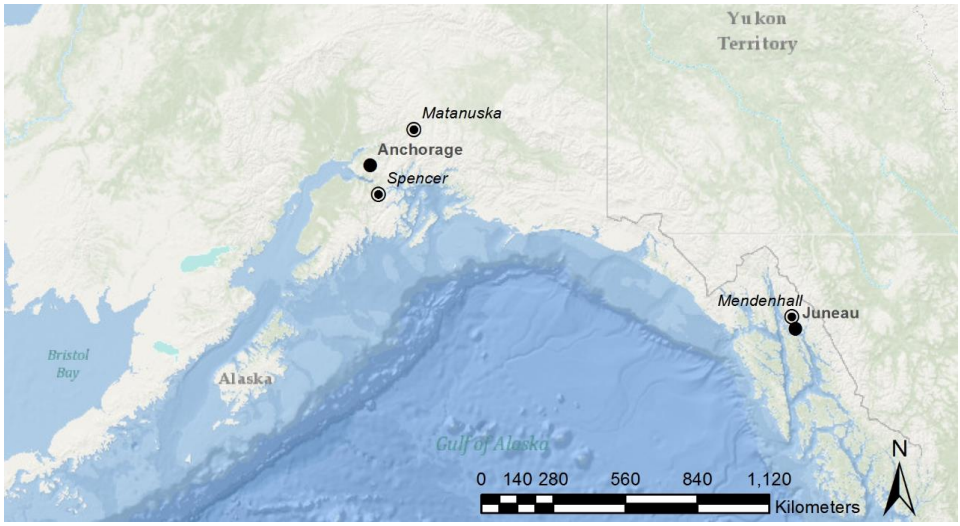

Figure 2.1: Map of glacier locations in southern Alaska

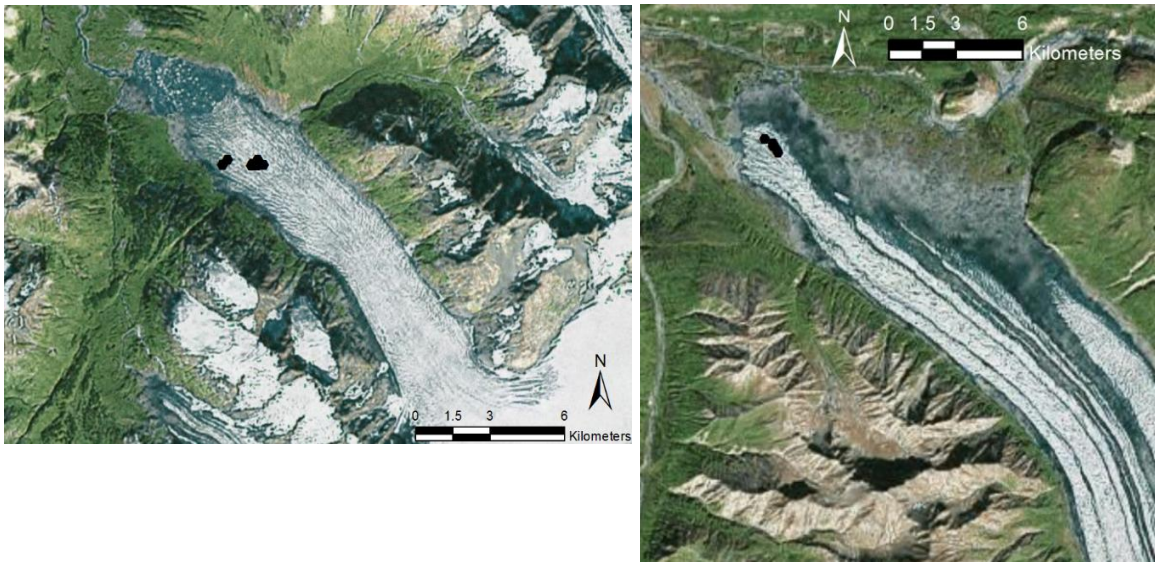

Figure 2.2: Maps of Spencer (left) and Matanuska (right) sample locations. Samples from Spencer and Matanuska were both collected from the toe of each glacier, without a great deal of special coverage. Therefore, the sample sites are not labeled.

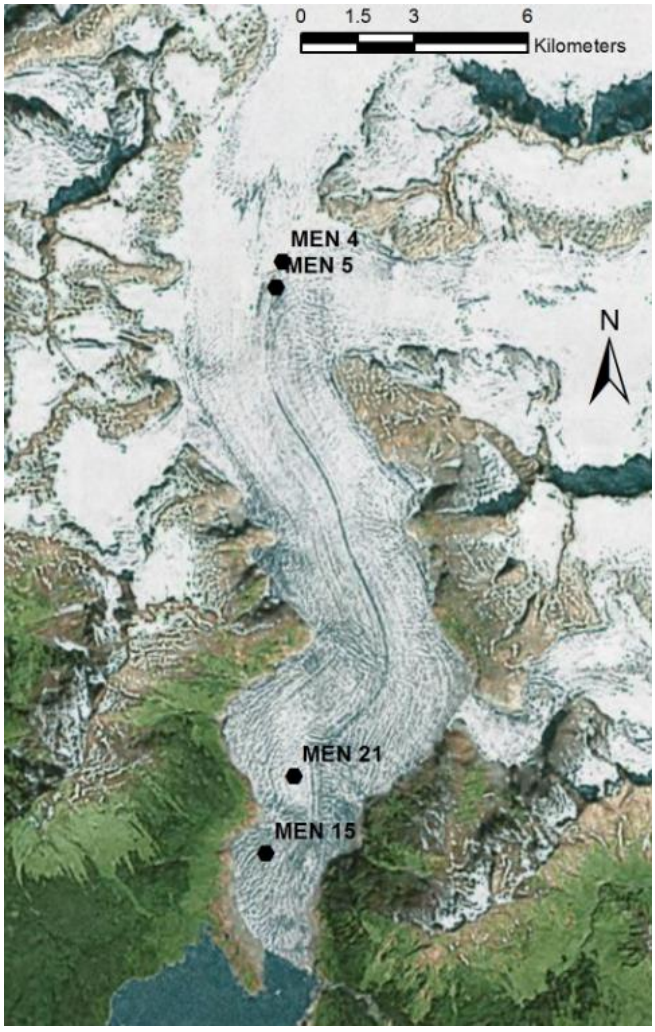

Figure 2.3: Map of Mendenhall glacier sample locations. At the time of sampling (July 2016), the sample MEN 15 was at the terminus (toe) of Mendenhall glacier, indicating greater glacial retreat than what is shown in this satellite image.

CHAPTER 3

STUDY 1: MATANUSKA AND SPENCER GLACIERS

3.1 Overview

The first study of this work seeks to determine if there is variation in cryoconite carbon composition and carbon source(s) to microorganisms within cryoconite between two glaciers in relatively close proximity, with similar geology and climate. Spencer and Matanuska glaciers are located in the Chugach Mountains in southern Alaska, about 138km from one another. In relation to Anchorage, the largest city in Alaska, Matanuska glacier is located almost due east, where Spencer is located due south.

Matanuska and Spencer glaciers lie in a transitional climatic zone in southern Alaska, which is characterized by a mixture of continental and maritime climates in different parts of the year. Typically, continental climates are described by low precipitation and large temperature changes between summer and winter, where maritime climates have high precipitation and low temperature variability between seasons. The region that both of these glaciers lie typically have four distinct seasons and precipitation levels between that of continental and maritime climates. Thus, this study seeks to determine the variation of cryoconite composition and microbial carbon usage between two glaciers in a similar climate.

When the glaciers were sampled in August of 2015, Spencer glacier was more weathered than Matanuska glacier. The surface of both glaciers was covered in

supraglacial debris, but the surface of Spencer was more weathered and had fewer typical cryoconite than Matanuska. Conversely, Matanuska had a clean ice surface, populated with cryoconite and supraglacial sediment in cracks and other formations. Sampling was confined to the terminal end, or toe of each glacier, with a maximum transect up each glacier of about 2 km, parallel to the medial moraine. In order to determine if there was variability between cryoconite on the termini of Matanuska and Spencer glaciers, the amount, elemental and isotopic composition of bulk organic carbon, as well as the extractable and non-extractable organic carbon components were analyzed. In addition, biomarker analyses were completed in order to determine carbon sources to the supraglacial environment.

3.2 Results

Bulk organic carbon properties

Overall, the bulk organic carbon content of supraglacial cryoconite material on 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 3.1). Since the carbon content was similar and within the standard deviation of the six samples from each of the two glaciers, 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 3.1). The nitrogen content of cryoconite was 0.06 ± 0.01% for Matanuska (n=2, Table 3.1) and 0.09 ± 0.03% for Spencer (n=3, Table 3.1). C/N in cryoconite on

Matanuska (n=2) was 11.7 ± 1.5, on Spencer (n=3) was 11.2 ± 2.2 and when combined (n=5) was 11.41 ± 1.74 (Table 3.1).

Solvent extractable carbon and composition

The solvent extractable carbon was present in the total lipid extract, or TLE. The TLE 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 total organic carbon for MAT 6 and Spencer 15, respectively).

Alkanes were extracted in order to determine the relative contribution of aquatic, microbial, and terrestrial leaf waxes in Spencer and Matanuska cryoconite. 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 (Figure 3.1). Spencer had a shorter average chain length (C_{23}) than that of Matanuska alkanes (C_{27}). Additionally, when the relative abundance of odd to even chain lengths were examined, both glaciers had a moderate odd to even (O/E) carbon preference. (O/E of 5 and 3, respectively).

The concentration of PLFA was 75.6 ± 48.9 µg/g sample for Matanuska glacier (n=6) and 127.2 ± 90.5 µg/g sample for Spencer glacier (n=6). When duplicate samples were compared over both glaciers, the total amount of PLFA was 94.9 ± 66.6 µg/g sample (n = 23, Table 3.1). Although the p value of a t-test determined that the amount of microbes on each glacier were statistically similar (p = 0.059), it appears there is a higher abundance of PLFA on Spencer glacier. When the PLFA concentrations were converted to cellular abundance, the abundance of microbial cells was $6.8 \times 10^9 \pm 4.8 \times 10^9$ cells/g sample.

