The Organic Matter of Lake Vida, Antarctica:
Biogeochemistry of an Icy Planetary World Analog

BY

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THESIS
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I dedicate this dissertation to my parents.

Thank you, with every fiber of my being, for giving me life and light.

And to all the spacefaring microbes or conscious inhabitants of other strange worlds:

you are not alone.

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I begin this acknowledgement by noting that my experience at the University of Illinois at Chicago has been, to say the least, life-changing—and it was only made possible with a multitude of individuals and organizations.

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Parts of my graduate studies were spent at summer courses and workshops, during which I was able to garner a wonderful network of science friends and collaborators. I thank the International Geobiology Course for introducing me to the wide expansiveness and applications of geobiology, and all the amazing young scientists that I met there who are now well on their way to conquering their respective fields. I also thank those that I have met and interacted with at summer courses in Spain, Iceland, Sweden, and even the warm and breezy corner of Pasadena, California, all of whom continue to motivate and inspire me to further scratch at the doors of new and exciting science.

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L.C.

CONTRIBUTION OF AUTHORS

The research conducted that resulted in this dissertation was only possible with contributions from numerous individuals. The following detail contributions from various co-authors of each chapter:

Chapter II is a reprint of a manuscript for which I am the primary author. The article was published in *Organic Geochemistry*. Dr. Fabien Kenig devised the project. I performed the experiments, analyzed the data, produced the figures, and wrote the manuscript. Dr. Fabien Kenig, Dr. Alison Murray (Desert Research Institute) and I interpreted the results. Dr. Kenig, Dr. Murray, Dr. Peter Doran (Louisiana State University), and Dr. Christopher Fritsen (Desert Research Institute) conceived the original idea and collected the samples from Antarctica. All authors contributed to the manuscript.

Chapter III is written in the form of a manuscript for which I am the primary author, to be submitted to *Geochimica et Cosmochimica Acta*. Dr. Fabien Kenig aided in the mass spectral interpretation. I performed the experiments, analyzed the data, interpreted the results, produced the figures, and wrote the manuscript. All authors contributed to the manuscript.

Chapter IV is written in the form of a manuscript for which I am the primary author, to be submitted to *Geobiology*. I performed the lipidomics experiments, analyzed the data, interpreted the results, produced the figures, and wrote the manuscript. Dr. Fabien Kenig and Dr. Alison Murray collected the samples for lipids and metagenomics analysis from Antarctica. Dr. Alison Murray granted permission for the usage of metagenomics data currently held in the JGI Integrated Microbial Genomes & Microbiomes repository. Dr. Fabien Kenig supervised the project.

Chapter I and **Chapter V** are introductory and conclusion chapters for which I am the primary author.

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LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BSTFA	*N, O*-bistrifluoroacetamide
COG	Clusters of Orthologous Groups
DCM	Dichloromethane
DIC	Dissolved Inorganic Carbon
DMDS	Dimethyldisulfide
DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
$DMSO_2$	Dimethylsulfone
DMSP	Dimethylsulfoniopropionate
DMTS	Dimethyltrisulfide
DOC	Dissolved Organic Carbon
DOS	Dissolved Organic Sulfur
DOM	Dissolved Organic Matter
DOXP	1-deoxy-*D*-xylulose 5-phosphate
EC	Enzyme Commissioner
ESI-FT-ICR-MS	Electrospray ionization Fourier transform-ion cyclotron resonance-mass spectrometry
FA	Fatty Acid
FAMES	Fatty Acid Methyl Esters
FESEM	Field Emission Scanning Electron Microscopy
GC	Gas Chromatography
GC×GC	Multidimensional Comprehensive Gas Chromatography
GDGT	Glyceroldialkylglyceroltetraether
HMG	β-Hydroxy β-methylglutaryl
HP	Hewlett Packard
IMG/M	Integrated Microbial Genomes and Microbiomes
IPP	Isopentenyl diphosphate
JGI	Joint Genome Institute
KEGG	Kyoto Encyclopedia of Genes and Genomes
LGM	Last Glacial Maximum

LV	Lake Vida
LVBr	Lake Vida brine
MeSH	Methanethiol
MDVs	McMurdo Dry Valleys
MEP	2-*C*-methyl-*D*-erythritol 4-phosphate
MVA	Mevalonate
MS	Mass Spectrometry
NASA	National Aeronautics and Space Agency
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
OSCs	Organic Sulfur Compounds
Pfam	Protein family
SAM	S-Adenosylmethionine
TC	Total Carbon
TIC	Total Ion Current
TLE	Total Lipid Extract
TOC	Total Organic Carbon
PTFE	Polytetrafluoroethylene
POM	Particulate Organic Matter
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
UCM	Unresolved Complex Mixture
VOCs	Volatile Organic Compounds
VOSCs	Volatile Organic Sulfur Compounds
XANES	X-ray Absorption Near Edge Structures
XEDS	X-ray Energy Dispersive Spectroscopy

SUMMARY

The biogeochemistry of Lake Vida, Antarctica, was investigated by studying the organic matter composition in a cold, isolated, subzero, hypersaline, anoxic, and aphotic brine environment. The brine of Lake Vida, encapsulated within the thick (27+ m) of Lake Vida ice, harbors an exclusively bacterial community that are metabolically active at maintenance level. Lake Vida is a unique analog for icy planetary worlds. Studying the organic biosignatures that are produced, processed, and transformed in Lake Vida can enhance our understanding of life in subzero habitats and inform our search for extant biosignatures of life on other icy worlds, such as Mars, Europa, or Enceladus.

The standing crop of organic biosignatures in Lake Vida brine contains an abundant legacy component. Analysis of the dissolved organic matter of Lake Vida brine revealed that legacy paleometabolites from past environmental conditions (i.e., chlorophyll-derivatives) remained in the brine after millennia of isolation. The presence of organic sulfones further supports the existence of a large legacy signature in the brine. To study the modern organic biosignatures, we analyzed the particulate organic matter in the brine. As with dissolved organic matter fraction, the particulate organic matter fraction also contains legacy components. Large, eukaryotic detritus and legacy lipids that were indicative of a different, possibly photosynthetic, community were detected. We performed targeted metagenomics analysis to verify that the modern microbial community does not contain the genetic

instructions necessary to produce the putative legacy lipids. Our results indicate that Lake Vida brine contains abundant legacy organic matter in both the dissolved and particulate fraction. However, metagenomics analysis suggests that some of the lipids observed in the particulate organic matter fraction could potentially be modern.

Ultimately, the presence of legacy biosignatures in the Lake Vida brine organic matter suggest that organic biosignatures of previous ecosystems may be preserved in isolated ecosystems for long timescales. The prevalence of legacy in Lake Vida brine is due to the slow-growing, cold-limited lifestyle of the modern microbial assemblage. We posit that the abundance of legacy in other cold-limited, slow-growing ecosystems, on Earth and on other icy worlds, is not likely trivial and may affect the standing crop of the organic biosignatures of an environment at a given time. Thus, the search for signs of extant life on other icy planetary worlds must be undertaken with caution as organic biosignatures of ancient life may also be present, or potentially overwhelming the organic biosignatures of the modern community. However, combined with metagenomics analysis, potentially achieved by space-capable sequencing technology (i.e., nanopore sequencing), may allow for an integrated analysis of organic biosignatures, providing an avenue for detecting modern biosignatures, and thus, the presence of extant life.

CHAPTER I

INTRODUCTION

Our fascination with the search for life in the universe begins with fundamental questions on how life began, flourished, evolved, and persisted on our planet. The answers to these critical questions would inform our scientific search for signs of life in the solar system and beyond. Efforts to look for *in situ* evidence of life began with the iconic NASA Viking missions dedicated to exploring the regolith of Mars for signs of active metabolisms and any organic constituent that could support life (Biemann et al., 1977). The findings from this mission triggered an enormous effort to improve our detection strategies for planetary exploration. On Earth, discoveries of life in extreme environments further paved the way for investigations of analogs of planetary conditions that could host life, such as hydrothermal vents, geothermal springs, acid and hypersaline lakes, arid and polar deserts, and the deep subsurface (see reviews by Rothschild and Mancinelli, 2001, and Edwards et al., 2012). Some

of the best interpretations we can make about the ubiquity of life elsewhere requires a rigorous examination of the pervasiveness of life here on Earth.

The most useful terrestrial analogs of planetary environments are places that we consider "extreme"—at least on the scale of human tolerance. Microbial life seems to persist, and even thrive, in a myriad of locations that are seemingly hostile to biological activities. The limitation of life, as we know it, imposed by physiological conditions that apply evolutionary stress to the inhabiting biological systems, is at the core of life's ubiquity on Earth and, most likely, elsewhere. However, concurrent with our increased understanding of the limitations of life, our knowledge of biosignatures must also increase.

Biosignatures are defined as molecules, elements, substances, or features that can be used as evidence for life (past or present) and are distinguishable from abiogenic background (Domagal-Goldman et al., 2016). Molecular biosignatures, synonymous to *biomarkers*, are compounds or metabolites that can be traced to a living organism or molecular fossils for which we can identify their biochemical counterparts (Summons et al., 2008). The NASA Astrobiology Roadmap highlights a set of priority goals and objectives for the search for life in the solar system using biosignatures (Des Marais et al., 2008). One of these goals is to identify biosignatures that can be used to characterize past or present life in extraterrestrial samples returned to Earth or *in situ*. The 2015 NASA Astrobiology Strategy further recommended areas of needed research in the exploration of habitable conditions on Earth that would inform the search for biosignatures elsewhere. For example, one of the key research questions "How can

we enhance the utility of biosignatures to search for life in the solar system and beyond?" (Section 5.4, pg. 100) specifically deems the recognition of extant biosignatures and how they are produced, processed, and preserved in environments as an important feat in astrobiological investigations, and suggest the strategies we can utilize to recognize them within the context of those particular environments (Hays et al., 2015).

