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Endolithic Microbial Carbon Cycling in East Antarctica

Natalie Tyler

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ENDOLITHIC MICROBIAL CARBON CYCLING IN EAST ANTARCTICA

by

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Submitted in Partial Fulfillment of the Requirements

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DEDICATION

To the Tyler family (Steve, Melody, Phyllis, Rynanne, and Karmin) and my partner, Alec. Your unwavering support and belief in my abilities made this a reality – thank you.

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ABSTRACT

Microbes adapt to inhospitable conditions by colonizing niches that protect them from severe environmental conditions, making them model organisms in the search for life on other planets. Endoliths are globally ubiquitous microbes that colonize structural cavities within rocks, which protect them from environmental stressors while maintaining access to nutrition and light sources. Despite harsh katabatic winds, extreme temperature fluctuations, harmful UV radiation and exposure to desiccation, endolithic communities have been observed in Antarctica. Previous studies have found viable and abundant microbial biomass through microscopy and the addition of isotopic labels. However, due to the slow metabolic activity of these microbes, very little is known about the in-situ activities of these communities – especially in regions outside the McMurdo Dry Valleys where endoliths are thought to be cycling carbon on centennial to millennial time scales. Here we show that East Antarctic endoliths are cycling carbon quickly and are therefore quite active. Through radiocarbon (^{14}C) analyses of the viable cell membrane (as PLFA), we found that the $\Delta^{14}\text{C}$ composition of these microbial communities were on average predominantly modern, with a few samples signaling older carbon in the system. These findings indicate that Antarctic endoliths are cycling carbon on decadal time scales rather than on a centennial or millennial scale. This work provides new insights into the potential variability of Antarctic endolith activities and demonstrates that, despite the climatic extremes that exist farther inland on the most inhospitable continent on Earth, indigenous life can thrive.

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CHAPTER 1

INTRODUCTION

Life as we know it generally exists within a confined set of parameters.

Extremophiles, defined as organisms that survive and even thrive in locations that experience one or more extreme (physical or geochemical), have long been studied to explore the extent of life on Earth with an eye on future astrobiological applications. After plants, microbes are the most abundant organisms on Earth (Bar-On et al., 2018) and, as a result of their ability to colonize advantageous niches, are often the only lifeform found in extreme environments.

Endolithic, or “rock-dwelling” microorganisms are specialized in that they can colonize the interior of any type of rock. They range from preferring temperate climates to thriving under harsh conditions and can be found across a wide variety of environments. In general, endoliths preferentially colonize rocks with naturally higher translucence and porosity (Friedmann, 1982), such as sandstone, in order to maximize photosynthesis. In addition, these microbes have been found to colonize rocks that have either been metamorphically altered to possess these traits (e.g. shocked gneisses) or have undergone significant weathering (Cockell et al., 2002). Endoliths are often the only form of life found in extreme terrestrial environments and their ability to survive has been attributed to the protection provided by their microhabitats from environmental stressors (Friedmann et al., 1976; Friedmann et al., 1982). Colonization of the structural cavities within ~1-2 mm of the rock surface is favorable as it can be up to 10 °C warmer in these

spaces than the surrounding air on a sunny day (McKay et al., 1993) and provides sufficient exposure to sunlight while sheltering inhabitants from harmful UV radiation (Hughes & Lawley, 2003). Despite harsh katabatic winds, arid conditions, and freezing temperatures, endoliths have been found in one of the most extreme locations on Earth - Antarctica.

Antarctica, a remnant of the ancient supercontinent Gondwana that broke up between 180-36 million years ago after the arrival of a mantle plume caused crustal rifting, is primarily divided into East Antarctica, an older continental craton, and the younger West Antarctica, which is actively rifting and includes the Antarctic Peninsula. The Antarctic Ice Sheet, which contains approximately 30 million km³ of ice (NSIDC, 2019) and covers 98% of the continent, is similarly considered to be comprised of two separate masses that are divided by the Transantarctic Mountains: The West Antarctic Ice Sheet, and the East Antarctic Ice Sheet. The East Antarctic Ice Sheet is the largest mass of ice on Earth and is extremely thick, reaching up to 5 km in thickness for some regions (NASA, 2014). The ice-free areas of Antarctica are usually limited to northern coastal areas, isolated rocky peaks (commonly called “nunataks”), and dry valleys extending above the ice and snow. Previous studies have found living endolithic microorganisms colonizing rocks located within these ice-free areas (Friedmann et al., 1976; Friedmann, 1985; Friedmann et al., 1988; Vestal, 1988; Johnston & Vestal, 1991; Wierzchos et al., 2001; Hughes & Lawley, 2003; Wierzchos et al., 2005; Büdel et al., 2008; Colesie et al., 2016; Brady et al., 2018).

Antarctic endolithic communities are considered one of the simplest known ecosystems, with cyanobacteria as the primary producers, fungi as the consumers, and

bacteria as the decomposers (Friedmann, 1982). In order to adapt to unfavorable conditions, it is thought that these microorganisms are able switch from active to dormant metabolic states (Friedmann et al., 1976). To partially compensate for the seasonally-induced light restriction, Antarctic endoliths preferentially colonize north-facing rocks to augment photosynthetic activity (Friedmann, 1982). Previous observations suggested that these communities are likely slow-growing due to short austral summers and long winters, where sunlight is limited and exposure to extremely cold temperatures is typical throughout the year.