The distribution of PLFA structures can indicate, broadly, the type of microorganisms present in the cryoconite environment (Table A.1). The most abundant structures in these samples were $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $brC_{16:0}$ (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 (Figure A.1) 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, but the relative abundance of the structures $C_{15:0}$, $brC_{14:0}$, $C_{16:1}$, and $C_{18:1}$ were higher in MAT 1 and Spencer 15 than in the rest of the samples. It should be noted that MAT 1 and Spencer 15 were samples closest to the terminus.

Isotopic composition of cryoconite carbon

The stable carbon (^{13}C) content of bulk organic carbon for Matanuska (n=6) was -26.2 ± 0.9‰ and for Spencer (n=6) was -26.6 ± 1.0‰. For all supraglacial samples on Matanuska and Spencer, the ^{13}C content of bulk organic carbon was -26.3 ± 0.9‰ (n=12, Table 3.2). The bulk organic carbon radiocarbon values for Matanuska ($\Delta^{14}C$ = -335 ± 189‰, n=6) and Spencer ($\Delta^{14}C$ = -277 ± 282‰, n=6) glaciers varied greatly. Since the variability of the $\Delta^{14}C$ data was so high and yet similar between both glaciers, we grouped the data from the two glaciers to yield an average bulk organic carbon radiocarbon value of -306 ± 231‰, (n=12), which corresponded to a bulk age of 3633 ± 4034 ^{14}C years old (Table 3.2).

The radiocarbon (^{14}C) content of extracted PLFA 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 3.2). MAT 2 and Spencer 5.1 were more depleted in PLFA ^{14}C relative to the rest of the samples (-233 ± 20‰ and -65 ± 20‰, respectively), while most of the PLFA contained carbon that was recently in equilibrium with the atmosphere. Comparing the ^{14}C content of PLFA to the ^{14}C content of the cryoconite bulk organic carbon from which the PLFA were extracted (Figure 3.2) showed that PLFA were significantly more modern than bulk organic carbon ($\Delta\Delta^{14}C_{PLFA-bulk}$ = 288 ± 128‰). Even though the PLFA ^{14}C of MAT 2 and Spencer 5.1 were far more depleted than the other PLFA ^{14}C samples, the $\Delta\Delta^{14}C_{PLFA-bulk}$ for these two samples were similar to the other samples ($\Delta\Delta^{14}C_{PLFA-bulk}$ = 377‰ ± 15 and 71‰ ± 15 for MAT 2 and Spencer 5.1, respectively).

Different compounds are present within the solvent extractable and non-solvent extractable organic carbon components of the bulk cryoconite organic carbon. In order to determine if the solvent extractable or non-extractable carbon was driving the old age of the bulk carbon, we investigated the radiocarbon content of the total extractable lipids. Radiocarbon content of the solvent extracted carbon (TLE) was -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 3.2). For Mat 6, the $\Delta^{14}C$ of the TLE was within the analytical uncertainty of the microbial lipids ($\Delta^{14}C$ PLFA: MAT 6 = 34 ± 20 ‰). Unfortunately, due to sample size limitations for ^{14}C analysis, no PLFA ^{14}C was determined for Spencer 15. Modern PLFA are only a small portion of the 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 (Equations 2 and 3), it was possible to determine the age of the organic carbon present in cryoconite that was not able to be solvent extracted (extracted residue).

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

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

Where the mass (M) of the bulk is composed of the mass fractions (M) of the extractable and non-extractable carbon. Using Equations 2 and 3, the $\Delta^{14}C$ of the non-extractable carbon was found to be -334 ± 15‰ and -848 ± 15‰ for MAT 6 and Spencer 15, respectively (Table 3.2). As expected, since the modern lipid extract is only a small fraction of the total organic carbon in the samples, the non-extractable carbon is driving the old age of the bulk supraglacial material.

3.3 Discussion

Supraglacial microbes are primarily eating young carbon

The microbial lipids quantified in this study (PLFA) contained predominately carbon that was recently in equilibrium with the atmosphere. Using the concept of 'you are what you eat', the radiocarbon content of microbial lipids can indicate the radiocarbon content of the carbon source to the microorganism from which the microbial

lipids were extracted (Boschker et al., 2002). Since PLFA degrade quickly after cell death, PLFA represent only the viable, living microbial community (White et al., 1979). The modern radiocarbon content of the microbial lipids on Spencer and Matanuska glaciers indicated the use of modern carbon as a carbon source to the microbes in the supraglacial environment. 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). Comparing the ^{14}C content of the microbial lipids to the ^{14}C content of the bulk cryoconite organic carbon from which the microbial lipids were extracted (Figure 3.2), it was apparent that microbial lipids were significantly more modern than bulk organic carbon ($\Delta\Delta^{14}C_{PLFA\text{-}bulk}$ = 288 ± 128‰) for all samples, which translates into microbes containing carbon that is thousands of years younger than the carbon they are living in. This difference in radiocarbon between bulk organic carbon and microbial lipids, regardless of sample location, illustrated that supraglacial microbes were using predominately young carbon instead of aged cryoconite organic carbon. The preferential use of young carbon over cryoconite carbon suggested that the bulk cryoconite organic carbon is not bioavailable as a carbon source to the microbes within cryoconite.

There were two microbial lipid samples, which were more depleted in ^{14}C relative to the other samples analyzed. These depleted PLFA ^{14}C values indicate that the microbes in these samples contain older carbon. This older carbon could be because the old bulk cryoconite carbon is more accessible to the heterotrophs in these samples. However, the bulk cryoconite organic carbon is still much older relative to the microbes in these

samples, suggesting that microbes did not solely use old cryoconite organic carbon and must also be using modern carbon sources.

Previous work has suggested that microbial communities on glaciers are either primarily autotrophic (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., 2012a). Autotrophic microorganisms use inorganic atmospheric CO_2 as a carbon source, where heterotrophic microorganisms use organic carbon present in their surroundings, which can be organic carbon that autotrophs fix into the cryoconite environment. In order to determine the types of microorganisms present, the distribution of microbial lipid compounds were analyzed. Different microorganisms make different microbial lipid structures, and thus identifying the different lipids within a sample can identify, broadly, the type of microorganism(s) present. Some microbial lipid compounds are characteristic to autotrophic organisms, other compounds are characteristic to heterotrophic organisms, while some compounds are ubiquitous to all microorganisms (Table A.1). In these cryoconite samples, the distributions of microbial lipid (PLFA) (Table A.2 and A.3) structures observed indicated, broadly, the presence of a mixture of autotrophic and heterotrophic metabolisms within the supraglacial environment. The PLFA structures observed in these samples are typical of microbial communities that are composed of cyanobacteria (autotrophs), gram positive and gram negative bacteria (heterotrophs), and algae (autotrophs) (Zelles et al., 1999; Dijkman et al., 2009; Green and Scow, 2000; Boschker et al., 2005). A cluster analysis was run on the distribution of microbial lipids, which determined that there were no systematic differences of microbial communities between sample locations on either glacier. Similar microbial lipid

distributions, and therefore the same attributed microbial communities, as this work were found in cryoconite samples from Greenland, Canada, and Antarctica (Pautler et al., 2013 and Xu et al., 2010). 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., 2012a). The abundance of microbial cells, estimated from the concentration of microbial lipids, was similar to what has been found in tundra soils (Kobabe et al., 2004). Although the amount of PLFA within a microbial membrane can vary depending on factors such as temperature and stress and therefore may not be directly comparable between different environments, this broadly indicated that microbes on Matanuska and Spencer glaciers were present in a high abundance (Frostegard et al., 2011). Glaciers that were previously considered microbially inactive are now shown to be capable of supporting a high abundance of both autotropic and heterotrophic microbial communities.

The overall modern radiocarbon content of the microbial lipids suggested modern carbon usage by both autotrophs and heterotrophs within cryoconite. Since the data presented here are the ^{14}C values of all the microbial lipids in the samples, and not lipids specific to autotrophic and heterotrophic organisms, this data represents the community as a whole. If we had measured the ^{14}C content of autotrophic and heterotrophic groups of compounds rather than all of the microbial lipids as a whole, our data may be able to distinguish more specifically what carbon heterotrophs versus autotrophs were consuming. However, due to sample size limitations and the high degree of uncertainty associated with ^{14}C of specific microbial lipid compounds, we only determined the radiocarbon content of all microbial lipids together. Additionally, the most abundant

PLFA ($C_{16:0}$), which was between 10% and 25% of the total PLFA in all samples, is produced by both autotrophic and heterotrophic organisms. Therefore $C_{16:0}$ specific ^{14}C would be unhelpful for separating autotrophic and heterotrophic carbon usage.

Modern carbon usage by microbes on the surface of Spencer and Matanuska glaciers suggested two ideas: 1) that autotrophy dominates in the supraglacial environment, or 2) that heterotrophy is as abundant as autotrophy, but heterotrophic microorganisms in cryoconite predominately use the modern carbon fixed by autotrophic microorganisms and not the old organic carbon in which they are living. These ^{14}C data indicated the presence of two distinct carbon pools on the surface of Spencer and Matanuska glaciers: a large microbial community, which is using modern carbon, living within and interacting minimally with a larger old carbon environment. The data presented here is consistent with a study that showed heterotrophic microbes on a glacier in Antarctica were using only the carbon fixed to them by autotrophic microorganisms (Smith et al., 2017). The use of previously fixed carbon by heterotrophs implied that autotrophic carbon is quickly recycled by heterotrophs on glacier surfaces and that other organic carbon within cryoconite may not be bioavailable to the microorganisms.