Our knowledge of extant (modern) biosignatures principally came from studies of robust ecosystems that pervade Earth's surface or oceans, which are typically rich in solar-derived energy (photosynthesis) or mineral-derived energy (chemolithotrophy). However, recent research has begun to recognize the importance of understanding life that inhabits low energy systems (Jones et al., 2018), especially life that is not directly in contact with surface processes, such as in the deep subsurface, oligotrophic marine sediments (Biddle et al., 2006) and ocean floor subsurface (Cowen et al., 2003; Edwards et al., 2012), as well as cold-limited ecosystems in icy environments (Murray et al., 2012). In all of these extreme environments, slow metabolism is dedicated mostly to cell maintenance and repair and, thus, the standing crop of metabolites from extant organisms may be limited. As a result, other competing reactions, such as abiotic processes, may influence the biosignature composition of a community at a given time. This enforces a critical constraint on the information that can be obtained from the current standing crop of metabolites, as it could be representing not only the extant community, but potentially an overwhelming signal from the past community that may have existed in a completely different environment, or other abiotic processes that

accompanied those biosignatures' transformations. Low energy systems are likely pervasive in the solar system, such as within the deep subsurface or under the ice caps of Mars, as well as in brine pockets and inclusions within ice or the deep oceans of Europa or Enceladus. Thus, a key impetus in characterizing organic biosignatures in low energy systems is to separate the past organic contributions from those of the present community, in an effort to distinguish past life biosignatures (dead) from extant life biosignatures (alive). Such characterization would inform exploration missions and help implement better detection methods planned for the search for extant life in the icy bodies of our solar system such as Mars, Europa, or Enceladus.

In this dissertation, I address three major questions:

1) **What biosignatures are produced, processed, and transformed in a hypersaline, subzero, and subsurface icy planetary analog environment?**
2) **What interpretations can we make regarding the presence of contemporary biosignatures, and thus, the occurrence of extant life, in these types of cold environments?**
3) **What are the current strategies that can facilitate the detection and identification of organic biosignatures in these types of environments using modern space-capable technology and instruments?**

These three questions drove the scientific approach and experimental design of this research. I attempted to answer these questions by, first, characterizing the metabolites extracted from a modern, subsurface, cold (-13 °C), aphotic, and anoxic interstitial brine under the thick ice of Lake Vida (East Antarctica). To do so, I employed the use of environmental metabolomics, the study of the interaction between organisms and their environments, to elucidate the biogeochemistry of the Lake Vida brine ecosystem. Second, I characterized novel organic sulfones that represent a fraction of the organic matter in Lake Vida brine and may hold clues to the modern or ancient processes that dominate the system. Lastly, I analyzed the total lipids (lipidome) extracted from the particulate organic matter collected on filters, which likely represent a majority of the modern biomass currently living in the Lake Vida brine ecosystem. I compared the lipidome to the metagenome of the Lake Vida brine microbial community in order to infer specific modern or ancient processes that contributed to the total organic carbon pool of the Lake Vida brine ecosystem.

1.1. Thesis outline

This dissertation consists of three research chapters, as well as introductory and conclusion chapters. It begins with this chapter, **Chapter I**, a general overview of the McMurdo Dry Valleys, Lake Vida brine as an endmember subzero liquid environment on earth, methods in environmental metabolomics, lipidomics, metagenomics, and organic geochemistry used in

this research, as well as an overview of the current state of knowledge and strategies currently implemented in the search of *in situ* organic matter on other icy planetary worlds. **Chapter II** of this dissertation addresses the practicality of using metabolomics as an analytical tool to elucidate the organic matter composition, and thus, biogeochemistry of the extant microbial community in Lake Vida brine This chapter is already published in the journal *Organic Geochemistry* (Chou et al., 2018). **Chapter III** describes the tentative identification of novel organic sulfones observed in the dissolved organic fraction of Lake Vida brine. The potential sources and transformations of these compounds are discussed. Chapter III will be submitted to *Geochimica Cosmochmica Acta*. **Chapter IV** details the stu dy of lipids (lipidomics) obtained from the particulate organic matter collected from Lake Vida brine, which, theoretically, represents modern organic matter of the Lake Vida brine microbial assemblage. This data is interpreted concurrently with a targeted metagenomics analysis to seek for the genetic potential for the Lake Vida brine microbial assemblage to produce those lipid biosignatures. I conclude this dissertation in **Chapter V**, where I synthesize the major findings of this research, and describe directions for future work that may be derived from the results of this study, as well as draw upon the scientific contribution of this research to the field of astrobiology.

1.2. Life in subzero conditions

The Earth's biosphere, including the deep ocean, is comprised of approximately 85% permanently cold habitats (temperatures below 5°C; Margesin and Miteva, 2011). Temperature is one of the most important constraints that limit the distribution and colonization of life on earth. Yet, even in environments that are consistently below 0°C, such as marine seafloors, permafrost soils and brine pockets (cryopegs), or high-altitude clouds, psychrophilic microorganisms, which are defined here as "cold-loving" (Boetius et al., 2014), are found to be either metabolically active (at maintenance level) or growing (Price, 1999; Price and Sowers, 2004). The imposition of low temperatures on biological activities results in slow kinetics of biochemical reactions and ultimately enforces physicochemical constraints that biomolecules experience at low temperatures (Siddiqui and Cavicchioli, 2006). Consequently, cold environments have selected for organisms that have adapted various strategies to overcome the stress factors caused by low temperatures. These strategies include the upregulation of cold-adapted enzymes, production of cryoprotectants, anti-freeze, or ice-binding proteins, upregulation of polyunsaturated and branched fatty acids, peptidoglycan, and other membrane proteins associated with transport of solutes, and the production of exopolysaccharides (Deming, 2002; De Maayer et al., 2014). Organisms in cold environments possess the ability to retain physical membrane flexibility in order to support structural integrity and allow for biochemical catalysis that occurs across cell membranes (Boetius et al., 2014). In addition to

the low temperature regime, psychrophiles often encounter additional stresses associated with cold environments, such as desiccation (permafrost soils or lithic communities), variable pH (sea ice), excessive UV and radiation (high-altitude clouds and alpine communities), high osmotic pressure (deep-sea brines, cryopegs, polar hypersaline lakes), and low availability of nutrients (oligotrophic marine environments). As a result, many cold-adapted psychrophiles possess mechanisms that can overcome these types of extreme conditions as well (D'amico et al., 2006).

1.3. McMurdo Dry Valleys, East Antarctica

The dry regions of Antarctica, such as the McMurdo Dry Valleys, in East Antarctica (Figure 1a), Ellsworth and Transantarctic Mountains, coastal Antarctic Peninsula in West Antarctica, as well as the Vestfold Hills, make up only 0.4% of the total area of Antarctica that is ice-free (Cary et al., 2010). Within these ice-free regions, the aquatic ecosystems can range from fresh to hypersaline, encompassing lacustrine systems that are subglacial, epiglacial, permanently ice-covered, or perennially ice-free, as well as those containing mixed or highly stratified water columns (De Maayer et al., 2014).

The largest of all ice-free regions in Antarctica is the McMurdo Dry Valleys (MDVs; Fountain et al., 1999), which are a network of glacially carved valleys that lay in generally west-to-east direction, located between the Polar Plateau and the Ross Sea in Southern Victoria Land

Antarctica (Figure 1b). The MDVs are an extreme cold desert environment, with mean annual temperatures ranging from -15 to -30 °C, and annual precipitation rate of less than 100 mm per year (Doran 2002). Because of this, biological communities in the MDVs are generally limited to microorganisms that are constantly exposed to environmental stresses such as extremely cold temperatures, desiccation, as well as extreme seasonal variation of sunlight (Priscu et al., 1999).

The MDVs host a variety of permanently ice-covered lakes (with the exception of an open hypersaline centimeters-deep pond, Don Juan Pond; Doran et al., 2002) that range from ~5 to 70 m in depth (Doran et al., 1994). These lakes vary from freshwater to hypersaline, can be fed by shallow groundwater or surface water (Chinn, 1993), and may be present in a closed-basin (endorheic), or in an open-basin (exorheic). In addition, the ice-covers attenuate light penetration as well as restrict wind mixing and gas exchange with the atmosphere, enhancing the stability of stratified water columns.

The history of the MDV lakes is well-documented (Kellogg et al., 1980; Doran et al., 1999, 2014; Hall et al., 2002; Wagner et al., 2006, 2011) and was reviewed in detail by Doran et al. (1994). Briefly, the present lakes such as Lake Vanda in Wright Valley (Hall et al., 2001), Lake Fryxell and Lake Bonney (Kellogg et al., 1980) in Taylor Valley (Figure 1b) are likely remnants of larger glacial lakes that occupied the valleys during the Last Glacial Maximum (LGM). During drier and colder conditions, the lakes may have evaporated to near dryness, leading to increasing concentrations of solutes. During warmer climatic regimes, glacial melt

input contributed freshwater into the lake basins. These freshwaters remained above the monimolimnion (the lower layer of a meromictic lake) due to the density contrast, creating the stratified lake conditions that are observed in most of the modern MDV lakes (Doran et al., 1994).

The perennial ice-covers of the MDV lakes can range from 3 to 6 m in thickness (Doran et al., 2004), forming a physical barrier of ice and limiting the lakes' interaction with the atmosphere. These ice covers, which form at the ice water interface, are ablated in the summer and ice loss can measure up to 1.62 m (Dugan et al., 2013). Moats can form around the lake perimeter and streams can flow into the lakes and beneath the ice cover, introducing sediments and nutrients, especially during rare high melt years when moats are significantly larger (Doran et al., 2004). While this is true for the perennially ice-covered lakes, Lake Vida (77°39'S, 161°93'E), in Victoria Valley (Figure 1c), seems frozen to the ground (up to 27 m of ice was measured; Dugan et al., 2015b) and its ice formed differently (Doran et al., 2004). During the summer, meltwater flows above the permanent ice instead of under it. This causes the thick lake ice to grow from the surface of the lake up. Lake Vida's thick ice (27+ m), makes it the thickest nonglacial lake ice on Earth (Dugan et al., 2015b). Below 15.8 m, the ice of Lake Vida contains an interstitial brine that is completely isolated from the atmosphere. This brine harbors a microbial community of extant bacteria (no archaea or eukaryotes were detected; Murray et al., 2012). The production, processing, and preservation of organic matter in Lake Vida's ice-sealed brine is the subject of this dissertation.