Despite previous studies, the viability and activities of Antarctic endolithic microorganisms remains uncertain. Both viability and activity are typically measured through culturing, microscopy, the addition of isotopic labels, and natural abundance radiocarbon (^{14}C) analysis. Previous culture studies were not successful (Friedmann, 1988) and were overall inadequate given the low culturability of most microorganisms (Amann et al., 1995). However, the remaining methods - microscopy, isotopic labelling and natural abundance ^{14}C analysis – have successfully shown that endoliths in Antarctica are viable. Microscopic analyses found that the physiological states of Dry Valley microbial cells vary as a function of elemental composition (Mulyukin et al., 2002) and fluorescence (Wierzchos, 2004), suggesting that these endoliths were metabolically active. Additional evidence of metabolically active endoliths was observed *in-situ* as exfoliative trace fossils left behind by still-growing communities (Friedmann & Weed, 1987). Carbon metabolism (primarily via photosynthesis) was observed over a wide range of temperatures, light intensities, and moisture regimes through the addition of various organic and inorganic compounds during in-situ and in-vitro studies (Vestal,

1988). Later, isotopically ^{14}C -labeled bicarbonate studies applied to lichen and cyanobacteria-dominated colonies found very low rates of photosynthetic carbon incorporation times and extremely long turnover times (Johnston & Vestal, 1991). While these studies were instrumental in understanding the viability of Antarctic endoliths, they have yet to address the *in-situ* activities of these communities.

The analysis of naturally abundant radiocarbon in certain biomarkers can be used to determine microbial carbon sources (Petsch et al., 2001; Slater et al., 2005), thus providing some indication of cycling rates in natural systems. Phospholipid-derived fatty acids (PLFA) are components of cellular membranes that hydrolyze within days of cell death (White et al., 1979) and are therefore considered a biomarker of the viable microbial community. In general, PLFA structures differ between various microorganisms and, although some types are more ubiquitous, can also be used to get a broad brushstroke of community composition. Using the concept of “you are what you eat”, the isotopic content of PLFA is considered representative of the isotopic content of carbon sources to the microbes (Petsch et al., 2001; Slater et al., 2005). Since Antarctic endoliths have been found to preferentially consume atmospheric CO_2 (Vestal, 1988) and the atmospheric composition of $^{14}\text{CO}_2$ is well known, the ^{14}C content of the PLFA can be used to determine the rate of carbon cycling (i.e. the activity) of an endolithic community (Ziolkowski et al., 2013), or how much time has passed since the PLFA carbon was last in equilibrium with the atmosphere. Recent work in the McMurdo Dry Valleys found younger ^{14}C ages in endoliths at lower elevations that were proximal to the coast, where warmer temperatures and greater water availability lead to higher productivity within a community (Colesie, et al., 2016). Isotopic analyses of PLFA have also been applied to

assess carbon sourcing and cycling by endoliths in the Dry Valleys (Brady et al., 2018). The radiocarbon ages of microbial PLFA were found to be younger than the total organic carbon (TOC) – comprising both dead and living biomass – ages of the rocks they were living in, suggesting fixation of modern atmospheric CO₂ (amid some mixing with older carbon sources). Therefore, using natural abundance radiocarbon to assess the microbial activity of endoliths found higher growth rates than previously thought. While this work has substantially contributed to ascertaining the activities of West Antarctic endolithic microorganisms, very little is known about communities residing elsewhere across the continent.

Recently, endoliths have been observed colonizing rocks within the Dronning Maud Land region of East Antarctica. To date, limited work has been done to assess the overall viability and carbon cycling within these communities. To address this knowledge gap, this study used ¹⁴C analysis of phospholipids to examine the activities (as carbon cycling) of endolithic microorganisms in East Antarctica.

CHAPTER 2

METHODS

2.1 STUDY SITE AND SAMPLE COLLECTION

Encompassing about 2,741,000 km² of the area in East Antarctica, Dronning Maud (or Queen Maud) Land comprises one sixth of the entire continent and lies roughly between 20° W and 45° E. Rock samples containing endoliths were collected from several nunataks and valleys in the Sør Rondane Mountains of East Dronning Maud Land, approximately 200 km inland from the coast (Figure 2.1). Field excursions were based out of the Belgian Princess Elisabeth Antarctic Station (PEAS), which is located along Utsteinen Ridge within East Dronning Maud Land. This area is geographically distinct from the McMurdo Dry Valleys in West Antarctica, with over 3,000 km of distance between the two stations, thus providing a unique contrast with which to assess local endoliths.

Utsteinen Nunatak, where PEAS is based, is approximately 1360 m.a.s.l and located at 71° 56' 42.26" S, 23° 20' 42" E. The mean annual air temperature, measured in 2009 and 2010, was approximately -19 °C (Gorodetskaya et al., 2013). Perlebandet Nuntak is about 1280 m.a.s.l and just under 21 km northwest of Utsteinen Ridge, while Dubois is about 1350 m.a.s.l and almost 9 km south of Utsteinen. The Dry Valley in this study is approximately 1670 m.a.s.l. and is located about 19 km southwest from Utsteinen. Many of these nunataks are sheltered from the katabatic winds that come

from the plateau, which provides a warmer and less hostile environment for the microbial communities. Due to its high elevation and proximity to the Antarctic plateau, the Dry Valley site was the windiest location and typically had little snow accumulation as a result. Endolithic colonies were identified by pigmentation that extended from the rock surface to approximately 1-2 mm into the subsurface (Figure 2.2). A total of 23 samples were collected over two austral summers during early 2017 and late 2017 through early 2018. Most samples consisted of highly weathered, extremely friable lithologic fragments. Rock fragments were broken free with a rock hammer and sealed in sterilized plastic bags. Samples were frozen immediately after collection and kept frozen until analysis back at the University of South Carolina. Of the 23 samples collected, a total of 11 rock samples were processed for PLFA analyses and five of those 11 samples were selected for isotopic analysis.