Cryoconite carbon on these glaciers is old, mineral bound organic matter

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 total lipid extract, or TLE. The amount of total solvent extractable carbon was low (<10%) for the two samples investigated in this manner, MAT 6 and Spencer 15. These two samples met the sample size requirements

needed to complete an additional TLE extraction and thus were chosen to investigate their TLE content. Additionally, the radiocarbon content of these two TLE samples was also far more modern in ^{14}C than the bulk organic carbon, which suggests that the solvent extractable organic carbon is composed of carbon that was recently in equilibrium with the atmosphere. Also, the alkanes extracted from these samples indicated the presence of fresh, terrestrial and microbial carbon entering, which is consistent with the TLE containing modern carbon. The alkane odd over even preference for both glaciers being much greater than one, indicates that alkane inputs were fresh and not highly degraded and therefore not aged. Based on the abundance, biomarker 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 the non-extractable carbon, or extracted residue, was greater than 90% of the total organic carbon within cryoconite and was driving the old age of the bulk cryoconite organic carbon.

Stable isotope ^{13}C and carbon to nitrogen ratios within the bulk organic carbon were also utilized to help pinpoint carbon sources to the supraglacial environment. The ^{13}C content of bulk cryoconite organic carbon on Matanuska and Spencer glaciers (-26.3 ± 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 et al., 2002; Peters et al., 2005), as well as fossil sources such as oil or carbon present in sedimentary rocks such as shale (Peters et al., 2005). Due to the limited dynamic range of ^{13}C, relative to ^{14}C, and the similarity in ^{13}C content from different end members, the precise determination of carbon sources from ^{13}C is restricted. We found that there was a strong relationship between the ^{13}C and radiocarbon (^{14}C) content of cryoconite organic carbon on

Matanuska and Spencer glaciers (r^2 = 0.76, Figure 3.3), which indicated that more modern ^{14}C bulk organic carbon was more depleted in ^{13}C. For example, the ^{13}C values observed on these glaciers is consistent with the input of fresh, and therefore modern, C_3 plant derived carbon. However, the input of fresh plant derived carbon does not explain the old age of the extracted residue, therefore the extracted residue carbon must be from another source other than fresh plant derived carbon.

On the other hand, carbon to nitrogen ratios could indicate microbial carbon and/or soil organic matter inputs to the supraglacial environment. C/N is the ratio of carbon to nitrogen in a system and can indicate carbon sources to the system. C/N in cryoconite on Matanuska and Spencer glaciers was 11.41 ± 1.74. C/N ratios between 5:1 and 15:1 suggest a microbial carbon source (Hedges and Oades, 1997), therefore the measured C/N indicated 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.

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 explored 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 because it is a sample of atmospheric particulate matter collected in a naval shipyard in

the Washington, D.C. area in 1976-1977. Radiocarbon analysis of black carbon in NIST 1649a was found to be primarily depleted in ^{14}C (Currie et al., 2002), which suggests that the material is from fossil fuel combustion. Additionally, the solvent extractable component of NIST 1649a had a ^{14}C content that was also depleted and significantly more depleted than the bulk organic carbon ($\Delta^{14}C_{bulk}$~ -400‰, NIST 1649a, $\Delta^{14}C_{TLE}$~ -700‰) (NIST SRM 1649a). 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 $\Delta^{14}C_{TLE}$ of the cryoconite material should have been much more depleted 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 old age of the non-extractable carbon is due to fossil fuel combustion. In addition, certain small polycyclic black carbon compounds have also been found to be solvent extractable into the lipid extract (Weidemeier et al., 2015). Since the ^{14}C content of the extracted cryoconite carbon was modern, it is unlikely that fossil fuel derived black carbon was a major part of the lipid extract and may not be present in these cryoconite samples.

If inputs from fossil fuels are unlikely to be driving the old age of the carbon, other sources are likely responsible for the aged carbon. One possible explanation could be that the non-extracted carbon that was modern carbon that was deposited to the glacier at some point in the past and 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. Further research should investigate if cryoconite carbon along a transect from to toe to the accumulation zone of the glacier have different ages.

Another explanation for the old age of the non-extractable carbon could be an aged geologic source. Some rock, such as shale, can contain organic carbon that is ^{14}C free ($\Delta^{14}C < -1000‰$), not readily solvent extractable and has a ^{13}C content typical of terrestrial organic matter. The inorganic material in cryoconite is typically of crustal origin (Wientjes et al, 2012) and therefore, it is likely that the local mountains are the primary source of material to the cryoconite. Since Spencer and Matanuska glaciers are situated in the Chugach Mountains, which are known to contain sedimentary rocks, it is possible that the old age of the cryoconite material could be from the crustal inputs from the local mountains. Only a small amount of ^{14}C free inputs are needed to drive the age of the non-extractable carbon much older. Therefore, we hypothesize that the geologic setting of these glaciers is driving the radiocarbon content of the cryoconite carbon.

Table 3.1: Cryoconite sample locations, %C, %N, C/N, PLFA concentrations from Matanuska (MAT) and Spencer glaciers collected in August 2015.

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

Table 3.2: Isotopic properties of bulk cryoconite sediment and extracted PLFA in per mil (‰) notation

Sample name	$\Delta^{14}C$ Bulk (±5‰)	^{14}C age BP	^{14}C age BP ±	$\delta^{13}C$ Bulk (±0.5‰)	$\Delta^{14}C$ PLFA (±20‰)	$\Delta\Delta^{14}C$ (‰)	$\Delta^{14}C$ TLE (±10‰)
MAT 2	-611	7580	30	-25.0	-234	377	--
MAT 3	-207	1860	25	-27.2	27	234	--
MAT Orange	-96	810	20	-26.3	--	--	--
MAT 4	-289	2740	25	-26.4	25	314	--
MAT 5	-500	5560	30	-25.2	22	522	--
MAT 6	-311	2990	30	-27.0	34	345	-30
Spencer 15	-839	14680	40	-24.8	--	--	-162
Spencer 14	-92	770	25	-27.5	--	--	--
Spencer 10	-175	1540	25	-26.3	47	222	--
Spencer 5.1	-136	1170	25	-27.3	-65	71	--
Spencer 8	-284	2680	30	-26.5	39	323	--
Spencer 9	-141	1220	25	-27.1	48	189	--

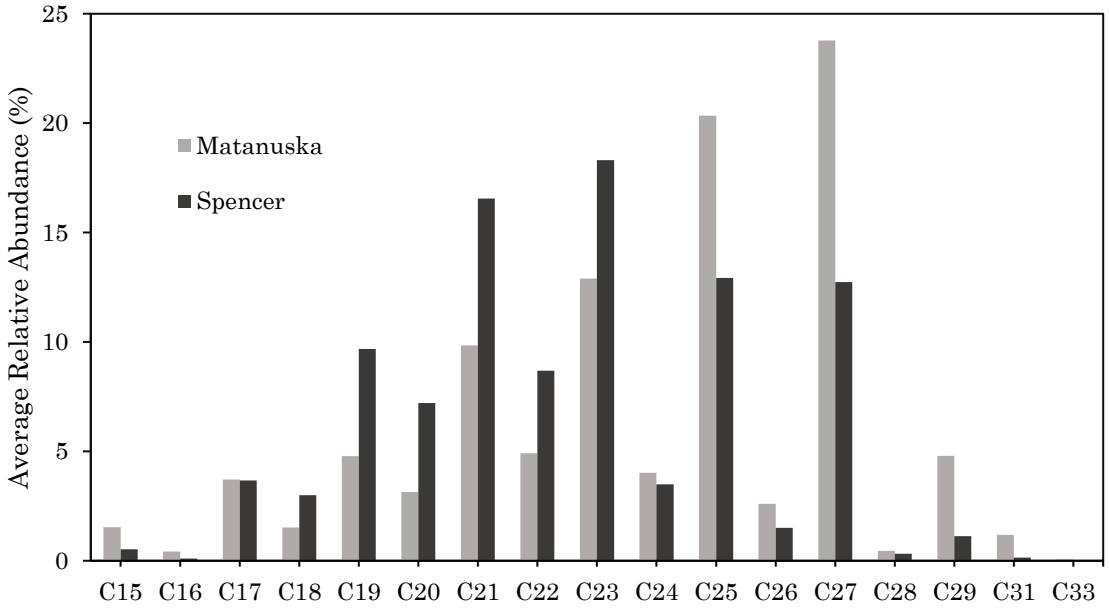

Figure 3.1: Distribution of alkane chain lengths on Matanuska and Spencer glaciers, represented as average relative abundance (%)