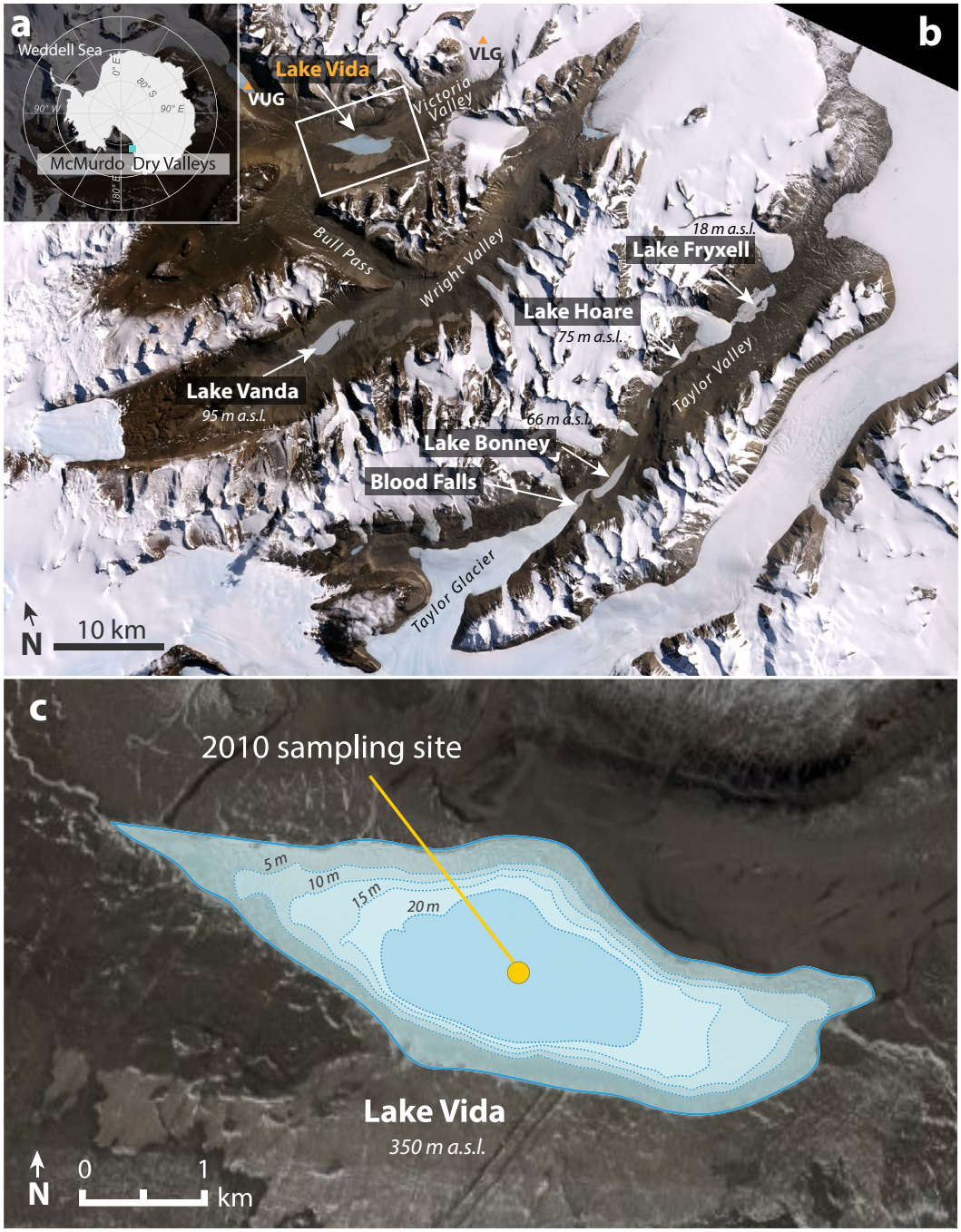

Figure 1: (a) Map of Antarctica showing the location of McMurdo Dry Valleys**. (b)** Satellite image of McMurdo Dry Valleys showing the location of major ice-covered lakes, as well as the subglacial brine Blood Falls. **(c)** Bathymetric lines and location of the 2010 sampling site (77.39ºS, 161.93ºE) for collecting Lake Vida ice core and Lake Vida brine. The orange triangles represent the Victoria Upper Glacier (VUG) and the Victoria Lower Glacier (VLG). The satellite image was acquired by Robert Simmon on December 18, 1999, based on data provided by the NASA GSFC Oceans and Ice Branch and the Landsat 7 Science Team. Bathymetric lines of the ice-cover were obtained from Dugan et al. (2015b).

1.4. Lake Vida brine

Lake Vida is located in a closed basin at the center of Victoria Valley (Figure 1c). It has a surface area of ~6.8 km^2, with a length of ~3.5 km and a width of ~1 km. Lake Vida overlays in part, basement rocks of Victoria Valley, such as the granite of the Orestes Pluton and crosscutting mafic dykes (Allibone et al., 1993; Turnbull et al., 1994), and in part, glacial drifts including abundant dolerite, derived from the glacial erosion of the Jurassic Ferrar Dolerites, which surrounds Lake Vida North, East and South. The quaternary glacial drifts cover an extensive portion of the surface of Victoria valley and date to 17,000 to 21,000 years ago (Webster et al., 1994).

The surface meltwaters of Victoria Valley are derived from several major glaciers (Figure 1b). The Victoria Lower Glaciers (East of Lake Vida) and the Victoria Upper Glaciers (West of Lake Vida) form meltwater streams that feed into Lake Vida during the Austral summer (Hall et al., 2002). Since Victoria Valley is a closed basin, all drainages eventually lead to Lake Vida. Groundwater movements in the MDVs are not yet fully understood, though the dynamics of this hydrological network is important to the physical properties and evolution of the region, as well as the stability of biological communities that persist within the subsurface. Subsurface imaging of Lake Vida revealed that the brine below is connected to an extensive network of interstitial fluids that either permeate into fractures of basement rock, or situates within unconsolidated sediments at the lake bottom (Dugan et al., 2015a). This subsurface

interstitial fluid, akin to groundwater, was also detected beneath glaciers, lakes, and permafrost in Taylor Valley (Mikucki et al., 2015), and may be related to the geological history of the MDV lakes where marine intrusions and subsequent drawdown of the paleolakes are likely related to climatic variation during the Last Glacial Maximum.

Drilling endeavors were carried out to explore the depth of Lake Vida in 1960's, but all attempts to penetrate the lake were largely unsuccessful beyond the depth of 11.5 m (Calkin and Bull, 1967). Because of this, it was thought that Lake Vida was likely frozen to its base. However, resistivity measurements conducted over a decade later revealed the presence of a low resistivities horizon at ~40 m below the center of the lake, indicative of a body of saline, unfrozen liquid, or lake sediments (McGinnis et al., 1973). Indeed, when Doran et al. (2003) led a team to conduct deep ice drilling of Lake Vida in 1996, they encountered wet saline ice at 15.8 m below the surface, showing the existence of a trapped, cold, hypersaline brine.

At present, the interstitial brine of Lake Vida is aphotic, anoxic, very cold (-13.4°C), with a salinity of 188 psu (Murray et al., 2012). The brine was observed directly down to 27 m in a borehole (Murray et al. 2012) and an airborne electromagnetic survey revealed that the brine network extends ~30 m down from the surface to a depth of 100 m (Dugan et al., 2015a). Radiocarbon ages of microbial organic matter obtained at 12 m within the ice suggest that Lake Vida brine has been isolated from the environment for at least ~2800 ^{14}C y (Doran et al., 2003).

The present day Lake Vida brine (LVBr) hosts a temperature-limited, solely bacterial community where the average generation time was estimated at 120 years (Murray et al., 2012).

This rate is comparable to the value predicted for maintaining metabolism at LVBr temperature (Price and Sowers, 2004). Due to the brine's unusual and complex geochemistry (i.e., high levels of reduced and oxidized nitrogen and sulfur species, dissolved metals like Fe and high levels of dissolved organic carbon), it is difficult to precisely determine which of the geochemical resources allow for the persistence of this very slow-growing microbial community.

1.5. Environmental metabolomics, metagenomics, and lipidomics

Environmental metabolomics is the study of the interaction between organisms and their environments using evidence obtained from all metabolites (metabolome) present in an organism or ecosystem (Viant, 2007; Bundy et al., 2009). The physiology of a given ecosystem is not only dependent on the genetic capacities inscribed in the total DNA of an ecosystem (metagenome) but is also highly constrained by the environmental conditions and fluxes of energy and nutrient necessary to carry out biological functions (Kido Soule et al., 2015). Thus, understanding how environmental changes can influence the growth, development, and survivability of organisms can shed light on the capacity for genetic adaptation and ultimately capacity for changes in the biological composition or diversity in environments of interest.

A metabolome is a library of low molecular weight molecules (<1000 Da) that are present in a biological system in a particular physiological state (Fiehn, 2002). Metabolites are

the initial reactants and final products of biological processes, and their abundances represent the state of a biological system, that is ultimately dictated by genomic potential and environmental influences. In general, metabolomes encompass compounds that participate in metabolic reactions that are crucial to cell maintenance, growth, and typical biological functions. Unlike other classes of biomolecules, metabolites are not made up of repeating units of monomers. Instead, they are various combinations of organic molecules such as amino acids, carbohydrates, fatty acids, vitamins, lipids, inorganic, and elemental species (Fiehn, 2002).