2.2 EXTRACTION AND IDENTIFICATION OF MEMBRANE LIPIDS

Colonized rocks were initially crushed with a solvent rinsed impact mortar and pestle and further homogenized with a solvent-rinsed ceramic mortar and pestle. For each sample, approximately 80 g of colonized rock was solvent extracted for PLFA twice using a modified Bligh & Dyer method (White et al., 1979). Homogenized and pulverized samples were sonicated for 2 minutes with a 1:2:0.8 dichloromethane (DCM):methanol (MeOH):phosphate buffer solution and allowed to extract overnight at room temperature. The extraction solution was gravity filtered through solvent rinsed glass fiber filters into a separatory funnel and the solvent ratios adjusted to 1:1:0.9 DCM:MeOH:H₂O through the addition of equal parts DCM and deionized water. The resulting solution was shaken, then allowed to equilibrate into aqueous (upper) and

organic (lower) phases. The organic phase was collected, concentrated under a stream of ultrahigh purity (UHP) nitrogen and silica gel chromatography was used to separate the total lipid extract into the neutral lipids fraction (DCM), glycolipid fraction (acetone), and phospholipid fraction (MeOH) (White et al., 1979). The neutral lipids and glycolipid fractions were stored for future analyses, while the phospholipid fraction was evaporated to dryness under a stream of UHP nitrogen then reacted to fatty acid methyl esters (FAME) through mild alkaline transesterification (Guckert et al., 1985). FAME were further purified through secondary silica gel chromatography with 5 mL hexane, 5 mL of DCM and 5mL MeOH. The FAME-containing DCM fraction was blown down to 1 mL under a stream of UHP nitrogen.

Fatty acid methyl esters were quantified and identified on an Agilent 7890B/5977A GC-MS, where 1 μ L of FAME were injected using splitless mode into an HP-5MS column using helium (He) as a carrier gas. The temperature program for separating FAME began at 50 °C, increased at 20 °C per minute up to 130 °C, then at 4 °C per minute up to 160 °C and held isothermal for 5 minutes, then increased at 8 °C per minute up to 300 °C and held isothermal for 5 minutes. Quantification of FAME were completed using total ion current and external standards. FAME were identified through comparison of mass fragmentation patterns and retention times to commercially available standards (Matreya Inc. Bacterial Standards and Supelco 37 FAME mix).

Fatty acids are composed of long chains of hydrogen and carbon and can either be saturated or unsaturated. Saturated fatty acids have a single bond between carbon atoms (resulting in saturation with hydrogen atoms) and unsaturated fatty acids have one or more double bonds. The naming convention for PLFAs is based on the number of carbon

atoms followed by how many double bonds are present with some indication of the bond location. For example, 14:0 is a saturated fatty acid that consists of 14 carbons with single bonds only, while 16:1 is an unsaturated fatty acid that consists of 16 carbon atoms with a single double bond. The observed FAME were grouped into five categories based on these structural differences: saturated, monounsaturated, polyunsaturated, branched, and cyclopropyl. Whereas monounsaturated FAME have only one double bond, polyunsaturated FAME have multiple double bonds (e.g. 16:2, 16:3). Branched chain fatty acids are typically saturated and have methyl branches on the carbon chain, the most common of which are iso and anteiso branched fatty acids. Cyclopropyl fatty acids are saturated with respect to hydrogen and contain a ring-like structure within the molecule.

2.3 TOTAL ORGANIC CARBON PREPARATION

Endolith samples for TOC analysis were homogenized and baked at 80 °C for 48 hours to remove any remaining water. Approximately 1 g of each sample was fumigated with hydrochloric acid for an additional 48 hours to remove any trace inorganic carbon. Finally, fumigated samples were baked again (post-acidification) at 80 °C for 48 hours to remove excess moisture.

2.4 ISOTOPIC ANALYSES

Radiocarbon analysis of the fatty acid methyl esters were performed as a compound class rather than on individual fatty acids. Extracted FAME with at least 50 µg C were transferred with 1 mL of DCM to prebaked 6mm quartz tubes and dried under a stream of ultra-high purity (UHP) nitrogen. Cupric oxide and silver wire were added to the tubes, and they were evacuated on the vacuum line prior to being flame-sealed. The flame-sealed evacuated tubes were baked at 900 °C for 2 hours. The evolved CO₂ was

purified and quantified before being sent to the University of Georgia's Center for Isotopic Analysis for ^{14}C analysis using a CAIS 0.5 MeV accelerator mass spectrometer and separate ^{13}C analysis using a stable isotope ratio mass spectrometer. The transesterification cleaves the polar head group from the molecule and replaces it with a methyl. The methanol used during transesterification was assumed to radiocarbon-free ($\Delta^{14}\text{C} = -1000\text{‰}$) and the following equation was used to correct for this:

$$\Delta^{14}\text{C}_{\text{PLFA}} = [(N+1) * \Delta^{14}\text{C}_{\text{measured}} - \Delta^{14}\text{C}_{\text{MeOH}}]/N, \quad (1)$$

where N is the number of carbon atoms within the individual fatty acid compounds, $\Delta^{14}\text{C}_{\text{MeOH}}$ is the $\Delta^{14}\text{C}$ composition of the methanol used in the transesterification (assumed to be -1000‰), $\Delta^{14}\text{C}_{\text{measured}}$ is the isotopic composition of the fatty acid methyl ester and $\Delta^{14}\text{C}_{\text{PLFA}}$ is the isotopic composition of the phospholipid fatty acid. The radiocarbon data is reported in $\Delta^{14}\text{C}$ notation and is expressed in per mill (‰), while the $\delta^{13}\text{C}$ values are reported in standard delta notation relative to Pee Dee Belemnite (PDB). In addition, $\Delta^{14}\text{C}$ values were corrected to $\delta^{13}\text{C}$ values of -25‰ to correct for isotopic fractionation. For the PLFA $\Delta^{14}\text{C}$ values, the error was estimated to be $\pm 20\text{‰}$. For the TOC $\Delta^{14}\text{C}$ values, the error was estimated to be $\pm 5\text{‰}$.