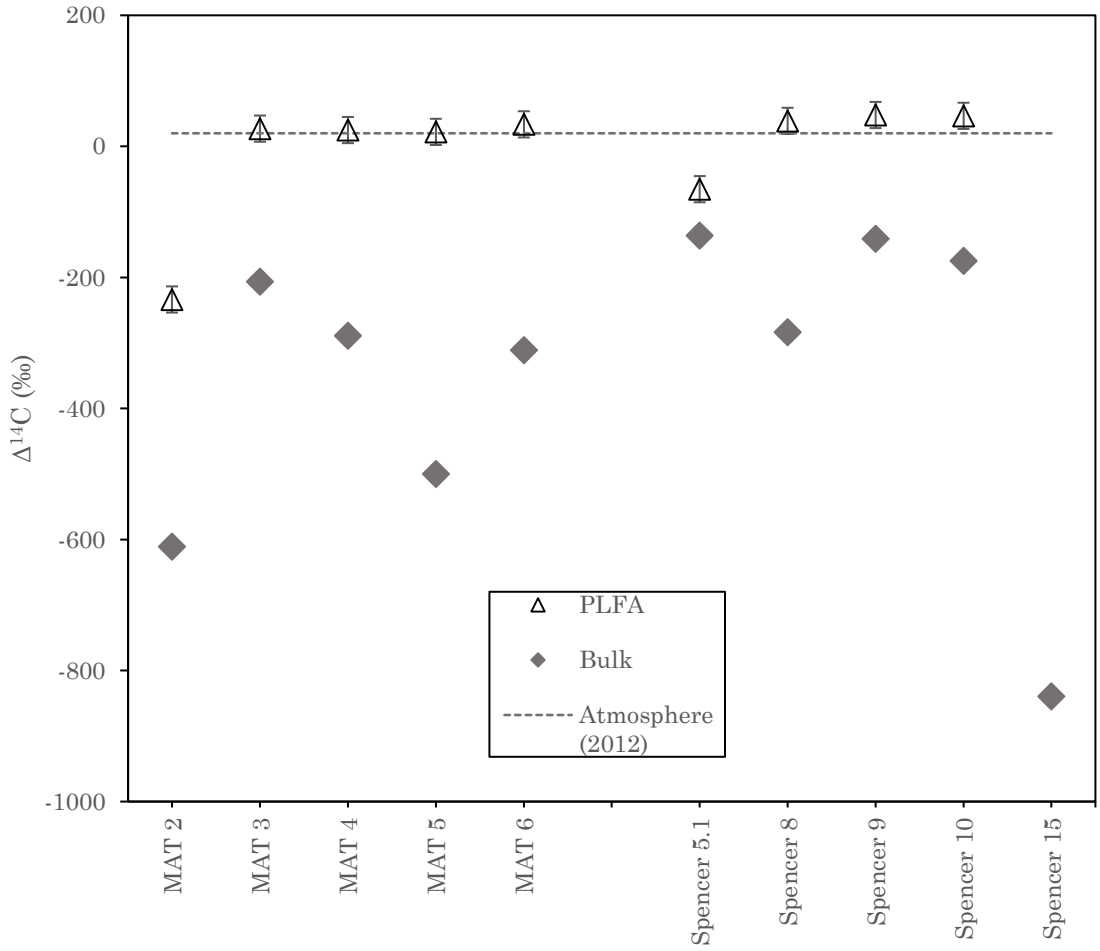

Figure 3.2. The radiocarbon (^{14}C) content of bulk cryoconite organic carbon and extracted phospholipid fatty acids (PLFA) in the per mil (‰) notation. The error associated is ±5‰ for $\Delta^{14}C_{bulk}$ and ±20‰ for $\Delta^{14}C_{PLFA}$. The error bars for bulk ^{14}C are smaller than the symbols. The ^{14}C content of the atmosphere (+20‰, dashed line) is included for reference (Levin et al., 2013). ^{14}C of radiocarbon-free organic carbon is -1000‰.

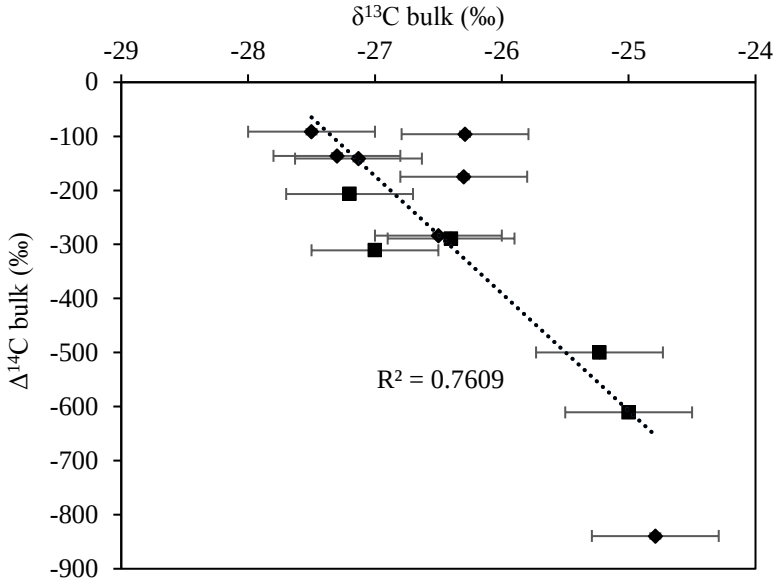

Figure 3.3: There is a strong relationship between the ^{13}C and ^{14}C content of Matanuska (squares) and Spencer (diamonds) bulk cryoconite organic carbon. Uncertainty for ^{14}C is ± 5‰ and uncertainty for ^{13}C is ± 0.5‰. Error bars for ^{14}C are smaller than the symbols.

CHAPTER 4

STUDY 2: MENDENHALL GLACIER

4.1 Overview

The focus of the second study was to determine the variation in carbon composition and microbial carbon usage on a single glacier. Study one surveyed the variability of carbon composition near the terminus or toe of two glaciers, but there is evidence that the composition of cryoconite material can change at increasing distances from the toe. For example, the amount of carbon on the K-transect of the Greenland Ice Sheet increases with increasing elevation up the glacier (Stibal et al., 2012b). Therefore, the goal of this study was to determine if the chemical composition and microbial community composition changed from the toe of the glacier to the snowline at the top of the ablation zone. To do so, samples on a transect of the ablation zone of Mendenhall Glacier were analyzed for radiocarbon (^{14}C) content of both extractable and non-extractable carbon, as well as biomarker analyses (alkanes, PLFA).

Mendenhall Glacier differs from Matanuska and Spencer glaciers from the first study in many ways. Mendenhall is located within city limits of Juneau, the capitol city of Alaska. Juneau has a very large tourism economy, which brings over a million visitors a year to the city (Alaska Department of Commerce), cruise ships to the harbor, and helicopters, sea planes, and dog-sledding to Mendenhall glacier. Overall, Mendenhall is the most "industrialized" or "urban" of the three glaciers, since both Spencer and

Matanuska do not receive the same flux of tourism, nor are they located in such a built up area. In addition, Mendenhall's location facing the Pacific Coast of North America facilitates the flow of air currents from Asia directly to the surface of the glacier (Jaffe et al., 1999; Yienger et al., 2000). As it has been theorized that combusted fossil carbon is deposited on glacier surfaces, Mendenhall Glacier is an ideal place to test this hypothesis, as there are many factors that would add combusted fossil carbon to the surface of the glacier.

Mendenhall glacier is located in an area characterized by a maritime climate, with a large amount of precipitation and few distinct seasons throughout the year. The amount of precipitation that Mendenhall glacier receives annually is much larger than that received by Spencer and Matanuska glaciers (Motyka et al., 2002).

4.2 Results

The amount of organic carbon within cryoconite on Mendenhall Glacier was 2.1 ± 0.6% (n=4 Table 4.1). There was no geospatial trend with the variation of total organic carbon in the cryoconite samples. The radiocarbon (^{14}C) content of bulk cryoconite organic carbon on Mendenhall was variable, yet always modern. The average ^{14}C content of Mendenhall bulk cryoconite organic carbon was 15 ± 22‰ (n=4, Table 4.1). There was a trend of increasingly modern carbon towards the snowline of the glacier. Stable carbon ^{13}C content of the bulk cryoconite organic carbon was -26.1 ± 0.7‰ (n=4, Table 4.1).

The amount of solvent extractable organic carbon within the total lipid extract (TLE) was 10.4 ± 3.9% (n=8, samples run in duplicate) of the total organic carbon, leaving about 88% of the organic carbon present as non-solvent extractable organic carbon. The average radiocarbon content (^{14}C) of the TLE was 69 ± 45‰ (n=4, Table 1), which is enriched relative to the modern atmosphere ($\Delta^{14}C_{atmosphere}$ = ~+20‰, Levin et al., 2013) and the bulk organic carbon. The enrichment of the samples compared to the modern atmosphere indicated the presence of bomb carbon from nuclear weapons testing in the 1950s. The radiocarbon content of the TLE became increasingly enriched further from the toe of the glacier, with MEN 4 an MEN 5 at the top of the glacier receiving the greatest bomb carbon influence.

The most abundant alkanes in the samples had a chain length of 27 carbons (C_{27}), which is consistent with freshly produced vascular plant material. There was also a major contribution of C_{23} alkane chain lengths, which is consistent with microbial or mossy inputs (Eglinton and Hamilton, 1967; Nott et al., 2000; Volkman et al., 1998). In all samples, the average chain length was 24.9 ± 0.8. The odd over even preference (O/E) was 2.9 ± 0.8.

Phospholipid fatty acids (PLFA) were also present in the TLE. The average PLFA concentration was 155.9 ± 109.3µg PLFA/g sample. When converted to cellular abundance, microorganisms were found to be present within cryoconite at a concentration of $1.1x10^{10} \pm 7.9x10^{9}$ cells/g sample, which are of a similar order of magnitude as Spencer and Matanuska glaciers (Chapter 3). The distribution of PLFA compounds is highlighted in Table A.4.

4.3 Discussion

Cryoconite carbon composition varies with location in the ablation zone

There is evidence that carbon composition within cryoconite can change with increasing elevation up a glacier ablation zone, with the amount of total organic carbon increasing up a peripheral glacier on Greenland (Stibal et al., 2012b). Therefore we examined a transect of the ablation zone of Mendenhall glacier in order to determine if there were any systematic changes of carbon composition. Overall, the total amount of organic carbon did not change systematically along the Mendenhall ablation zone, but the radiocarbon content of the organic carbon as well as the microbial complexity and abundance did vary along the transect.

The radiocarbon content of the organic carbon within cryoconite was measured in order to determine the carbon age composition on the surface of Mendenhall glacier. The radiocarbon (^{14}C) content of the bulk cryoconite organic carbon on Mendenhall was overall modern, with a trend of increasingly younger carbon with increasing distance from the toe of the glacier (Figure 4.1). For example, at the top of the glacier near the snowline, one sample, MEN 5, had organic matter that was ~20‰ enriched in ^{14}C relative to the 2012 atmosphere ($\Delta^{14}C_{MEN5} = +39 \pm 5‰$, vs $\Delta^{14}C_{atmosphere} \sim +20‰$, Levin et al., 2013), which indicated the presence of bomb carbon as a component of the total organic carbon at this location. Whereas, at the toe of the glacier, the organic carbon was significantly more depleted than the modern atmosphere ($\Delta\,^{14}C_{MEN15} = -19 \pm 5‰$). Even though the samples near the toe were depleted relative to today's atmosphere, the oldest bulk organic carbon measurement correspond to carbon that was fixed during the early 1900s. Therefore, the carbon in cryoconite on Mendenhall contains carbon fixed over the

past century, with the oldest carbon at the terminus and the youngest carbon near the snowline.