Due to structural and chemical diversity, the detection of metabolites has been traditionally difficult since they cannot be sequenced like linear nucleic acids or proteins. Identification of metabolites is typically done by gas chromatography coupled to mass spectrometry (GC-MS), or other chromatography techniques coupled to MS, such as high performance liquid chromatography (HPLC) or matrix-assisted laser desorption ionization (MALDI). Gas chromatography is considered the golden standard in organic geochemistry, metabolomics, and many other fields such as pharmaceutical science and natural products science. GC is used to analyze thermally stable compounds though some molecules require chemical derivatization in order to increase volatility and thermal stability (i.e., amino acids and sugars). For example, derivatization can achieved by reacting with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MBSTFA) to form trimethylsilyl esters from exchangeable protons (Dunn et al., 2013).

Multidimensional comprehensive gas chromatography-time of flight-mass spectrometry (GC×GC-TOF-MS) can enhance sensitivity and analytical resolution via the addition of a secondary chromatographic dimension. This method uses a cryogenic modulation mechanism to inject a complex mixture of compounds eluting from a first capillary column, typically a 30 m capillary column with an apolar phase, into a second capillary column with different properties, typically a 2 m long column with a polar phase). The resulting data, a suite of secondary column gas chromatograms, can be treated to provide a chromatographic surface from which mass spectra can be obtained. Ultimately, compounds that were not separated by the first capillary column can be separated on the second capillary column (Figure 2). We utilized GC×GC-TOF-MS to study the total solvent-extractable organic matter in Lake Vida brine as GC×GC allowed for the deconvolution of the complex low molecular weight (<1000 Da) organic molecules that were preserved in Lake Vida brine; this complex mixture was not resolvable using GC alone.

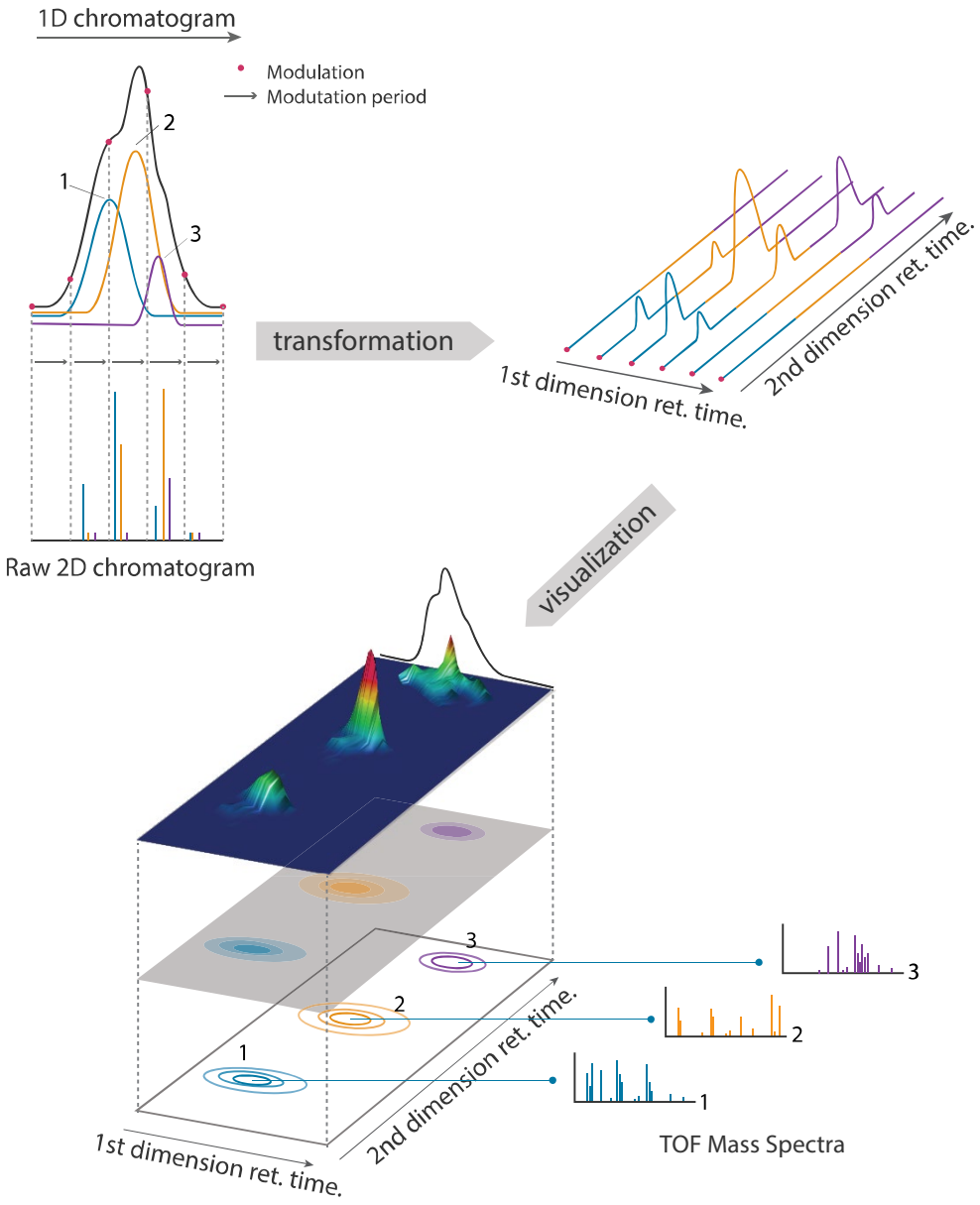

Figure 2: Schematic diagram visualizing GC×GC-TOF-MS data acquisition, chromatogram modulation, data transformation, data visualization, and mass spectra acquisition.

Metabolomics studies can be enhanced by validating information obtained from metagenomics. Metagenomics is a culture-independent method involving high throughput sequencing of environmental DNA which includes information on not only the abundance and diversity of the microbial communities but also the genetic potential for carrying out functions that are important to the ecosystem (Turnbaugh and Gordon, 2008). It has been used to investigate the infrastructure of metabolic pathways and to reconstruct contextual overviews of complex interactions that otherwise would have been difficult to ascertain using culture-dependent methods alone. Taken together with metabolomics, metagenomic sequences, especially those of novel biosynthetic pathways, can be used to guide metabolomics analysis and potentially new metabolite discovery (Tang, 2011). Similarly, lipidomics, the study of all lipids obtained from an ecosystem, is characterized by the system-level analysis of cellular lipid profiles, their functional roles, including interactions with other biomolecules and the genetic capacity for the synthesis and catabolism of those lipids (Wenk, 2005; Pearson, 2013).

Investigating a cold, subzero, hypersaline habitats such as Lake Vida brine, requires the contextual interpretation of multi "-omics" data. The unusual and complex geochemistry of Lake Vida brine, which contains high levels of reduced and oxidized inorganic species (N, and S), high levels of dissolved organic carbon, and high dissolved Fe, along with the likelihood that Lake Vida brine autochthonous sources are being processed extremely slowly suggest that organic matter obtained for metabolomics or lipidomics analysis alone are not sufficient to explicate extant biological activity. Combined with metagenomics, however, we can

confidently assess the presence of metabolites and lipids and determine whether the biogeochemistry of Lake Vida brine inferred from metabolomics and lipidomics is biologically significant to the modern inhabiting microbial community.

1.6. Lipid biomarkers as organic geochemical proxies

Biomarkers, or molecular fossils, are remnants of biomolecules that were produced, deposited, and preserved in the geologic environments (Peters et al., 2005). Despite the fact that only a minute amount of the organic matter that is produced in an ecosystem is ultimately preserved in a sedimentary setting, lipid biomarkers themselves can provide a wide variety of information about their origins and the environmental conditions during organic matter deposition and preservation. In lacustrine ecosystems, contributions from both allochthonous and autochthonous sources of organic matter can complicate the interpretation of bulk geochemical analyses (Castañeda and Schouten, 2011), such as total organic carbon(TOC), or carbon to nitrogen (C:N) ratios. Consequently, studying specific compounds that can be assigned to an organism, groups of organisms, or a specific process, can reveal the levels of interaction between those organisms within the ecosystem or allow for the reconstruction of particular biogeochemical processes that took place in that environment.

Lacustrine lipid biomarkers are also sensitive recorders of the local climate, providing valuable archives for past environmental conditions (Meyers and Ishiwatari, 1993; Castañeda

and Schouten, 2011). Some lakes may have a seasonal or permanent anoxic water column, further increasing the potential for organic matter preservation (Sobek et al., 2009). In addition, lacustrine systems host primary producers, such as cyanobacteria and algae (i.e., diatoms, dinoflagellates) that have diagnostic biomarkers (Volkman et al., 1998), allowing for shifts in community structures to be investigated and ecosystem dynamics to be reconstructed.

For high-latitude lakes, the physical (i.e., size, depth, morphology) and chemical (temperature, pH, salinity, oxygen, dissolved solutes) characteristics not only vary among each other (Quesada and Vincent, 2006), but also from other lakes around the world. These attributes are controlled by modern processes such evaporation, precipitation, ice-cover ablation, contribution of freshwater from melts, or biological activities responsible for cycling the lake organic matter, which can vary between lakes and their occupying valley. More important for the MDV lakes is the significant contribution of legacy, a carryover of past ecosystem conditions that remain persistent in the environment (Knoepfle et al., 2009; Doran et al., 2014). Thus, organic geochemical proxies that are typically applied to other lacustrine systems may not be straightforward for the MDV lakes. However, the biological diversity of these lacustrine systems is simple, with no predation or burrowing of sediments affecting the sediment deposition. Furthermore, the absence of modern vascular plants or limited anthropogenic input means that their contribution can essentially be ignored.

1.7. Lake Vida as an astrobiological analog of icy planetary worlds

Subzero brines in extreme environments have garnered much interests ever since the predicted presence of contemporary liquid brines on the surface (Cull et al., 2010; Martin-Torres et al., 2015; Ojha et al., 2015) and the subsurface of Mars (Orosei et al., 2018). Cold brines may also exist in the icy shells of the Europa (McCord et al., 2002) or Enceladus (Zolotov, 2007), expanding the potential habitable conditions that could host life in our solar system (Figure 3). The presence of concentrated solutes in these ecosystems depresses the freezing point of water and allows for liquid to remain at extremely low temperatures. Because of this, life in cold, hypersaline conditions not only would have to adapt to the temperatures well below 0°C, but also high osmotic pressures associated with the low water activity. Understanding how terrestrial life has adapted to these conditions in planetary analogs may be an important feat in developing strategies to search for life in those extraterrestrial environments.