Figure 2.1: Study area map showing the location of the Sør Rondane Mountains in East Antarctica and sites where endolith samples were collected: Utsteinen, Dubois, Dry Valley, and Perlebandet.

Figure 2.2: Near-surface pigmentation of an endolith sample, indicating microbial colonization, with rock hammer for scale.

CHAPTER 3

RESULTS

3.1 TOTAL ORGANIC CARBON OF EAST ANTARCTIC ROCKS

The TOC content within the rock fragments ranged from 0.2 to 1.1% (Table 3.1). The $\Delta^{14}\text{C}$ values of the TOC within these samples were overall depleted relative to the recent atmosphere, ranging from -164 to $-4 \pm 5\text{‰}$. In four of the five total organic carbon samples, $\Delta^{14}\text{C}_{\text{TOC}}$ was significantly depleted relative to the current atmosphere: Dry Valley 1 ($-164 \pm 5\text{‰}$), Dubois ($-138 \pm 5\text{‰}$), Perlebandet ($-90 \pm 5\text{‰}$) and Dry Valley 2 ($-70 \pm 5\text{‰}$). The corresponding ages for these TOC samples range from 1,435 to 579 years BP in uncalibrated ^{14}C years. $\Delta^{14}\text{C}_{\text{TOC}}$ from Utsteinen were the least depleted ($-4 \pm 5\text{‰}$), indicating the presence of decadal-aged carbon - about 33 years BP in uncalibrated ^{14}C years. The $\delta^{13}\text{C}$ values of the TOC were depleted and ranged from -34 to $-26 \pm 0.5\text{‰}$. $\delta^{13}\text{C}_{\text{TOC}}$ from Dry Valley 1 was the most isotopically depleted ($-34 \pm 0.5\text{‰}$), followed by Utsteinen ($-32 \pm 0.5\text{‰}$), and Dubois ($-31 \pm 0.5\text{‰}$). $\delta^{13}\text{C}_{\text{TOC}}$ values from Dry Valley 2 ($-27 \pm 0.5\text{‰}$) and Perlebandet ($-26 \pm 0.5\text{‰}$) were the least isotopically depleted among the endolithic TOC samples analyzed.

3.2 PHOSPHOLIPID-DERIVED FAME: ABUNDANCE AND ISOTOPIC COMPOSITION

Phospholipid fatty acid concentrations ranged from 0.9 to 6.9 $\mu\text{g/g}$ (Table 3.2). The highest abundance of PLFA was observed at Utsteinen (6.9 $\mu\text{g/g}$), followed by

Dubois (3.6 $\mu\text{g/g}$), Dry Valley 2 (2.8 $\mu\text{g/g}$), and Dry Valley 1 (1.3 $\mu\text{g/g}$). Perlebandet had the lowest abundance of PLFA at 0.9 $\mu\text{g/g}$. A generic conversion factor of 2×10^4 cells pmol^{-1} (Green & Scow, 2000) was used to convert PLFA concentrations to viable cell estimates. The calculated cell abundances ranged from 6.5×10^7 to 5.0×10^8 cells/g of sample, where the greatest number of viable cells were observed at Utsteinen (5.0×10^8 cells/g) and were an order of magnitude higher than the estimate for Perlebandet (6.5×10^7 cells/g). While great care was taken to collect areas with the greatest colonization (i.e. more pigment), it is very possible that uncolonized zones within the rocks were incorporated during the sampling process – resulting in diluted lipid concentrations and cell abundances. It is therefore possible that the actual PLFA and viable cell values are higher than what was measured.

Overall, the distribution of FAME varied at each location – indicating variability in microbial community composition (Figure 3.1). The most abundant FAME were polyunsaturated fatty acids (PUFA), predominantly 18:2 and 18:3, which are fairly ubiquitous types of FAME among many different microorganisms. However, they are generally known to be indicative of fungi (Frostegard & Bååth, 1996; Zelles, 1999), algae (Boschker & Middleburg, 2002), and cyanobacteria (Dijkman et al., 2010; Zelles, 1999) within a microbial community. PUFA comprised 64% of the FAME for Dubois, 63% of the FAME for Utsteinen, 56% of the FAME for Dry Valley 2, 15% of the FAME for Perlebandet, and 9% of the FAME for Dry Valley 1 (Table 3.2). Monounsaturated fatty acids (MUFA) were the second most abundant type of FAME observed in every sample, mostly 18:1 and 16:1, are generally considered to be indicative of gram-negative bacteria (Boschker et al., 2005; Zelles, 1999) or methanotrophs (Boschker & Middleburg, 2002;