The modern radiocarbon content of cryoconite organic carbon on Mendenhall is unlike other alpine glacier radiocarbon data found within the literature. Although there have been no other direct radiocarbon measurements of cryoconite particulate organic carbon (POC) on this glacier, it has been found that dissolved organic carbon (DOC) exported from Mendenhall and glacial watersheds adjacent to Mendenhall was old (Hood et al., 2009; Fellman et al., 2015; Stubbins et al., 2012). DOC and POC are not directly comparable, but their relative radiocarbon content as it relates to glacial carbon cycling will be discussed in Chapter 5. One continental glacier study found modern POC within surface ice along the K transect of the Greenland Ice Sheet and was hypothesized to originate from microorganisms on the ice surface (Wientjes et al., 2012). The fact that the particulate organic carbon becomes younger higher in the ablation zone on Mendenhall glacier suggested that the physical process of exposure age could be controlling the composition of the carbon.

To further investigate the composition of the organic carbon, we measured the ^{14}C content of the total extractable lipids. The total lipid extract (TLE) constituted less than 15% of the total organic carbon present within Mendenhall cryoconite, but was found to be larger portion of the organic carbon than other supraglacial cryoconite (Chapter 3). The radiocarbon content of the extractable lipids was more enriched in ^{14}C than the bulk cryoconite organic carbon and was, on average, ~50‰ enriched relative to the modern atmosphere. While all of the extractable carbon samples were enriched in ^{14}C, the greatest enrichment was furthest from the toe of the glacier at the snowline. Samples

MEN 4 and MEN 5, near the snowline at the top of the ablation zone, were enriched in ^{14}C by about 85‰ compared to the 2012 atmosphere. This ^{14}C enrichment in nature indicates bomb carbon was a major component of the extractable carbon at these sample locations. There are two ways bomb carbon could have been introduced into the cryoconite environment: 1) The carbon was deposited in this location when bomb carbon was introduced in the 1950s and has stayed in the supraglacial environment until sampling, or 2) the carbon was kept in another reservoir before recently coming to the cryoconite. An example reservoir could be the uptake of bomb carbon by surrounding vegetation, which has subsequently become part of the cyroconite material, therefore adding ^{14}C-enriched organic carbon to cryoconite.

Since the radiocarbon content of both the TLE and bulk organic carbon showed a trend of more enriched ^{14}C with increasing distance from the toe of the glacier, these results can provide insight into carbon cycling in these systems. For example, samples near the toe were close to the modern day atmosphere, whereas samples from near the snow line contained carbon that was last in equilibrium with the atmosphere decades ago. This variation in ^{14}C through the ablation zone implies that carbon is more slowly cycled at the top of the ablation zone compared to the toe of the glacier. This difference in carbon cycling was attributed to seasonal exposure variations at different elevations on the glacier. During the winter, an ice lid forms over cryoconite and snow will accumulate on the glacier. Those samples higher on the glacier will be covered with snow for a greater portion of the year (Benn and Evans, 2010), and will receive less direct interaction with the atmosphere. The longer snow coverage in the higher portions of the

ablation zone could slow carbon cycling in these upper glacier cryoconite locations and allow carbon to reside for a longer period of time.

Microorganisms have been found to play an important role in the cycling of carbon in cryoconite, and therefore we examined how the microbial community changed along the transect on Mendenhall glacier. The extraction, quantification, and identification of microbial lipids (PLFA) allowed for the broad classification of microbial communities present within Mendenhall cryoconite. Different microorganisms make different microbial lipids and therefore examining the variation of microbial lipid structures across sample locations could indicate if microbial community composition broadly changed on Mendenhall glacier. There was a systematic change of microbial lipid structure relative distributions along the transect, with a shift from mostly saturated PLFA near the snowline to mostly monounsaturated PLFA at the toe (Figure 4.2). These findings suggested that the microbial community is becoming more complex with increased atmospheric exposure time (Zelles et al., 1999). Another study showed a higher relative contribution from saturated fatty acids in Sweden cryoconite and more monounsaturated fatty acids in Svalbard cryoconite, which was linked to a higher contribution of cyanobacteria on Svalbard than in Sweden cryoconite (Lutz et al., 2016). Overall, cryoconite microbial communities estimated in the past via PLFA as well as genetic identification have revealed diverse microbial communities on alpine glacier surfaces (Xu et al., 2010; Pautler et al., 2013; Anesio et al., 2009; Anesio et al., 2010; Hodson et al., 2008; Lutz et al., 2016), with the simplest communities being found in the ice covered cryoconite on Antarctica (Pautler et al., 2013). In addition to adding complexity, exposure time was also responsible for increased abundance of microbes.

The abundance of microorganisms also varied along the transect on Mendenhall glacier. The highest concentration of microbial lipids was found near the toe of the glacier and decreased in concentration towards the snow line (Figure 4.3). Therefore, this data suggests that the relative contribution of the viable microbial community increases with longer exposure times. Since the lower ablation zone is open to the atmosphere for a longer portion of the year, the growing season for microorganisms at the toe is longer than for those at the top of the glacier, which allowed for the highest abundance of microorganisms at the toe. As microbes increased in concentration, the ^{14}C content of the TLE increased, indicating that carbon is cycled more quickly in cryoconite, which contain a higher abundance of microorganisms (Figure 4.4). This suggests that seasonal variation of the ablation zone may impact both the carbon composition of cryoconite material and how quickly the carbon is cycled within cryoconite.

The age of the organic carbon within cryoconite on Mendenhall becomes increasingly modern with increased elevation on the glacier, where the microbial communities present within cryoconite decrease in abundance and complexity with increased elevation on the glacier, all of which are linked to longer exposure time at the toe of the glacier. Seasonal variation could explain the reason why some carbon may be more quickly cycled in different parts of the ablation zone, but cannot account for the source of this modern carbon overall.

Sources of the modern carbon

These results have shown that the cryoconite organic carbon on Mendenhall is modern. However, it is still unclear what the carbon sources to the cryoconite are. It has

been hypothesized that fossil carbon from the combustion of fossil fuels was a major component of organic carbon on Mendenhall's surface (Stubbins et al., 2012), however, based on the ^{14}C results from this work, fossil carbon is not a major contributor to cryoconite carbon on Mendenhall. If fossil carbon was a major component of the bulk cryoconite particulate organic carbon, the radiocarbon content of the bulk organic carbon as well as the solvent extractable and non-extractable carbon components would contain older carbon. It is possible there could be a small contribution of fossil combusted carbon to Mendenhall cryoconite, but its old ^{14}C content is overwhelmed by other modern inputs. Therefore, the sources of carbon to Mendenhall cryoconite must be predominantly modern. Three possible sources of modern carbon to the surface of Mendenhall glacier could have been 1) in situ biological production, 2) modern aerosol delivered carbon, or 3) local, terrestrial carbon. Below we explore each of these possible carbon sources.

One possible source of modern carbon to cryoconite on Mendenhall glacier could be the addition of carbon by microbes living in cryoconite. Our results show that the longer the cryoconite are exposed, the more microbes there are in the cryoconite and the more modern the sample is, suggesting that increased microbial abundance increases carbon cycling of modern carbon. Although for this study we did not measure the radiocarbon content of microbial lipids directly in order to determine carbon sources to microbes on Mendenhall glacier, it was assumed that the microbial lipids contained modern carbon, since the radiocarbon content of the TLE was modern. As with the work in Chapter 3, microbes on the surface of Mendenhall glacier were likely using modern sources of carbon, including atmospheric CO_2 and the modern carbon fixed by autotrophs. Therefore, the microbes on Mendenhall were adding modern carbon to the

cryoconite environment. The source of all modern carbon within cryoconite could be the combination of living and dead microorganisms, however, it is believed that microbial carbon inputs to the surface of this glacier are small, in comparison to other potential modern carbon sources to alpine glaciers.

Alkanes within Mendenhall cryoconite contained a high abundance of both C_{27} alkanes and C_{23} alkanes, which indicated vascular plant and mossy or microbial carbon sources, respectfully (Eglinton and Hamilton, 1967; Nott et al., 2000; Volkman et al., 1998) (Figure 4.5). Microbial carbon has been found to be a major carbon input to continental glacier surfaces, such as Greenland and Antarctica (Musilova et al., 2017; Smith et al., 2017), but alpine glaciers have additional organic carbon inputs not available to continental glaciers, such as surrounding vegetation, soils, and rocks. Microbial carbon is a component of the bulk modern organic carbon, but its contribution is low relative to microbial carbon inputs to continental glacier surfaces.

A second hypothesis for the source of modern carbon to the surface of Mendenhall glacier is wind-blown aerosol delivered carbon, which could originate from either the ocean, the forests, or from fossil fuel combustion. Stable isotope ^{13}C content of Mendenhall cryoconite organic carbon (~ -26‰) is too depleted to originate from a marine source, which normally has a ^{13}C content of about -21‰ and is within the range of terrestrial or fossil carbon (Williams and Gordon, 1970; Peters et al., 2005). Since fossil carbon is not likely a major component of Mendenhall cryoconite carbon, aerosol delivered carbon is likely terrestrial in nature.

Previous work has found lignin material on Mendenhall glacier (Hood et al., 2009). Lignin, which is a structural material only found in wood, suggested that terrestrial

plant material can be transported to the glacier. However, because we examined the sediment material on the glacier, we inherently examined large particle sizes. Large particles do not travel as far as aerosol delivered carbon, and therefore they likely originated from the local forests. This is corroborated by alkane distributions from Mendenhall cryoconite. Mendenhall cryoconite organic carbon contained predominately C_{27} and C_{23} chain length alkanes (average chain length 24.9) and a moderate odd over even ratio (Figure 4.5). This suggested that the source of these alkanes was fresh vascular plants, mosses, microbes, or a combination thereof (Eglinton and Hamilton, 1967; Nott et al., 2000; Volkman et al., 1998). Additionally, as explained previously, terrestrial carbon could be responsible for transporting enriched bomb carbon to Mendenhall's surface. Therefore, terrestrial organic carbon is likely a major source of modern carbon to the surface of Mendenhall glacier, but it is unlikely to be delivered as aerosols.