Thus, this study on characterizing the cold, hypersaline, anoxic, and aphotic brine of Lake Vida and the history of Lake Vida itself has broader implications for astrobiology with respect to several major applications. First, this study will inform on the types of biosignatures expected in cold, hypersaline environments. Second, our understanding of the contribution of previous ecosystems in a low energy, slow-growing environment to the pool of contemporary biosignatures will be increased, allowing for a more robust interpretation of organic

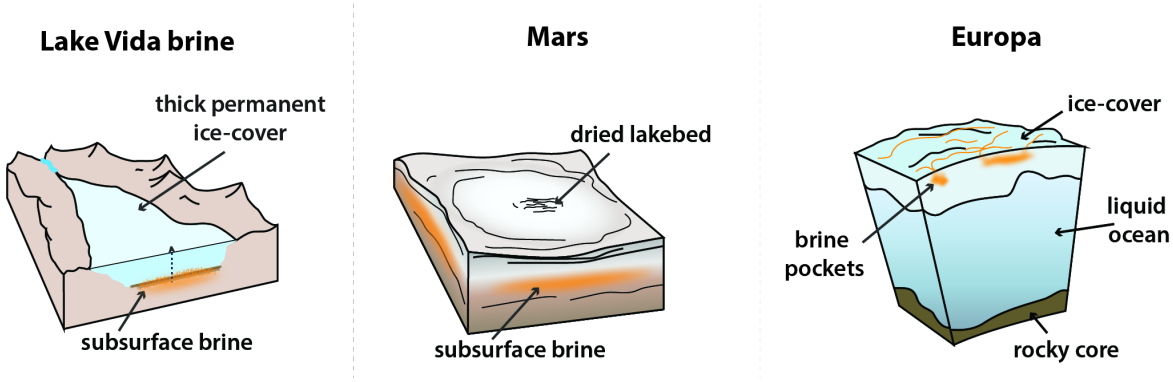

Figure 3: Lake Vida brine as an icy planetary analog of extraterrestrial environments such as the subsurface brines of Mars, or putative brine pockets within the ice shell of Europa.

geochemical investigations of icy worlds, should we plan an exploration mission to those places. And third, we may be able to resolve some of the challenges we are currently facing in modern exploration endeavors, especially on Mars, where brines may be pervasive, both in the surface and subsurface. Ultimately, this research seeks to reveal the enigmatic nature of the Lake Vida brine microorganisms, an ecosystem that lives on the brink of existence—at the edge of biogeochemical extreme, and in one of the only places on Earth that could illuminate our understanding what life could be out there, especially on or within our icy neighboring worlds.

Work Cited

Allen, A.D., Gibson, G.W., 1962. Geological investigations in Southern Victoria Land, Antarctica, Part 6: Outline of the Geology of the Victoria Valley Region. New Zealand Journal of Geology and Geophysics 5, 234–242.

Allibone, A.H., Cox, S.C., Smillie, R.W., 1993. Granitoids of the Dry Valleys area, southern Victoria Land: Geochemistry and evolution along the early Paleozoic Antarctic Craton margin. New Zealand Journal of Geology and Geophysics 36, 299–316.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proceedings of the National Academy of Sciences of the United States of America 103, 3846–3851.

Biemann, K., Oro, J., Toulmin, P., Orgel, L.E., Nier, A.O., Anderson, D.M., Simmonds, P.G., Flory, D., Diaz, A. V, Rushneck, D.R., Biller, J.E., Lafleur, A.L., 1977. The search for organic substances and inorganic volatile compounds in the surface of Mars. Journal of Geophysical Research 82, 4641–4658.

Boetius, A., Anesio, A.M., Deming, J.W., Mikucki, J., Rapp, J.Z., 2014. Microbial ecology of the cryosphere : sea ice and glacial habitats. Nature Reviews Microbiology 13, 677–690.

Bundy, J.G., Davey, M.P., Viant, M.R., 2009. Environmental metabolomics: A critical review and future perspectives. Metabolomics.

Calkin, P.E., Bull, C., 1967. Lake Vida, Victoria Valley, Antarctica. Journal of Glaciology 6, 833–836.

Cary, C., McDonald, I.R., Barrett, J.E., Cowan, D.A., 2010. On the rocks: the microbiology of Antarctic Dry Valley soils. Nature Reviews Microbiology 8, 129–138.

Castañeda, I.S., Schouten, S., 2011. A review of molecular organic proxies for examining modern and ancient lacustrine environments. Quaternary Science Reviews 1–41.

Chinn, T.J., 1993. Physical Hydrology of the Dry Valley Lakes, in: Physical and Biogeochemical Processes in Antarctic Lakes, Antarctic Research Series. pp. 1–51.

Chou, L., Kenig, F., Murray, A.E., Fritsen, C.H., Doran, P.T., 2018. Effects of legacy metabolites from previous ecosystems on the environmental metabolomics of the brine of Lake Vida, East Antarctica. Organic Geochemistry. 122, 161-170.

Cowen, J.P., Giovannoni, S.J., Kenig, F., Johnson, H.P., Butterfield, D., Rappe, M.S., 2003. Fluids from Aging Ocean Crust That Support Microbial Life. Science 299, 120–123.

Cull, S.C., Arvidson, R.E., Catalano, J.G., Ming, D.W., Morris, R. V, Mellon, M.T., Lemmon, M., 2010. Concentrated perchlorate at the Mars Phoenix landing site: Evidence for thin film liquid water on Mars. Geophysical Research Letters 37, L22203–L22203.

D'amico, S., Collins, T., Marx, J.-C., Feller, G., Gerday, C., 2006. Psychrophilic microorganisms: challenges for life. EMBO reports 7, 385–389.

De Maayer, P., Anderson, D., Cary, C., Cowan, D.A., 2014. Some like it cold: Understanding the survival strategies of psychrophiles. EMBO Reports 15, 508–517.

Deming, J.W., 2002. Psychrophiles and polar regions. Current Opinion in Microbiology 5, 301–309.

Des Marais, D.J., Nuth, J.A., Allamandola, L.J., Boss, A.P., Farmer, J.D., Hoehler, T.M., Jakosky, B.M., Meadows, V.S., Pohorille, A., Runnegar, B., Spormann, A.M., 2008. The NASA Astrobiology Roadmap. Astrobiology 8, 715–730.

Domagal-Goldman, S.D., Wright, K.E., Adamala, K., Arina de la Rubia, L., Bond, J., Dartnell, L.R., Goldman, A.D., Lynch, K., Naud, M.-E., Paulino-Lima, I.G., Singer, K., Walter-Antonio, M., Abrevaya, X.C., Anderson, R., Arney, G., Atri, D., Azúa-Bustos, A., Bowman, J.S., Brazelton, W.J., Brennecka, G.A., Carns, R., Chopra, A., Colangelo-Lillis, J., Crockett, C.J., DeMarines, J., Frank, E.A., Frantz, C., de la Fuente, E., Galante, D., Glass, J., Gleeson, D., Glein, C.R., Goldblatt, C., Horak, R., Horodyskyj, L., Kaçar, B., Kereszturi, A., Knowles, E., Mayeur, P., McGlynn, S., Miguel, Y., Montgomery, M., Neish, C., Noack, L., Rugheimer, S., Stüeken, E.E., Tamez-Hidalgo, P., Walker, S.I., Wong, T., 2016. The Astrobiology Primer v2.0. Astrobiology 16, 561–653.

Doran, P., Kenig, F., Knoepfle, L.J., Mikucki, A.J., Lyons, W.B., Doran, P.T., Kenig, F., Knoepfle, J.L., Mikucki, J.A., Lyons, W.B., 2014. Radiocarbon distribution and the effect of legacy in lakes of the McMurdo Dry. Limnology and Oceanography 59, 811–826.

Doran, P.T., Berger, G.W., Lyons, W.B., Wharton, R.A., Davisson, M.L., Southon, J., Dibb, J.E., 1999. Dating Quaternary lacustrine sediments in the McMurdo Dry Valleys, Antarctica. Palaeogeography, Palaeoclimatology, Palaeoecology 147, 223–239.

Doran, P.T., Fritsen, C.H., McKay, C.P., Priscu, J.C., Adams, E.E., 2003. Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "'ice-sealed'" east Antarctic lake. Proceedings of the National Academy of Sciences 100, 26–31.

Doran, P.T., Priscu, J.C., Lyons, W.B., Powell, R.D., Andersen, D.T., Poreda, R.J., 2004. Paleolimnology of extreme cold terrestrial and extraterrestrial environments, in: R. Pienitz,

M.S.V.D. and J.P.S. (Ed.), Long-Term Environmental Change in Arctic and Antarctic Lakes. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 475–507.

Doran, P.T., Wharton, R.A., Lyons, W.B., 1994. Paleolimnology of the McMurdo Dry Valleys, Antarctica. Journal of Paleolimnology 10, 85–114.

Dugan, H.A., Doran, P.T., Tulaczyk, S., Mikucki, J.A., Arcone, S.A., Auken, E., Schamper, C., Virginia, R.A., 2015a. Subsurface imaging reveals a confined aquifer beneath an ice-sealed Antarctic lake. Geophysical Research Letters 42, 96–103.

Dugan, H.A., Doran, P.T., Wagner, B., Kenig, F., Fritsen, C.H., Arcone, S.A., Kuhn, E., Ostrom, N.E., Warnock, J.P., Murray, A.E., 2015b. Stratigraphy of Lake Vida, Antarctica: hydrologic implications of 27 m of ice. The Cryosphere 9, 439–450.

Dugan, H.A., Obryk, M.K., Doran, P.T., 2013. Lake ice ablation rates from permanently ice-covered Antarctic lakes. Journal of Glaciology 59, 491–498.