Zelles, 1999). MUFA comprised 48% of the FAME for Perlebandet, 30% of the FAME for Dry Valley 1, 16% of the FAME for Dubois, 14% of the FAME for Dry Valley 2 and 12% of the FAME for Utsteinen. Branched chain fatty acids (BCFA) were nearly as abundant as MUFA and found in every sample, predominantly i-16:0, and are thought to represent gram-positive (Green & Scow, 2000; Zelles, 1999) and gram-negative bacteria (Zelles, 1999) in the microbial community. BCFA comprised 38% of the FAME at Perlebandet, 36% of the FAME at Dry Valley 1, 14% of the FAME at Dry Valley 2, 8% of the FAME at Dubois, and 7% of the FAME at Utsteinen. Saturated fatty acids (SFA), mostly 16:0, are ubiquitous FAME and found in many different organisms, including cyanobacteria (Dijkman et al., 2010). SFA were less abundant than PUFA, MUFA, or Branched, and was only found in four of the five endolith samples, comprising 17% of the FAME at Utsteinen, 16% of the FAME at Dry Valley 1, 12% of the FAME at Dubois, and 11% of the FAME at Dry Valley 2. The least abundant category of FAME - cyclopropyl fatty acids (cyclo) - were only detectable in one sample and consisted predominantly of cyclo-17:0, which may be indicative of gram-negative bacteria (Zelles, 1999) within the microbial community. Cyclo represented about 9% of the FAME at Dry Valley 1.

The $\Delta^{14}\text{C}$ values of the viable microbial membranes (as PLFA) ranged from -111 to $62 \pm 20\%$ (Table 3.1). $\Delta^{14}\text{C}$ of PLFA from Perlebandet were the most depleted in radiocarbon ($-111 \pm 20\%$), followed by Dry Valley 1 ($-14 \pm 20\%$). The corresponding ages for these two PLFA samples ranged from 944 to 114 years BP in uncalibrated ^{14}C years – indicating the presence of aged carbon as a source within the endolithic system. $\Delta^{14}\text{C}$ of PLFA were enriched within the remaining three samples: Dry Valley 2 ($31 \pm$

20%), Dubois ($33 \pm 20\%$), and Utsteinen ($62 \pm 20\%$). These signals correspond to modern radiocarbon ages and the presence of bomb carbon, demonstrating some variability in carbon sources to Antarctic endoliths.

The $\delta^{13}\text{C}$ values of PLFA ranged from -39 to $-35 \pm 1\%$ (Table 3.1). $\delta^{13}\text{C}_{\text{PLFA}}$ of endoliths from Dry Valley were the most isotopically depleted (-39 to $-36 \pm 1\%$), followed by Perlebandet ($-38 \pm 1\%$), then Utsteinen ($-36 \pm 1\%$). $\delta^{13}\text{C}_{\text{PLFA}}$ of endoliths from Dubois were the least isotopically depleted ($-35 \pm 1\%$) among the samples analyzed.

Table 3.1: East Antarctic endolith data showing % total organic carbon (TOC), $\delta^{13}\text{C}$ values for TOC and viable membrane lipid (PLFA), $\Delta^{14}\text{C}$ values for TOC and PLFA, concentrations of PLFA ($\mu\text{g/g}$), and estimated cell abundance (# cells/g sample) based on PLFA concentrations.

Sample ID	Area	TOC (%)	TOC $\delta^{13}\text{C}$ (‰)	TOC $\Delta^{14}\text{C}$ (‰)	TOC Uncal. ^{14}C Age (Yrs BP)	PLFA $\delta^{13}\text{C}$ (‰)	PLFA $\Delta^{14}\text{C}$ (‰)	PLFA Uncal. ^{14}C Age (Yrs BP)
PES 25	Perlebandet	0.7	-26	-90	758	-38	-111	944
PES 2	Utsteinen	1.1	-32	-4	33	-36	62	modern
PES 16	Dry Valley 1	0.2	-33	-164	1435	-39	-14	114
PES 3A	Dry Valley 2	0.4	-27	-70	579	-36	31	modern
PES 7A	Dubois	0.5	-31	-138	1192	-35	33	modern

Table 3.2: East Antarctic endolith data showing PLFA concentrations ($\mu\text{g/g}$), estimated cell abundance (# cells/g sample) based on PLFA concentrations, and the distributions of different types of PLFA (in mol %) for each sample.

Sample ID	Area	PLFA Conc. ($\mu\text{g/g}$)	Est. # cells/g	<u>PLFA Distributions (mol %)</u>				
				Saturated	MUFA	PUFA	Branched	Cyclo
PES 25	Perlebandet	0.9	6E+07	0%	48%	15%	38%	0%
PES 2	Utsteinen	6.9	5E+08	17%	12%	63%	7%	0%
PES 16	Dry Valley 1	1.3	1E+08	16%	30%	9%	36%	9%
PES 3A	Dry Valley 2	2.8	2E+08	11%	14%	56%	14%	0%
PES 7A	Dubois	3.6	3E+08	12%	16%	64%	8%	0%

Figure 3.1: Distribution of different types of fatty acid methyl esters (FAME) in mol % for individual endoliths.

CHAPTER 4

DISCUSSION

4.1 ENDOLITHIC MICROBES ARE PRIMARILY USING RECENT ATMOSPHERIC CARBON

Radiocarbon analyses of lipids from the viable microbial community (as PLFA) show that, overall, East Antarctic endoliths are cycling carbon faster than previously thought. The most recent $\Delta^{14}\text{C}$ values of atmospheric CO_2 for East Antarctica were $16 \pm 3\text{‰}$ in 2016 and $12 \pm 3\text{‰}$ in 2017 (I. Levin, pers. communication, March 25, 2019), where it is steadily declining over time at a rate of about 5‰ per year due to the combustion of radiocarbon-dead fossil fuel carbon and the uptake of bomb carbon by the biosphere and ocean. East Antarctic endoliths appear to be incorporating carbon that was in equilibrium with the recent atmosphere. The $\Delta^{14}\text{C}_{\text{PLFA}}$ of endoliths from Dry Valley 2 ($31 \pm 20\text{‰}$) and Dubois ($33 \pm 20\text{‰}$) were consistent with recent atmospheric $\Delta^{14}\text{C}$ values (Table 3.1). The simplest explanation for the modern carbon in the membrane lipids is that these microbial communities are cycling carbon over yearly or decadal time scales. One way this could occur is through autotrophic fixation of atmospheric carbon and heterotrophic consumption of modern bioavailable carbon. If this carbon had been fixed hundreds or thousands of years ago, it would have undergone radioactive decay and the resultant composition of the PLFA would be much more depleted in radiocarbon. Therefore, for two of the samples in this study, the most plausible explanation for the radiocarbon content of the viable membrane lipids is that these microbes are actively