Overall, all carbon components of bulk cryoconite particulate organic carbon (POC) on Mendenhall have been modern. However, it has been shown that mountain glaciers, including Mendenhall, export old dissolved organic carbon, or DOC (Stubbins et al., 2012; Fellman et al., 2015; Bhatia et al., 2013; Hood et al., 2009). The difference in radiocarbon content of POC and DOC from Mendenhall suggested that the carbon sources to these carbon pools are not the same. This discrepancy, as well as further sources of modern carbon, will be discussed in Chapter 5.

Table 4.1: Bulk and isotopic properties of cryoconite carbon on Mendenhall glacier.

Sample name	Distance from toe (km)	%C	$\Delta^{14}C$ Bulk (±5‰)	$\delta^{13}C$ Bulk (±0.5‰)	$\Delta^{14}C$ TLE (±10‰)	% extractable C	[PLFA] µg/µgC
MEN 15	0	1.83	-15	-27.1	33	8.42	0.17
MEN 21B	1.19	2.43	19	-25.6	29	17.71	0.11
MEN 5	7.83	2.58	39	-25.5	94	6.23	0.08
MEN 4	8.14	1.39	18	-26.1	119	15.38	0.07

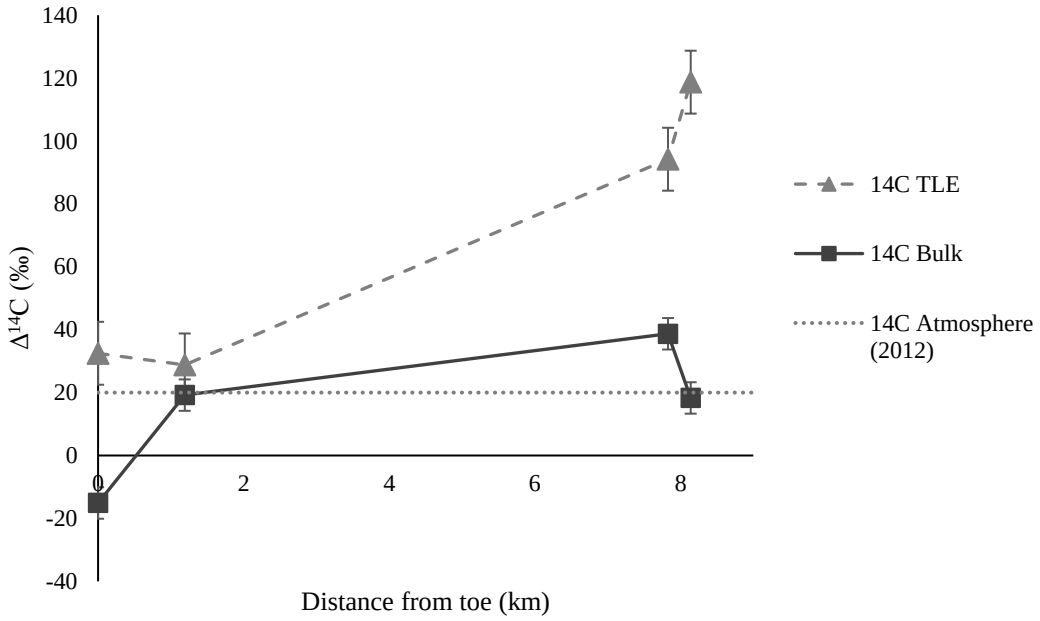

Figure 4.1: The radiocarbon (^{14}C) content of bulk cryoconite organic carbon and the solvent extractable carbon present in the total lipid extract (TLE), versus distance from the toe along the transect of Mendenhall glacier. The error associated is ±5‰ for bulk ^{14}C and ±10‰ for TLE ^{14}C. The radiocarbon content of the current atmosphere is +20‰ and included for reference (Levin et al., 2013).

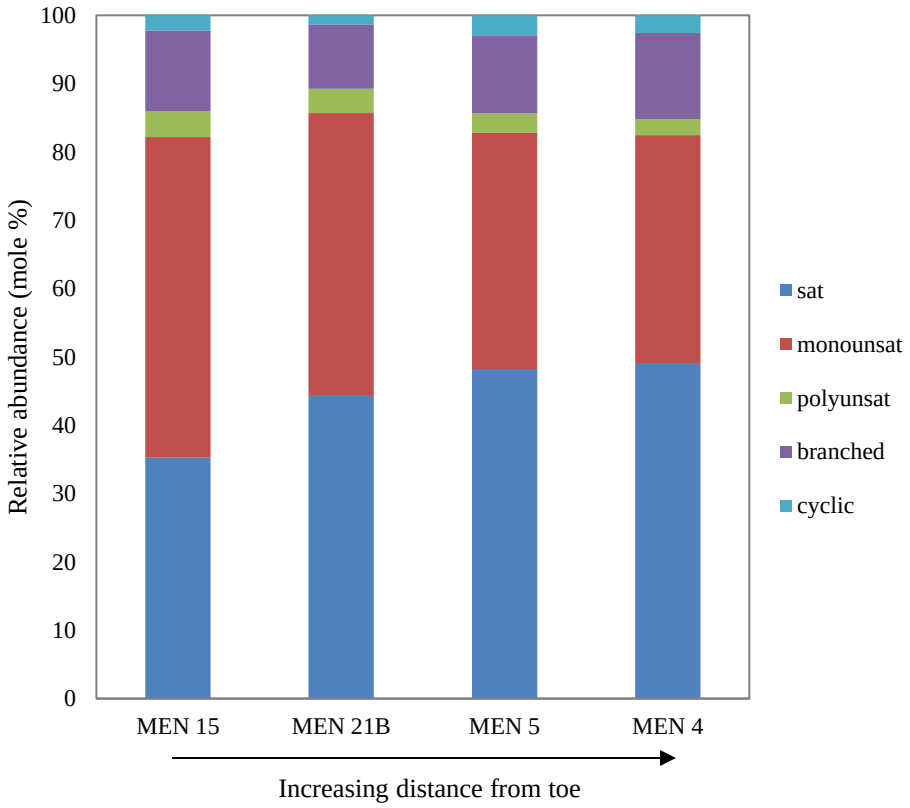

Figure 4.2: PLFA structure variations along the transect on Mendenhall glacier. From left to right, MEN 15 is located at the toe of the glacier and MEN 4 is located at the snowline. There is a slight shift from predominately monounsaturated PLFA at the toe to mostly saturated PLFA at the snowline. Sat = saturated, monounsat = monounsaturated, polyunsat = polyunsaturated PLFA compounds.

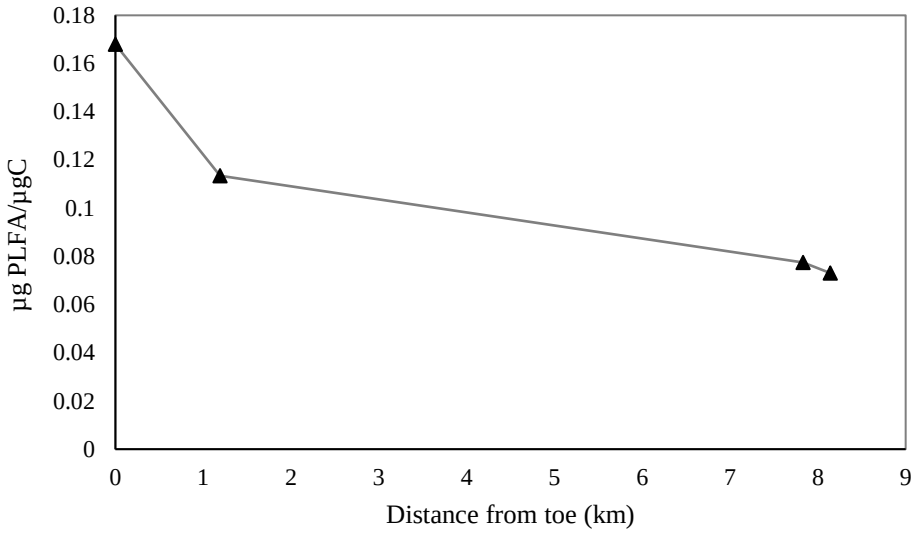

Figure 4.3: Concentration of PLFA normalized to the amount of total organic carbon (µg PLFA/µg C) in cryoconite samples versus distance from the toe of Mendenhall glacier.

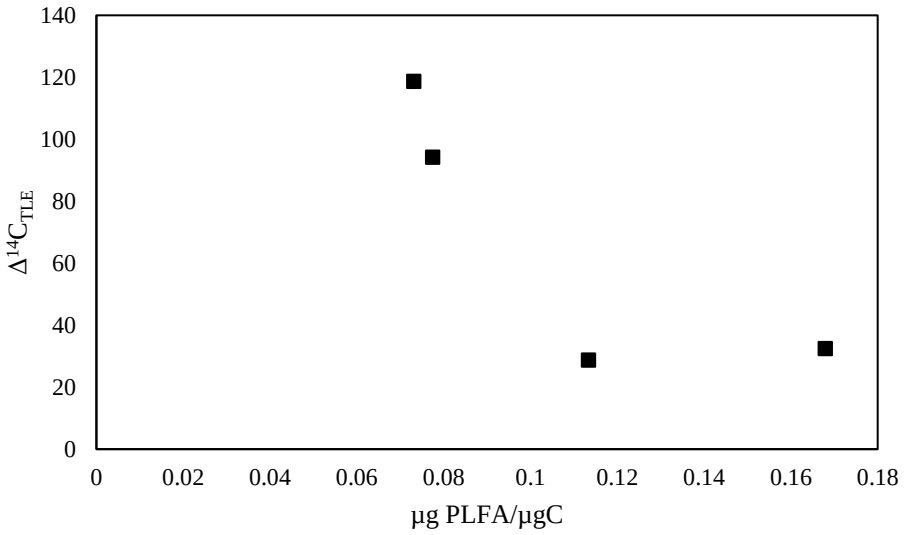

Figure 4.4: Concentration of PLFA compared with the ^{14}C content of the total lipid extract (TLE) shows that there are more microbes in locations with a lower ^{14}C content of the TLE. This could indicate that carbon is cycled more quickly where there are more microorganisms present.