Dunn, W.B., Erban, A., Weber, R.J.M., Creek, D.J., Brown, M., Breitling, R., Hankemeier, T., Goodacre, R., Neumann, S., Kopka, J., Viant, M.R., 2013. Mass appeal: Metabolite identification in mass spectrometry-focused untargeted metabolomics. Metabolomics 9, 44–66.

Edwards, K.J., Fisher, A.T., Wheat, C.G., 2012. The deep subsurface biosphere in igneous ocean crust : frontier habitats for microbiological exploration. Frontiers in Microbiology 3, 1–11.

Fiehn, O., 2002. Metabolomics – the link between genotypes and phenotypes. Plant Molecular Biology 48, 155–171.

Fountain, A.G., Lyons, W.B., Burkins, M.B., Dana, G.L., Doran, P.T., Lewis, K.J., McKnight, D.M., Moorhead, D.L., Parsons, A.N., Priscu, J.C., Wall, D.H., Wharton, R. a., Virginia, R. a., 1999. Physical Controls on the Taylor Valley Ecosystem, Antarctica. BioScience 49, 961.

Hall, B.L., Denton, G.H., Overturf, B., 2001. Glacial Lake Wright, a high-level antarctic lake during the LGM and early holocene. Antarctic Science 13, 53–60.

Hall, B.L., Denton, G.H., Overturf, B., Hendy, C.H., 2002. Glacial Lake Victoria, a high-level Antarctic lake inferred from lacustrine deposits in Victoria Valley. Journal of Quaternary Science 17, 697–706.

Hays, L., Achenbach, L., Bailey, J., Barnes, R., Baross, J., Chen, I., Ciesla, F., Laine, P., Lloyd, K., Lyons, T., Meadows, V., Mojzsis, S., Muller, U., Pasek, M., Powell, M., College, J., Robinson, T., Rosenzweig, F., Vance, S., Welander, P., Williams, L., Wordsworth, R., 2015. Astrobiology roadmap 2015.

Jones, R.M., Goordial, J.M., Orcutt, B.N., 2018. Low energy subsurface environments as extraterrestrial analogs. Frontiers in Microbiology 9, 1–18.

Kellogg, D.E., Stuiver, M., Kellogg, T.B., Denton, G.H., 1980. Non-marine diatoms from late Wisconsin perched deltas in Taylor Valley, Antarctica. Palaeogeography, Palaeoclimatology, Palaeoecology 30, 157–189.

Kido Soule, M.C., Longnecker, K., Johnson, W.M., Kujawinski, E.B., 2015. Environmental metabolomics: Analytical strategies. Marine Chemistry 177, 374–387.

Knoepfle, L.J., Doran, T.P., Kenig, F., Lyons, W.B., Galchenko, V.F., 2009. Particulate organic and dissolved inorganic carbon stable isotopic compositions in Taylor Valley lakes , Antarctica : the effect of legacy. Hydrobiologia 632, 139–156.

Margesin, R., Miteva, V., 2011. Diversity and ecology of psychrophilic microorganisms. Research in Microbiology 162, 346–361.

Martin-Torres, F.J., Zorzano, M.-P., Valentin-Serrano, P., Harri, A.-M., Genzer, M., Kemppinen, O., Rivera-Valentin, E.G., Jun, I., Wray, J., Bo Madsen, M., Goetz, W., McEwen, A.S., Hardgrove, C., Renno, N., Chevrier, V.F., Mischna, M., Navarro-Gonzalez, R., Martinez-Frias, J., Conrad, P., McConnochie, T., Cockell, C., Berger, G., Vasavada, A.R., Sumner, D., Vaniman, D., 2015. Transient liquid water and water activity at Gale crater on Mars. Nature Geoscience 8, 357–361.

McCord, T.B., Teeter, G., Hanse, G.B., Sieger, M.T., Orlando, T.B., 2002. Brines exposed to Europa surface conditions. Journal of Geophysical Research 107, 5004.

McGinnis, L.D., Nakao, K., Clark, C.C., 1973. Geophysical identification of frozen and unfrozen ground, Antarctica, in: Proceedings 2nd International Conference on Permafrost. Yakutsk, Russia, pp. 136–146.

Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry--an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry 20, 867–900.

Mikucki, J.A., Auken, E., Tulaczyk, S., Virginia, R.A., Schamper, C., Sørensen, K.I., Doran, P.T., Dugan, H., Foley, N., 2015. Deep groundwater and potential subsurface habitats beneath an Antarctic dry valley. Nature Communications 6, 1–9.

Mosier, A.C., Murray, A.E., Fritsen, C.H., 2007. Microbiota within the perennial ice cover of Lake Vida, Antarctica. FEMS Microbiology Ecology 59, 274–288.

Murray, A.E., Kenig, F., Fritsen, C.H., Mckay, C.P., Cawley, K.M., Edwards, R., Kuhn, E., Mcknight, D.M., Ostrom, N.E., Peng, V., Ponce, A., Priscu, J.C., Samarkin, V., Townsend, A.T., Wagh, P., Young, S.A., To, P., Doran, T.P., 2012. Microbial life at − 13 ° C in the brine of an ice-sealed Antarctic lake. Proceedings of the National Academy of Sciences 109, 20626–20631.

Ojha, L., Wilhelm, M.B., Murchie, S.L., McEwen, A.S., Wray, J.J., Hanley, J., Masse, M., Chojnacki, M., 2015. Spectral evidence for hydrated salts in recurring slope lineae on Mars. Nature Geoscience 8.11 (2015): 829.

Orosei, Flamini, E., Mattei, E., Pajola, M., Soldovieri, F., Cartacci, M., Cassenti, F., Frigeri, A., Guippi, S., Martifu, R., Masdea, A., Mitri, G., Nenna, C., Noschese, R., Restano, M., Seu, R., 2018. Radar evidence of subglacial liquid water on Mars. Science 7268, 1–9.

Pearson, A., 2013. Lipidomics for Geochemistry, 2nd ed, Treatise on Geochemistry: Second Edition. Elsevier Ltd. doi:10.1016/B978-0-08-095975-7.01022-6

Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker Guide: Biomarkers and Isotopes in the Environment and Earth History, Cambridge University Press.

Price, P.B., 1999. A habitat for psychrophiles in deep Antarctic ice. Proceedings of the National Academy of Sciences 97.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proceedings of the National Academy of Sciences 101, 4631–4636.

Priscu, J.C., Wolf, C.F., Takacs, C.D., Fritsen, C.H., Laybourn-Parry, J., Roberts, E.C., Sattler, B., Lyons, W.B., 1999. Carbon Transformations in a Perennially Ice-Covered Antarctic Lake. BioScience 49, 997–1008.

Quesada, A., Vincent, W.F., 2006. 11 . Landscape Control of High Latitude Lakes in a Changing Climate, in: Bergstrom, D.M., Convey, P., Huiskes, A. (Eds.), Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer, pp. 221–252.

Siddiqui, K.S., Cavicchioli, R., 2006. Cold-Adapted Enzymes. Annual Review of Biochemistry 75, 403–433.

Sobek, S., Durisch-kaiser, E., Zurbru, R., 2009. Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and sediment source. Limnology and Oceanography 54, 2243–2254.

Summons, R.E., Albrecht, P., McDonald, G., Moldowan, J.M., 2008. Molecular biosignatures. Space Science Reviews 135, 133–159.

Tang, J., 2011. Microbial Metabolomics. Current Genomics 12, 391–403.

Turnbaugh, J.P., Gordon, L.J., 2008. An Invitation to the Marriage of Metagenomics and Metabolomics. Cell 134, 708–713.

Turnbull, I.M., Allibone, A.H., Forsyth, P.J., Heron, D.W., 1994. Geology of the Bull Pass-St Johns Range area, southern Victoria Land, Antarctica, scale 1:50,000, Institute. ed. Institute of Geological & Nuclear Sciences.

Viant, R.M., 2007. Metabolomics of aquatic organisms: the new 'omics' on the block. Marine Ecology Progress Series 332, 301–306.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin{, F.O., 1998. Microalgal biomarkers: A review of recent research developments. Organic Geochemistry 29, 1163–1179.

Wagner, B., Melles, M., Doran, P.T., Kenig, F., Forman, S.L., Pierau, R., Allen, P., 2006. Glacial and postglacial sedimentation in the Fryxell basin, Taylor Valley, southern Victoria Land, Antarctica. Palaeogeography, Palaeoclimatology, Palaeoecology 241, 320–337.

Wagner, B., Ortlepp, S., Doran, P.T., Kenig, F., Melles, M., Burkemper, A., 2011. The Holocene environmental history of Lake Hoare, Taylor Valley, Antarctica, reconstructed from sediment cores. Antarctic Science 23, 307–319.

Webster, J.G., Brown, K.L., Vincent, W.F., 1994. Geochemical processes affecting meltwater chemistry and the formation of saline ponds in the Victoria Valley and Bull Pass region, Antarctica. Hydrobiologia 281, 171–186.

Wenk, M.R., 2005. The emerging field of lipidomics. Nature Reviews Drug Discovery 4, 594–610.

Zolotov, M.Y., 2007. An oceanic composition on early and today's Enceladus. Geophysical Research Letters 34, 1–5.