cycling carbon. However, because the history of atmospheric $^{14}\text{CO}_2$ is complex, there could be another explanation for the “modern” ^{14}C composition of the membrane lipids. The radiocarbon composition of atmospheric CO_2 can be altered by variability in cosmic ray flux (natural production) or through artificial production, such as high-energy weapons testing. Nuclear weapons testing in the late 1950’s and early 1960’s introduced an excess of artificial radiocarbon into the atmosphere, resulting in very enriched $\Delta^{14}\text{C}$ values shortly thereafter. This “pulse” has been used as an isotopic tracer for ecosystem uptake of bomb carbon. Thus, bomb carbon can be used to determine the activity of microbes that are slowly growing over decades. If the $\Delta^{14}\text{C}$ of the PLFA is enriched relative to today’s atmosphere, it can be assumed that bomb carbon is present and that the microbes are likely cycling carbon on a decadal scale. Therefore, while the $\Delta^{14}\text{C}_{\text{PLFA}}$ of the endoliths from Dry Valley 2 and Dubois ($31 \pm 20\text{‰}$ and $33 \pm 20\text{‰}$, respectfully) were within error of the $\Delta^{14}\text{C}$ of modern atmospheric CO_2 , it is also possible that these isotopic values could also be due to the mixing of old carbon from very slowly cycling cells and bomb carbon taken up by microbes that cycle on decadal time scales. Similarly, the more enriched Utsteinen $\Delta^{14}\text{C}_{\text{PLFA}}$ ($62 \pm 20\text{‰}$) indicates the incorporation of bomb carbon. The fact that these microbial lipids contain carbon that is within error of the recent atmosphere is indicative that East Antarctic endoliths are cycling carbon on a decadal scale or less.

While most of the microbial PLFA contained carbon that was recently in equilibrium with the atmosphere, several PLFA samples contained older carbon. Perlebandet and Dry Valley 1 had the most depleted $\Delta^{14}\text{C}_{\text{PLFA}}$ values in the study ($-110 \pm 20\text{‰}$ and $-14 \pm 20\text{‰}$, respectfully), indicating the presence of aged carbon consistent

with ages of 945-115 BP in uncalibrated ^{14}C years. There are several scenarios that could explain the aged carbon in the PLFA: 1) the endoliths are slowly-growing on a hundred-year scale (i.e. not very active), 2) heterogeneous distribution of microbial colonization and carbon sources within the rock itself, or 3) the endoliths are actively recycling older carbon in the system. While previous studies have argued that these extremophilic communities are likely slow-growing in order to survive in such a harsh environment (Friedmann, 1982; Johnston & Vestal, 1991), the predominantly modern $\Delta^{14}\text{C}_{\text{PLFA}}$ values seen in this study, coupled with recent work in the McMurdo Dry Valleys (Brady et al., 2018; Colesie et al., 2016; Zazovskaya et al. 2016), suggests that the scenario in which the microbes are growing on a hundred-year scale may be unlikely. Therefore, another explanation for the aged carbon within the PLFA is needed.

Evidence of the second scenario, where heterogeneous distribution of microbial colonization and carbon sources within the rocks could result in aged ^{14}C values, two samples from the same site yielded different results. Low concentrations preclude the ability to have multiple samples within the same rock, so – rather than sampling the same rock multiple times – two rocks were sampled from the same location: Dry Valley. While viable membrane lipids from Dry Valley 2 contained modern carbon ($\Delta^{14}\text{C}_{\text{PLFA}} = 31 \pm 20\text{‰}$), Dry Valley 1 revealed the incorporation of older carbon ($\Delta^{14}\text{C}_{\text{PLFA}} = -14 \pm 20\text{‰}$). The contrast between these two samples could be due to a greater abundance of active microorganisms within the sampled region from one rock (Dry Valley 2) versus the sampled region from the other (Dry Valley 1). Another potential cause for this difference is a recent mechanical loss of stabilized (i.e. preserved) organic carbon (both living and dead biomass) from one or both of the Dry Valley samples. As endoliths colonize and

continue to grow within the structural cavities of a rock, they biogenically weather away lithologic flakes (Friedmann, 1982) – likely resulting in the loss of stored carbon from various areas of the rock. Therefore, the aged ^{14}C value from Dry Valley 1 could be due to less weathering and the resultant accumulation of older carbon in the system.