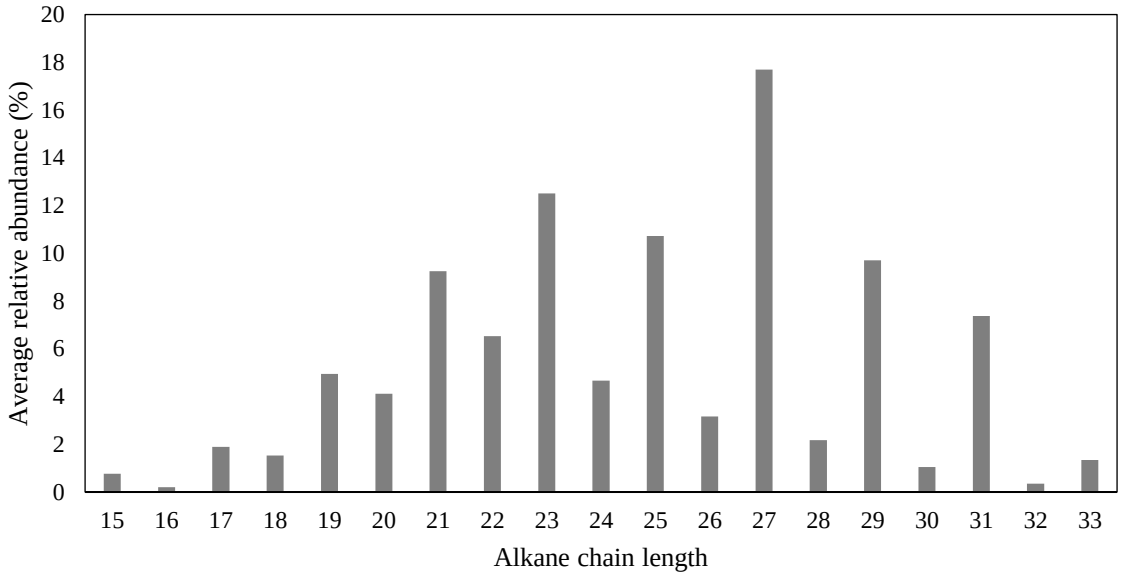

Figure 4.5: Alkane distributions within cryoconite on Mendenhall glacier. Alkanes here have an average chain length (ACL) of 24.9 and a high odd over even preference (3.5). The high abundance of C_{27} alkanes likely originates from a terrestrial vascular plant source. The second highest abundance is C_{23} alkanes, which likely originate from mosses or microorganisms within cryoconite.

CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

Using radiocarbon, we studied the carbon composition of cryoconite material on three mountain glaciers in southern Alaska. We were able to determine the age of the extractable and non-extractable components of bulk cryoconite organic carbon, as well as specific carbon sources to microorganisms in the supraglacial environment of the ablation zone. Matanuska and Spencer glaciers were chosen in the first study in order to compare cryoconite composition between two glaciers under similar environmental conditions, such as geology and climate. The second study assessed variation on a single glacier, by comparing cryoconite composition on a transect from the toe of Mendenhall glacier to the snowline.

The radiocarbon content of extracted microbial lipids (PLFA) on all three glaciers was modern. This suggested that microorganisms on the surfaces of Matanuska, Spencer, and Mendenhall glaciers were using modern carbon as a carbon source. The source of this modern carbon was likely atmospheric carbon usage by autotrophs, who then fixed modern carbon to be used by heterotrophs. This is consistent with a study that found heterotrophic microorganisms were using modern carbon fixed by autotrophs within a supraglacial stream in Antarctica, via ^{13}C labeling experiments (Smith et al., 2017).

The extractable carbon component (TLE) was more modern than the bulk organic carbon at every sample location on Spencer, Matanuska, and Mendenhall glaciers, and

included a significant enrichment by bomb carbon on Mendenhall glacier. Alkane biomarkers within the TLE suggested that fresh microbial carbon was a component of Spencer cryoconite, fresh terrestrial plant carbon was a carbon source to Matanuska, and both were carbon sources to Mendenhall cryoconite. In addition, C/N ratios on Spencer and Matanuska indicated microbial carbon inputs to the cryoconite on these glaciers. Overall, there was evidence suggesting modern carbon sources to cryoconite, however, the radiocarbon content of cryoconite organic carbon on Spencer and Matanuska glaciers was old.

The radiocarbon content of bulk cryoconite organic carbon varied between the two studies. Bulk organic carbon on Matanuska and Spencer glaciers was depleted in ^{14}C and was thousands of years old, whereas bulk organic carbon on Mendenhall glacier was modern, and at one location was enriched relative to the modern atmosphere. Since the solvent extractable carbon was modern, other carbon inputs were responsible for the depleted radiocarbon content of Matanuska and Spencer cryoconite carbon. In addition, less than 15% of the total organic carbon was solvent extractable, and therefore there must be other carbon inputs responsible for greater than 85% of non-extractable modern carbon on Mendenhall. Since the radiocarbon content of the non-extractable organic carbon varied between glacier locations, the sources of carbon to the surfaces of these glaciers must be different.

Previous studies have found that dissolved organic carbon (DOC) on the surface and within the outflow of Mendenhall glacier, the export of other glacial watersheds adjacent to Mendenhall, glacial watersheds near Spencer, and export from Greenland was old (Hood et al., 2009; Stubbins et al., 2012; Fellman et al., 2015; Bhatia et al., 2013).

This study quantified the radiocarbon content of particulate organic carbon (POC) on the surface of Spencer, Matanuska, and Mendenhall glaciers. Differentiation between DOC and POC is operationally defined, where all substances that pass through a GF/F filter are considered dissolved compounds. As the radiocarbon content of POC on Mendenhall's surface is modern and DOC on the surface and within Mendenhall's export is old, there must be different sources of DOC vs POC to the surface of Mendenhall glacier.

DOC within glacier export can reflect the DOC content of both supra- and sub-glacial runoff, where POC within cryoconite only reflects carbon sources to the supraglacial environment. Old DOC has been hypothesized to originate from at least two different sources: old microorganisms (Hood et al., 2009) or fossil combusted carbon (Stubbins et al., 2012). Old microorganisms are either those that have been stuck in glacial ice and are now being exposed due to glacier melt, or microorganisms that look old due to their carbon source. There is evidence that microorganisms in a proglacial stream (export) use old DOC as a carbon source (Fellman et al., 2015), which they in turn release as old DOC. Fossil combusted carbon can be carried to the surface of these glaciers as aerosol carbon. As we did not measure the radiocarbon content of DOC, only POC, combusted fossil carbon may be a component of the overall organic carbon on the surface of these glaciers. However, due to the fact that no combusted carbon compounds were present in the total lipid extract, as indicated by its modern carbon content in all samples, other carbon sources besides fossil carbon predominate in the supraglacial environment of Spencer, Matanuska, and Mendenhall glaciers.

Finally, organic carbon could have come to the surface of Spencer, Matanuska, and Mendenhall glaciers from surrounding geology. Spencer and Matanuska glaciers are

situated in the Chugach mountain range in southern Alaska, which is composed of sedimentary geology, comprised of rocks such as shale (Burns et al., 1991; Eckhart 1992). Sedimentary rocks contain ^{14}C-free carbon ($\Delta^{14}C$ = -1000‰). The addition of mineral-bound organic carbon from these fossil carbon bearing sedimentary rocks to the surface of Spencer and Matanuska glaciers could account for the old age of supraglacial POC at these glacier locations, as well as for the fact that this old carbon was not solvent extractable. In contrast, southeastern Alaska, where Mendenhall lies, is characterized by metamorphic geology (Gehrels and Berg, 1994). During metamorphic processes, most organic carbon is released from rocks as CO_2. Therefore, there is little to no fossil carbon, or little carbon overall, added to the surface of Mendenhall glacier from surrounding rocks, which is consistent with the modern radiocarbon content of bulk cryoconite POC on Mendenhall's surface. In order to account for the greater than 85% organic carbon that was mineral bound within Mendenhall cryoconite, there must be another carbon source to the glacier surface. Modern, mineral bound carbon can originate from surrounding soil. Components of soil are mineral bound (Kleber et al., 2007) and the top layer of soil is generally modern (Trumbore 1993). Therefore, the addition of POC from surrounding soil is likely a major component of Mendenhall cryoconite organic carbon.

It is apparent that glacial ecosystems are complex and dynamic. Abundant and diverse microorganisms populate glacier surfaces all around the world, using and adding modern carbon to the supraglacial ecosystem. Their modern carbon sources seem to be independent from the POC environment in which they live, which highlights two distinct carbon pools on the surface of alpine glaciers: a large microbial community, living in and interacting minimally with a larger, sometimes old carbon environment. Ultimately, these

studies highlight the isotopic heterogeneity of supraglacial organic carbon. Because of this heterogeneity, it is expected that cryoconite carbon composition will vary with glacier location, depending on the relative inputs from varying surrounding vegetation, geology, and microorganisms. As exported carbon has major implications for downstream ecosystems, and as a warming climate could increase the surface area on glaciers for cryoconite to form, identifying carbon sources to glaciers around the world is significant.