CHAPTER II

EFFECTS OF LEGACY METABOLITES FROM PREVIOUS ECOSYSTEMS ON THE ENVIRONMENTAL METABOLOMICS OF THE BRINE OF LAKE VIDA, EAST ANTARCTICA

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Abstract

Lake Vida, located in a closed basin in the McMurdo Dry Valleys, East Antarctica, permanently encapsulates an interstitial anoxic, aphotic, cold (-13°C), brine ecosystem within 27+ m of ice. Metabolically active, but cold-limited, slow-growing bacteria were detected in the brine. Lake Vida brine is derived from the evaporation of a body of water that occupied the same basin prior to ~2800 years ago. The characteristics of this body of water changed over time and, at one point, likely resembled other modern well-studied perennial ice-covered lakes of the dry valleys. We characterized the dichloromethane-extractable fraction of the environmental metabolome of Lake Vida brine in order to constrain current and ancient biogeochemical processes. Analysis of the dichloromethane-extract of Lake Vida brine by gas chromatography-mass spectrometry and comprehensive multidimensional gas chromatography-time of flight-mass spectrometry reveals the presence of legacy compounds (i.e. diagenetic products of chlorophylls and carotenoids) deriving from photosynthetic algae and anaerobic, anoxygenic photosynthetic bacteria. This legacy component dilutes the environmental signal of metabolites deriving from the extant bacterial community. The persistence of legacy metabolites (paleometabolites), apparent in Lake Vida brine, is a result of the slow turnover rates of the extant bacterial population due to low metabolic activities caused by the cold limitation. Such paleometabolites may also be preserved in other cold-limited or nutrient-depleted slow-growing ecosystems. When analyzing ecosystems with low metabolic

rates, the presence of legacy metabolites must first be addressed in order to confidently recognize and interpret the environmental metabolome of the extant ecosystem.

Keywords: Geomicrobiology, Paleometabolites, Legacy, Limnology, Cryosphere, Environmental metabolomics, Brine

2.1. Introduction

Environmental metabolomics encompasses a subset of the field of metabolomics that is used to elucidate the relationship between living organisms and their environment by characterizing the global pool of metabolites obtained directly from that environment (Viant, 2007; Bundy et al., 2009). The biological interaction between a community and its environment is expressed in a suite of metabolites that contributes to the total organic carbon pool of the ecosystem, and is a reflection of both the current environmental conditions and the genetic potential of that community (Kido Soule et al., 2015). In lacustrine ecosystems, microbial activity is largely responsible for the degradation and reworking of dissolved organic material (Meyers and Ishiwatari, 1993). Applying environmental metabolomics to lacustrine ecosystems can provide insight into the current metabolic activities of its microbial community. Combined with other meta-"omics" platforms, such as metagenomics or metatranscriptomics, environmental metabolomics can illuminate the effects of ecosystem stressors such as temperature, salinity, or nutrient limitation on metabolic pathways (Bundy et al., 2009), allowing for an unprecedented view of the biogeochemistry of the ecosystem of interest, thus providing constraints on the processes active in a community under specific environmental conditions.

We hypothesize that the standing crop of metabolites in a cold-limited, slow-growing ecosystem may contain both the metabolites derived from the active community as well as

metabolites derived from past environmental conditions (a legacy component). The co-occurrence of metabolites corresponding to current biological processes with those corresponding to legacy potentially complicates the interpretation of the environmental metabolomics data as the metabolites must first be recognized as part of a legacy component, a modern component, or as part of both. However, if metabolites corresponding to legacy can be distinguished from those deriving from current biological activity, legacy metabolites (paleometabolites) may provide useful information on past biological processes that otherwise may not be preserved in the limnological record. The contribution of legacy metabolites is likely to be observable in the cryosphere where fluxes of new metabolites may be minute relative to the amount of metabolites inherited from previous ecosystems.

All microbial communities in the McMurdo Dry Valleys (MDVs), East Antarctica (Figure 1A), are challenged by environmental stress imposed by cold temperatures, and seasonal variation of sunlight (Priscu et al., 1999). Aquatic ecosystems in the MDVs are typically dominated by microorganisms and are influenced by bottom up controls such as availability of resources and environmental conditions, rather than by top down processes such as predation and competition (Moorhead et al., 1999). For example, in perennially ice-covered lakes, the spatial and temporal variation in light, oxygen, salinity and nutrients significantly affects the community structure. In addition, in the bottom waters of these lakes, the biological activity is strongly constrained by the chemical inventory, notably dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC), that persists in the environment but is derived

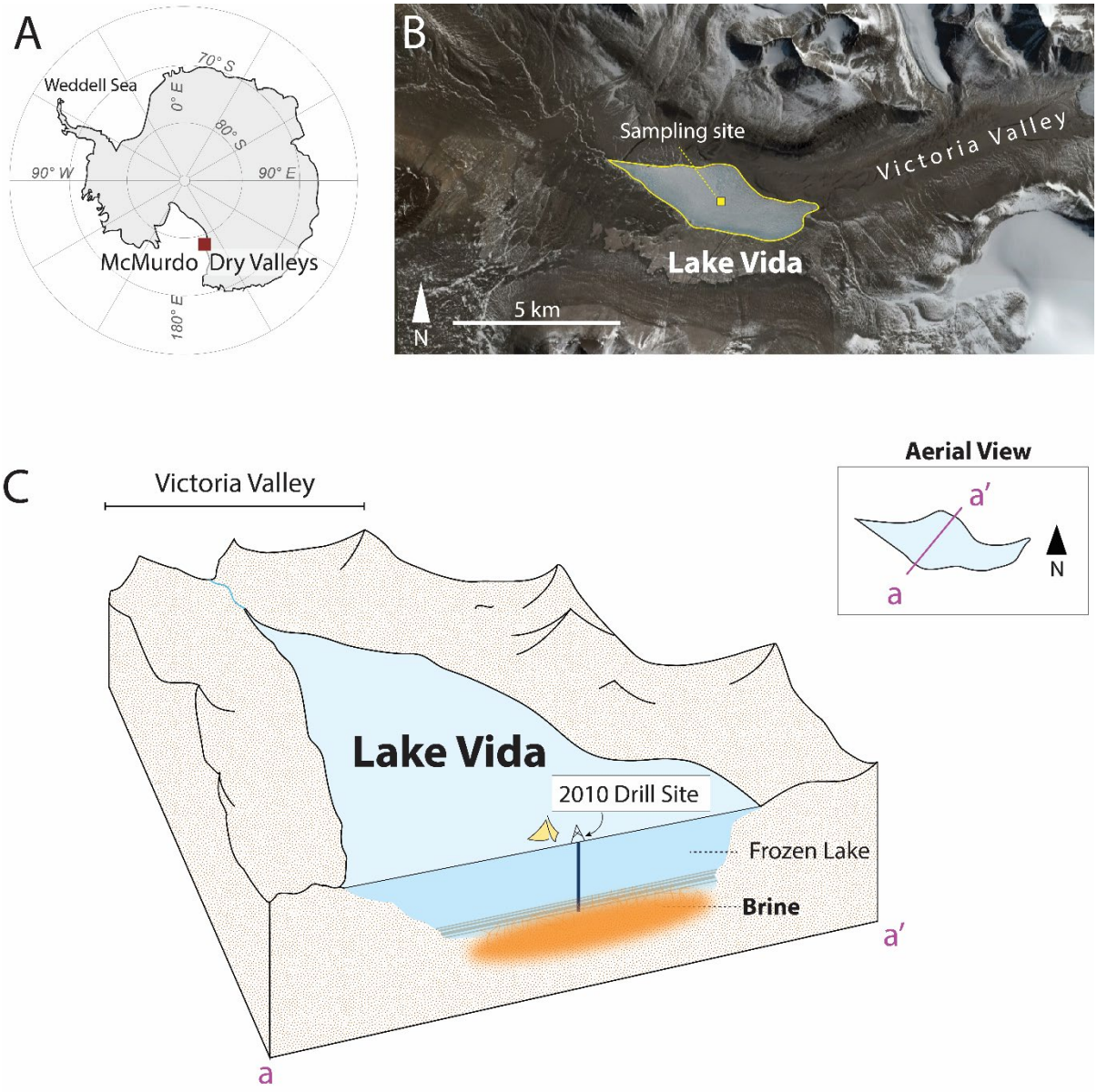

Figure 1: (A) Map of Antarctica showing the location of the McMurdo Dry Valleys. **(B)** Satellite image of Victoria Valley showing the location and outline of Lake Vida (taken by Landsat 7 on December 18, 1999). The yellow square indicates the borehole from which brine was sampled during the 2010 expedition. **(C)** Cross-section schematic of Lake Vida showing the location of the interstitial brine underneath the frozen lake body and the >10 cm sediment layers.

from processes associated with previous ecosystem conditions (Moorhead et al., 1999; Lyons et al., 2000; Knoepfle et al., 2009).

Lake Vida, located in Victoria Valley, the northernmost valley in the MDVs (Figure1B), permanently encapsulates an aphotic, anoxic, very cold (-13.4°C), interstitial brine (salinity 188) within its >27 m of ice (Figure 1C; Murray et al., 2012; Dugan et al., 2015b). Subsurface imaging of Lake Vida revealed that the brine network may extend up to 100 m deep (Dugan et al., 2015a). Radiocarbon ages of microbial organic matter obtained at 12 m beneath the surface of the ice cover suggest that Lake Vida brine (LVBr) has been isolated from the environment for at least ~2800 ^{14}C y (Doran et al., 2003). The total concentration of DOC in LVBr is high (580 mg-C·L^{-1}; Cawley et al., 2016). LVBr dissolved organic matter (DOM) has older radiocarbon ages (2955 to 4150 ^{14}C years BP; Cawley et al., 2016) than the microbial mat sampled at a depth of 12 m in the ice cover (2770±120 ^{14}C years BP; Doran et al., 2003). Spectroscopic and elemental characterization of LVBr DOM revealed low aromaticity, with predominantly hydrophilic microbial exudates (proteins, carbohydrates, amino acids, or fatty acids) that are high in N and S, suggesting that most of the organic material of LVBr is derived from ancient microbial production and have been further altered in the brine (Cawley et al., 2016). Isotopic analyses of iron and sulfur species in LVBr suggest the presence of a tightly coupled nitrogen-iron-sulfur cycle driven by both biotic and abiotic redox reactions (Proemse et al., 2017).