Evidence of the third scenario, where East Antarctic endoliths could be actively recycling relict carbon from within the system, a variety of carbon sources to these microorganisms exist. Potential sources include CO_2 from the atmosphere, CO_2 within the rock itself, carbonate, and stabilized organic carbon comprised of both living and dead biomass (Figure 4.4). Endolithic communities within the McMurdo Dry Valleys were found to use significantly variable carbon sources, where $\Delta^{14}\text{C}_{\text{PLFA}}$ values may reflect a mixture of more than one source (i.e. different isotopic compositions) (Brady et al., 2018). The availability of carbon sources to endoliths is likely a function of location, where local environmental conditions can impact the stability of carbon within the rock. For example, a horizontally-oriented rock is better for preserving endolithic organic matter as opposed to a vertical rock face, which is subject to enhanced physical weathering due to gravity (Zazovskaya et al. 2016). As weathered lithologic fragments flake away, carbon stored within the rock is gradually lost and may result in heterogeneous distribution of both biomass and carbon sources within the subsurface. Although the rocks collected for this study were generally friable, it is still very possible that relict carbon remains within the East Antarctic endolithic systems and is being actively recycled. The $\Delta^{14}\text{C}_{\text{PLFA}}$ values that resemble modern atmosphere (Utsteinen, Dry Valley, and Dubois) may be a result of isotopically-depleted (i.e. aged) carbon being mixed with

isotopically-enriched bomb carbon, indicating that endoliths are cycling carbon on a decadal scale, rather than yearly, scale.

The radiocarbon content of the viable microbial membranes from East Antarctic endoliths reported here appears to differ from those of West Antarctic endoliths (i.e. McMurdo Dry Valleys). In the McMurdo Valleys, $\Delta^{14}\text{C}_{\text{PLFA}}$ of samples from University Valley (UV) had values that ranged from -199 to $-74 \pm 20\text{‰}$, which correspond to uncalibrated ^{14}C ages of 1780 to 660 years BP while $\Delta^{14}\text{C}_{\text{PLFA}}$ from Farnell Valley (FV) was modern ($40 \pm 20\text{‰}$) (Brady et al., 2018). Overall, we found that East Antarctic endoliths are incorporating younger carbon (ranging 944 years BP to modern) than those in the McMurdo Valleys. This could indicate that East Antarctic endoliths are more active (i.e. cycling carbon faster) than West Antarctic endoliths. The difference in microbial activities may be a result of geographic location, where the East Antarctic study site is positioned at a lower latitude than the McMurdo Dry Valleys. Given the proximity of the Dry Valleys to the south pole, it's possible that endoliths in this region are exposed to greater seasonal extremes in the form of longer austral winters and shorter austral summers. Therefore, East Antarctic endoliths may be growing under more amenable conditions than those in West Antarctica and are more active as a result.

Another possible explanation for the differences between East and West Antarctic endoliths could be the elevations at which the samples were collected, where UV is located at a higher elevation (1,800 m.a.s.l) than FV (1,700 m.a.s.l) (Brady et al., 2018) and all of the East Antarctic sites (ranging 1280-1670 m.a.s.l). Previous work found that endolithic biomass was older at higher elevations as the cold, desiccating conditions allow for greater preservation of organic material, but inhibit community

productivity (Colesie et al., 2016). Therefore, the simplest explanation for the older ^{14}C ages of PLFA within the McMurdo Valleys could be that there is greater preservation of old carbon coupled with slower-growing endoliths.

4.2 TOTAL ORGANIC CARBON IS OLDER THAN VIABLE BIOMASS

The total organic carbon (TOC) – comprised of both living and dead biomass – in East Antarctic endolithic systems are generally more depleted than the carbon within the viable microbial membranes (Figure 4.1). In general, the $\Delta^{14}\text{C}_{\text{TOC}}$ of endolithic systems are less depleted at lower elevations and near the coast, where temperatures are warmer and water is more readily available (Colesie et al., 2016). While the East Antarctic Sør Rondane mountains are more distal from the coast than the McMurdo Dry Valleys, the $\Delta^{14}\text{C}_{\text{TOC}}$ values from this study are comparable to those from the Dry Valleys (Colesie et al., 2016; Brady et al., 2018) and from several coastal oases in East Antarctica (Zazovskaya et al. 2016). The $\Delta^{14}\text{C}_{\text{TOC}}$ at Utsteinen ($-4 \pm 5\text{‰}$), Dry Valley 1 ($-164 \pm 5\text{‰}$), Dry Valley 2 ($-70 \pm 5\text{‰}$), and Dubois ($-138 \pm 5\text{‰}$) were significantly more negative than the corresponding $\Delta^{14}\text{C}_{\text{PLFA}}$, which ranged from -14 to $62 \pm 20\text{‰}$. Overall, the depleted TOC relative to PLFA suggest that the microbes are incorporating carbon that is younger than the stabilized organic carbon within the endolithic system. However, the $\Delta^{14}\text{C}_{\text{TOC}}$ from Perlebandet ($-90 \pm 5\text{‰}$) was surprisingly younger than the $\Delta^{14}\text{C}_{\text{PLFA}}$ ($-111 \pm 20\text{‰}$), suggesting that the stabilized organic carbon (living and dead biomass) within the endolithic system is younger than the microbial PLFA. One explanation is that ancient PLFA could have been preserved within the endolithic system, as was thought to be the case for McMurdo Valley endoliths (Brady et al, 2018). While the preservation of ancient PLFA under extremely cold and dry conditions remains poorly understood and is

worth consideration, it is unlikely. Therefore, another explanation for the aged PLFA and young TOC existing within the same system is needed.

Of all the sampling sites, Perlebandet is at the lowest elevation (1,280 m.a.s.l) and is the nearest location to the coast. In general, ice-free areas in East Antarctica are considered to have moist coastal climates and are very different from dry valleys – which were previously thought to be the most common type of environment on the Antarctic continent (Balks et al. 2013; Zazovskaya et al. 2016). Recent work in the McMurdo Dry Valleys found a strong correlation between the isotopic composition of TOC, elevation, and proximity to the coast (Colesie et al., 2016). Given Perlebandet's proximity to the coast and elevation, it is possible that the climate in this area was more favorable to the mechanical weathering of the rocks compared to the other sampling sites. In addition, due to its distance from other nuntataks, Perlebandet receives some of the strongest katabatic winds in the area. As a result, older (isotopically-depleted) stabilized carbon that may have been in the process of being recycled by some of the endoliths could have been lost, resulting in a younger TOC signal while the microbes were still cycling “out” the older carbon on a decadal scale.