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APPENDIX A: SUPPLEMENTAL INFORMATION

Table A.1: PLFA structures and associated microbial class

Microbial class	**Structures**	**Source**
Cyanobacteria	14:0, 16:0, 14:1, 16:1, 16:2, 18:1	Dijkman et al. 2009; Zelles et al., 1999
Gram positive bacteria	16:1ω7, cy17:0, 18:1ω7, cy19:0, branched	Zelles et al., 1999, Green and Scow 2000
Gram negative bacteria	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0, a18:0	Zelles et al., 1999
Fungi	18:2ω6	Zelles et al., 1999
Green algae	18:2, 16:3	Zelles et al., 1999, Boschker et al. 2005
Both Algae and bacteria	14:0, 15:0, 16:0, 16:1ω7c, 18:0, 18:1ω9c, 18:2	Boschker et al. 2005, Zelles et al., 1999

Table A.2: PLFA structure distributions from Matanuska cryoconite in mole %

FAME	MAT 1	MAT 2	MAT 3	MAT 4	MAT 5	MAT 6	MAT Orange
C8:0	3.61	2.42	3.74	1.49	1.35	1.56	0
C9:0	20.28	0	2.38	4.09	0	0	0
C10:0	0	0	2.17	4.98	0	0	0
C11:0	0	0	0	0	0	0	0
C12:0	0	0	1.93	5.47	0	0	0
C13:0	0	0	0	0	0	0	0
C13:0	0	0	0	0	0	0	0
brC13	0	0	1.71	0	0	0	0
C14:1	0	0	0	0	0	0	0
C14:0	5.62	4.48	2.04	2.45	2.86	2.93	0
C15:0	6.14	4.65	3.24	2.61	2.66	2.98	17.92
brC14	6.51	4.82	3.30	2.96	2.95	3.85	20.64
brC14	0	0	2.22	2.40	2.46	2.43	0
brC15	0	0	6.20	0	2.40	0	0
C16:3	0	10.53	0	5.31	6.00	0	0
C16:1	0	0	0	5.28	5.42	2.95	0
C16:1	14.68	13.62	7.84	8.40	13.41	13.54	37.52
C16:1	0	0	6.38	5.29	0	3.14	0
C16:1	0	10.08	6.73	5.47	5.94	3.64	0
C16:1	0	0	0	0	0	0	0
C16:0	20.51	21.39	20.13	10.90	18.73	27.97	23.90
brC16:1	0	0	0	0	0	0	0
brC16	0	0	3.42	0	0	0	0
brC16	0	0	3.33	0	0	0	0
brC16	0	0	3.43	3.30	3.32	0	0
brC16	0	0	0	0	0	0	0
cyC17	0	0	3.66	0	0	4.14	0
C17:0	0	0	0	0	0	0	0
C18:2	0	9.40	0	4.83	5.47	5.43	0
C18:1	0	0	0	5.06	8.61	0	0
C18:1	0	0	0	4.81	0	0	0
C18:1	0	0	5.72	4.97	0	8.64	0
C18:1	12.14	9.72	5.78	5.69	6.71	10.71	0
C18:0	10.47	8.84	4.55	4.12	4.80	6.02	0
C20:0	0	0	0	0	3.67	0	0
C22:0	0	0	0	0	3.14	0	0
C24:0	0	0	0	0	0	0	0

Table A.3: PLFA structure distributions from Spencer glacier cryoconite in mole %.

FAME	Spen 5.1	Spen 6	Spen 7	Spen 8	Spen 9	Spen 10	Spen 12	Spen 13	Spen 14	Spen 15
C8:0	0.91	1.45	0.38	1.15	0.96	1.34	1.99	0.50	0.05	0
C9:0	0.11	0	0.03	0	0.11	0.37	0	0	0.09	0
C10:0	0.02	0	0.01	0	0.05	0.28	0	0	0.04	0
C11:0	0	0	0	0	0.01	0	0	0	0	0
C12:0	0.02	0	0.01	0	0.06	0.25	0	0	0.06	0
C13:0	0	0	0	0	0.02	0	0	0	0.02	0
C13:0	0	4.48	0	0	0.02	0.24	0	0	0.02	0
brC13	0.04	0	0.02	3.22	1.39	0.92	1.53	4.33	1.02	9.52
C14:1	0	0	0	0	0	1.33	0	0	0	0
C14:0	2.94	0	1.81	3.67	2.73	2.92	6.77	4.67	2.87	9.52
C15:0	2.47	6.41	1.53	4.72	3.68	3.15	7.18	5.74	3.41	10.31
brC14	3.12	7.32	1.66	5.41	4.71	5.04	7.71	6.51	4.33	10.65
brC14	1.52	5.82	1.29	4.42	2.08	2.06	0	0	2.11	0
brC15	1.38	0	1.60	4.21	1.66	2.15	0	0	2.01	0
C16:3	0	0	4.18	0	0	0	0	0	0	0
C16:1	1.33	0	4.06	5.65	2.39	2.84	7.55	6.28	3.29	0
C16:1	10.23	8.85	9.77	9.64	8.95	7.45	10.20	8.83	9.86	12.05
C16:1	4.48	7.49	0	0	2.34	2.46	0	6.43	2.34	0
C16:1	6.10	7.58	4.67	6.52	4.15	5.91	7.66	6.73	3.69	10.93
C16:1	0	0	0	0	0	2.31	0	0	0	0
C16:0	25.76	17.60	26.76	17.44	23.92	20.64	16.50	16.88	21.18	14.55
brC16:1	0	0	0	0	0	2.23	0	0	0	0
brC16	0	0	0	0	1.91	2.55	0	0	2.74	0
brC16	0	0	0	0	1.60	2.15	0	0	2.06	0
brC16	2.16	0	1.78	0	1.96	2.74	0	0	2.33	0
brC16	0	0	0	0	1.67	2.21	0	0	2.26	0
cyC17	2.99	10.64	2.68	8.18	3.50	3.77	0	0	3.57	0

C17:0	2.74	0	2.57	0	2.38	3.23	0	0	2.96	0
C18:2	4.38	0	4.88	5.76	3.65	2.97	0	0	2.74	0
C18:1	0	0	0	0	0	0	0	0	0	0
C18:1	0	0	0	0	0	0	0	0	0	0
C18:1	11.60	7.47	17.34	7.51	9.52	5.74	17.06	13.80	9.25	22.43
C18:1	7.70	7.45	5.74	6.80	7.29	4.45	15.80	12.67	4.50	0
C18:0	2.78	7.38	2.35	5.62	2.74	2.65	0	6.56	5.25	0
C20:0	1.85	0	1.73	0	1.61	1.83	0	0	2.01	0
C22:0	1.70	0	1.56	0	1.45	1.68	0	0	1.98	0
C24:0	1.57	0	1.47	0	1.33	0	0	0	1.82	0

Table A.4: PLFA structure distributions from Mendenhall glacier cryoconite in mole %.

FAME	MEN 4	MEN 5	MEN 21B	MEN 15
C8:0	0.72	0	1.11	0.45
C9:0	0.87	0	1.24	0.16
C10:0	0.03	1.30	0.54	0.16
C12:0	0.23	1.45	0.58	0
brC13:0	2.07	1.55	1.33	1.83
C14:0	3.81	4.07	2.45	2.47
C15:0	5.30	5.05	4.78	4.57
brC14:0	5.93	4.54	4.98	5.62
C15:0	1.52	1.37	0.80	1.58
brC15:0	1.58	1.39	1.16	1.63
C16:1	3.61	3.28	3.24	5.05
C16:1	8.65	9.61	9.17	9.96
C16:1	3.40	2.81	1.21	4.25
C16:1	4.57	4.01	5.90	5.88
C16:1	2.58	2.34	1.60	3.64
C16:0	23.78	25.28	26.86	18.00
brC16:0	2.09	1.85	1.25	1.99
brC16:0	1.60	1.41	0.59	1.50
C17:0	2.22	1.91	1.54	1.91
C17 cyc	2.80	2.66	1.31	2.18
C18:2	2.64	2.39	3.58	3.75
C18:1	6.28	6.67	14.13	9.90
C18:1	5.30	5.73	6.68	7.26
C18:0	4.02	3.85	2.25	2.27
C20:0	2.29	1.91	0.93	1.41
C22:0	1.96	1.90	0.65	1.27
C24:0	0	1.56	0	1.17

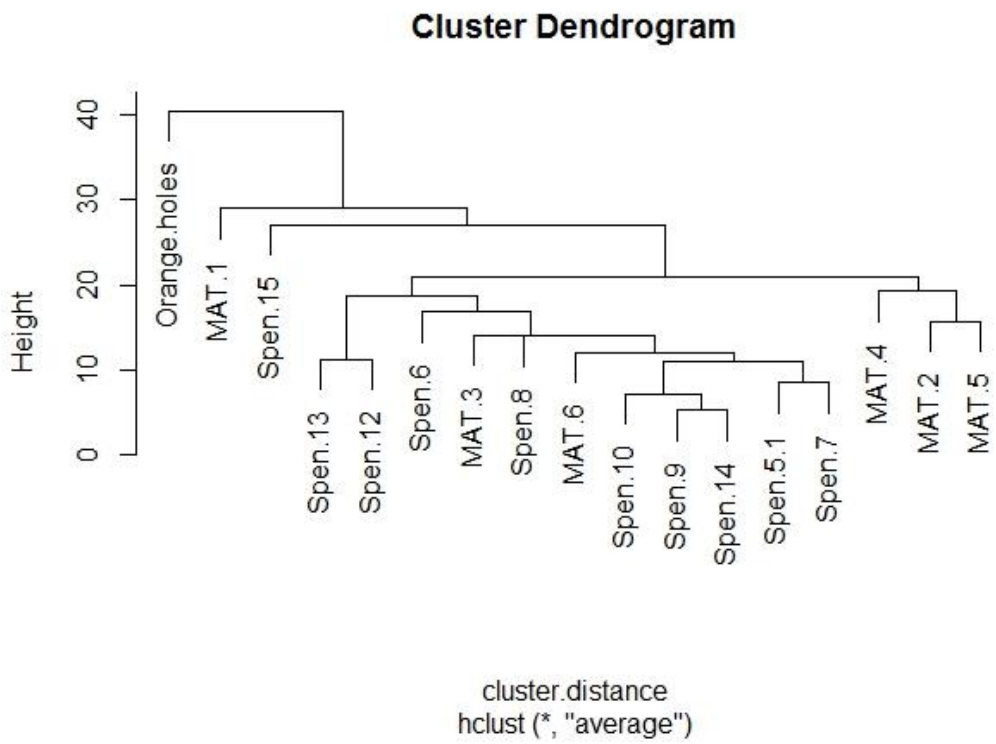

Figure A.1: A cluster dendrogram of all PLFA structure distributions on all extracted samples (37 PLFA structures across 17 samples), created using R. Samples clustered close to a height of zero are most similar and samples clustered furthest from zero are most dissimilar.