4.3 MICROBIAL COMMUNITY COMPOSITION

There appear to be strong correlations between microbial community composition and $\Delta^{14}\text{C}_{\text{PLFA}}$. In general, PLFA structures differ between different life forms. However, some PLFA are ubiquitous and can indicate a suite of organisms. Therefore, identifying the different PLFA structures within an endolithic sample is helpful in determining a broad brushstroke of microbial community composition. To explore the relationship

between community makeup and $\Delta^{14}\text{C}_{\text{PLFA}}$, radiocarbon signals were plotted against different types of PLFA (Figure 4.3).

There was a substantial statistical relationship between the radiocarbon content of the PLFA and the type of PLFA present, thus indicating that microbial community composition plays a role in how quickly microbes are cycling carbon (Figure 4.3). In Utsteinen, Dry Valley 2, and Dubois endolith samples, PUFA comprise over 50% of the PLFA identified. In contrast, endolith samples with a higher abundance of MUFA and branched PLFA had the least modern $\Delta^{14}\text{C}_{\text{PLFA}}$ signals. In Perlebandet and Dry Valley 1, the combined MUFA and Branched comprise over 50% of the PLFA identified. A strong statistical relationship ($R^2=0.91$) exists between the relative abundance of PUFA and $\Delta^{14}\text{C}_{\text{PLFA}}$, while equally strong negative correlations ($R^2=0.92$ and $R^2=0.95$, respectfully) are seen between the relative abundances of MUFA and Branched fatty acids and $\Delta^{14}\text{C}_{\text{PLFA}}$. These infer that autotrophy-dominated communities are using relatively young carbon and are therefore cycling carbon quite quickly.

4.4 VIABLE CELL ABUNDANCES WITHIN ENDOLITHIC SYSTEMS

A strong correlation ($R^2 = 0.72$) exists between PLFA-derived cell abundances and $\Delta^{14}\text{C}_{\text{PLFA}}$, where larger cell abundances correlate to younger carbon within the microbial membrane (Figure 4.4). Similar to $\Delta^{14}\text{C}_{\text{TOC}}$ values, biomass appears to vary as a function of coastal proximity and elevation, where larger biomasses (comprised of living and dead cells) correlate to higher elevations and increased distance from the coast (Colesie et al., 2016). Calculated cell abundances were 10^7 to 10^8 cells/gram of sample, which are comparable to recent estimates from the McMurdo Valleys (Brady et al., 2018). Utsteinen had the highest cell abundance (5.0×10^8 cells/g), followed by Dubois

(2.6×10^8 cells/g), and Dry Valley 2 (2.0×10^8) (Table 1). Dry Valley 1 (9.7×10^7 cells/g) and Perlebandet (6.5×10^7 cells/g) had the lowest overall cell abundances in the study. If cell abundances are considered representative of living biomass, the endolithic communities that colonized the Utsteinen, Dubois, and Dry Valley samples are more bountiful than those that colonized the from Dry Valley 1 and Perlebandet samples.

Figure 4.1: $\Delta^{14}\text{C}$ ‰ values for the TOC (circles) and PLFA (triangles) of endolithic communities in Dronning Maud Land, East Antarctica. The dashed line represents modern atmospheric CO_2 (about 40‰). Error bars are displayed for all points, where TOC errors are too small to be visible. This shows that Utsteinen has the youngest $\Delta^{14}\text{C}$ age for PLFA.

Figure 4.2: Schematic from Ziolkowski et al., 2013 showing carbon pathways and sources to endolithic microbial communities.

Figure 4.3: The correlation between PLFA abundance (in mol %) and the $\Delta^{14}\text{C}$ of the viable microbial membrane (in ‰; error $\pm 20\%$) for saturated, polyunsaturated (PUFA), monounsaturated (MUFA), and branched fatty acids.

Figure 4.4: Plot showing the strong correlation between $\Delta^{14}\text{C}$ (in ‰; error $\pm 20\text{‰}$) and PLFA-derived cell abundances (in cells/g).

CHAPTER 5

CONCLUSIONS

Antarctic endoliths were previously thought to be some of the slowest-growing (e.g. geological time scales) microbial communities on Earth due to climatic extremes including desiccation, katabatic winds, and freezing temperatures. However, recent work using natural abundance radiocarbon analysis of biomarkers found that West Antarctic endoliths were likely cycling carbon on centurial to decadal scales instead of millennial scales (Brady et al., 2018), but very little was known about extremophilic communities residing in East Antarctica. Using natural abundance radiocarbon analysis of the viable microbial membrane (as PLFA), this work illustrates that endoliths from the Dronning Maud Land region of East Antarctica are cycling carbon on decadal scales or less, which is much faster than previous estimates of endolithic microbial activity. The cellular abundances in this study were comparable to those from the McMurdo Dry Valleys and had a robust statistical relationship to radiocarbon age, indicating that endolith communities with larger biomass are likely more active. Certain FAME distributions also had a strong relationship to radiocarbon age – indicating a greater abundance of photosynthetic microorganisms in PLFA samples containing younger carbon. This work demonstrates that Antarctic endoliths may be more active on a continental scale than previously thought and possesses some astrobiological implications. If life ever existed on Mars (or other planets), then evidence of such may be found in one of the last refugium available – inside the rocks.

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