The history of the lake in the Vida basin in the last 5000 years BP is not well constrained but may have included the drawdown of a lake larger than the current Lake Vida into a brine and the cryoencapsulation of that brine in ice ~2800 years BP (Doran et al., 2003; Dugan et al., 2015b). The existence of a body of water occupying the Vida basin that was exposed to sunlight and the atmosphere is likely, given our current understanding of the age and composition of the brine organic material (Cawley et al., 2016) as well as Lake Vida's recent hydrological history (Dugan et al., 2015b). It is important to note that ice of Lake Vida accreted from the top of the ice cover, as it is regularly (but not annually) covered by additional ice formed from meltwater derived from the nearby alpine glaciers and ice sheet terminus (Dugan et al., 2015b; Doran et al., 2003). Under past light-penetrating conditions, the organisms responsible for autotrophic carbon fixation that would have inhabited the ancient Lake Vida were likely dominated by phototrophic communities, much like most other modern perennially ice-covered lakes in the MDVs (Priscu et al., 1999).

The present day LVBr hosts a bacterial community (neither eukaryotes nor archaea were detected) that is temperature limited, such that the average generation time was estimated at 120 years (Murray et al., 2012). This rate is comparable to the value predicted for maintaining metabolism at LVBr temperature (Price and Sowers, 2004). Due to the brine's unusual and complex geochemistry (i.e. high levels of reduced and oxidized nitrogen species present as NH_4^+, NO_3^-, NO_2^-, and N_2O, dissolved metals like Fe, and elevated amounts of sulfur dominated by SO_4^{2-} and a very high load of dissolved organic carbon), it is difficult to precisely determine

which of the geochemical resources allow for the persistence of this very slow-growing microbial community.

In subzero ecosystems such as LVBr, the carbon inventory is being processed extremely slowly. As a result, the total pool of organic compounds in the brine should reflect a combination of past and present day biological processes. The past, or legacy, contribution would be sourced from organisms that lived in the lake's previous ecosystem, as well as the capacities of past conditions to result in biotic or abiotic alteration of the organic material. In addition, organic matter from the brine should also contain a molecular signal from the active microbial assemblage, expressed as "modern metabolites". Since the amount of accumulated legacy material in a given system is dependent on the metabolic rate and capacity for the extant community to further degrade existing organic material, we speculate that other cold-limited or nutrient-limited ecosystems with slow metabolic rates, such as cryopegs (permafrost brine lenses), or deep subsurface environments, may also contain legacy compounds.

Permafrost constitutes a major portion of the cryosphere and comprises habitats that host microbial communities at subzero temperatures (see review by Jansson and Taş, 2014). Cryopegs are pockets of brine lenses in permafrost that are characterized by a high dissolved-solids content, which prevents the liquid reservoir from freezing (temperature of -6 to -11°C and salinity of 115 to 300 ppt; Gilichinsky et al., 2005; Colangelo-Lillis et al., 2016). Bacteria isolated from a Siberian cryopeg have been shown to be metabolically active in the laboratory at -10°C at very low rates (at ~7×10^{-4} per hour; Bakermans et al., 2003). *In situ* activity or direct

observation of cell reproduction in cryopegs have yet to be demonstrated. Nevertheless, bacterial communities in isolated cryopegs likely remained viable for thousands of years by limiting their biological activities to sufficient levels for cellular repair such as DNA damage from background radiation or amino acids racemization (Bakermans et al., 2003; Gilichinsky et al., 2003).

The "deep subsurface" harbors vast reservoirs of microbial life (see review by Edwards et al., 2012). The continental subsurface is oligotrophic and energy limited, a significant difference from LVBr. However, various forms of energy, such as organic matter for chemoorganoheterotrophy, are bioavailable in sedimentary rocks or brought from the surface by recharging groundwater. In addition, in the continental subsurface, carbon source such as CO_2 or CH_4, and redox species such as H_2, Fe^{3+}, or SO_4^{2-} have been shown to support chemolithoautotrophy (Chapelle and Lovley, 1990; Amend and Teske, 2005). On the other hand, the deep marine subsurface habitats consist of subseafloor sediments and crustal basement rocks with energy deriving from organic matter accumulated during marine burial, methane hydrates, or hydrogen produced by water-rock reactions (Chapelle et al., 2002; D'Hondt et al., 2002). Nevertheless, the metabolic rates of microbial life in both continental and marine settings are fundamentally limited by the fluxes of these forms of energy over time, which may be very slow due to diffusion-limited processes (Pedersen, 2000).

Price & Sowers (2004) compared metabolic rates of microbial communities from various cold surface, deep subsurface, and aeolian environments in order to calculate the

dependence of metabolic rate on temperature. They found that the organisms living in the deep subsurface such as deep aquifers (up to 388 m; Chapelle & Lovley, 1990), deep subsurface marine sediments (up to ~400 m; D'Hondt et al., 2002) and deep ice cores (~3000 m; Tison et al., 1998) are likely only using their energy towards survival (i.e. repair of DNA and protein damage) rather than for growth or maintenance (i.e. osmotic regulation, cellular pH maintenance, or motility). The metabolic rates of deep subsurface communities are ~1 to 2 orders of magnitude lower than that measured for LVBr microbes (Price and Sowers, 2004).

In this paper, we describe a liquid-liquid dichloromethane (DCM) extract of LVBr that was analyzed by gas chromatography-mass spectrometry (GC-MS) and comprehensive two-dimensional gas chromatography-time of flight-mass spectrometry (GC×GC-TOF MS). We discuss the presence of legacy compounds in this fraction of the LVBr metabolite collection. We assess the origin of the compounds detected on the basis of their structure and inferred biological origin, and attempt to elucidate some of the past metabolic processes they represent. Finally, we discuss the importance of discerning legacy metabolites from those produced by current biological processes, not only for the environmental metabolome of LVBr microbial assemblage, but also other environments where energy limitation, cold temperatures, or nutrient limitation results in slow-growing ecosystems.

2.2. Sample Collection and Methods

2.2.1 Brine collection

During the 2010 expedition to Lake Vida, brine samples were obtained at a depth of 16 m in a 18.5 m borehole in the >27 m lake ice (Figure1C; Murray et al., 2012; Dugan et al., 2015b). The clean access sampling strategy of the brine is described in Doran et al. (2008). LVBr was collected using a stainless steel submersible pump using polytetrafluoroethylene (PTFE) tubing, and stored in sterile PTFE bottles spiked with $HgCl_2$ (4 mM) to prevent further biological activities during storage at 4˚C.

2.2.2 Solvent-based environmental metabolome extraction

The total DCM-extractable fraction of LVBr (200 mL) was obtained by liquid-liquid extraction. Milli-Q® water was first extracted three times with DCM. This clean Milli-Q® water (100 mL) was then added to the brine to enhance the density difference between the salty aqueous phase and the denser DCM phase. The DCM-soluble fraction of the brine was then extracted three times using 100 mL of DCM per extraction. The extract was then rotary-evaporated to near dryness and subsequently dried under a low flow of N_2. Samples were then dissolved with cyclohexane (1 mg per mL of extract) and injected (1 µl) into the GC.

2.2.3 GC-MS and GC×GC-TOF MS

For GC-MS, a Hewlett Packard 6890 GC coupled to a HP-5973 mass selective detector, with an electron ionization mode at 70 eV and helium (>99.999% ultra-high purity, Praxair®) as a carrier gas with constant flow at 1 mL·min^{-1}, was used for the initial characterization of the DCM-soluble LVBr extract. The chromatographic column used in the GC was a 30 m long Agilent HP-5 MS (polydimethylsiloxane-95%/phenyl-5%; 0.25 mm I.D., 0.25 μm film thickness). The range of the masses scanned was *m/z* 40 to *m/z* 650 at a rate of three scans per second. The GC injector was operated in pulsed splitless mode at 320°C (40 psi). The oven temperature was kept at 60°C for 2 minutes, then ramped at 10°C·min^{-1} to 150°C and further ramped at 3°C·min^{-1} to 320°C and kept at 320°C for 20 minutes.

Due to the complexity of the DCM-soluble LVBr extract, we employed the use of a GC×GC-TOF MS, which provides a higher chromatographic resolving power and detection sensitivity that is suitable for separating low molecular weight analytes. The GC×GC-TOF MS used is a Leco Pegasus 4D system, which consists of an Agilent 7890 GC with a split/splitless injector, two capillary columns, a liquid nitrogen-cooled pulsed jet modulator, and a time-of-flight mass spectrometer. The DCM-soluble LVBr extract described above was dissolved in cyclohexane at 1 mg/mL and injected (1uL) into the GC×GC in pulsed splitless mode at 250°C (60 sec purge time). The first capillary column was a nonpolar 5% phenyl polydimethylsiloxane (SGE BPX-5, 24.47 m; 0.25 mm ID; 0.25 μm film thickness). The oven temperature for the first column was kept at 40°C for 1 minute, then ramped at 3°C·min^{-1} to 300°C, and held for 10

mins. The secondary capillary column was a medium-polarity 50% phenyl/50% polydimethylsiloxane (SGE BPX-50; 1.65 m; 0.1 mm ID; 0.1 μm film thickness). The oven temperature of the second column was programmed to remain 10°C hotter than that of the first oven. A 0.21 m, 0.1 mm ID BPX-50 was used as a transfer line to the TOF detector. Helium (>99.999% ultra-high purity, Praxair®) was used as the carrier gas in constant flow of 1 $mL \cdot min^{-1}$. The GC×GC modulation period was 6 s with a hot pulse time of 0.5 s and cool time of 2.5 s. The system was coupled to a mass spectrometer with an electron ionization mode at 70 eV and an ion source temperature of 200°C. The solvent delay was 660 seconds and the spectra were collected from *m/z* 30 to *m/z* 500 at a rate of 200 spectras per second. ChromaTOF, a Leco software package, was used for data processing, which included deconvolution algorithm and baseline correction. Mass spectra of reported compounds were characterized using the National Institute of Standards and Technology (NIST) reference library and comparison to spectra published in the literature.

2.3. Results & Discussion

Upon GC-MS, the total ion current (TIC) of the DCM-extractable environmental metabolome of LVBr displayed an unresolved complex mixture (UCM) in which several compounds can be resolved (Figure 2). This UCM consisted mainly of coeluting low molecular weight compounds (C_5 to C_{16}). The compounds that could be resolved were tentatively

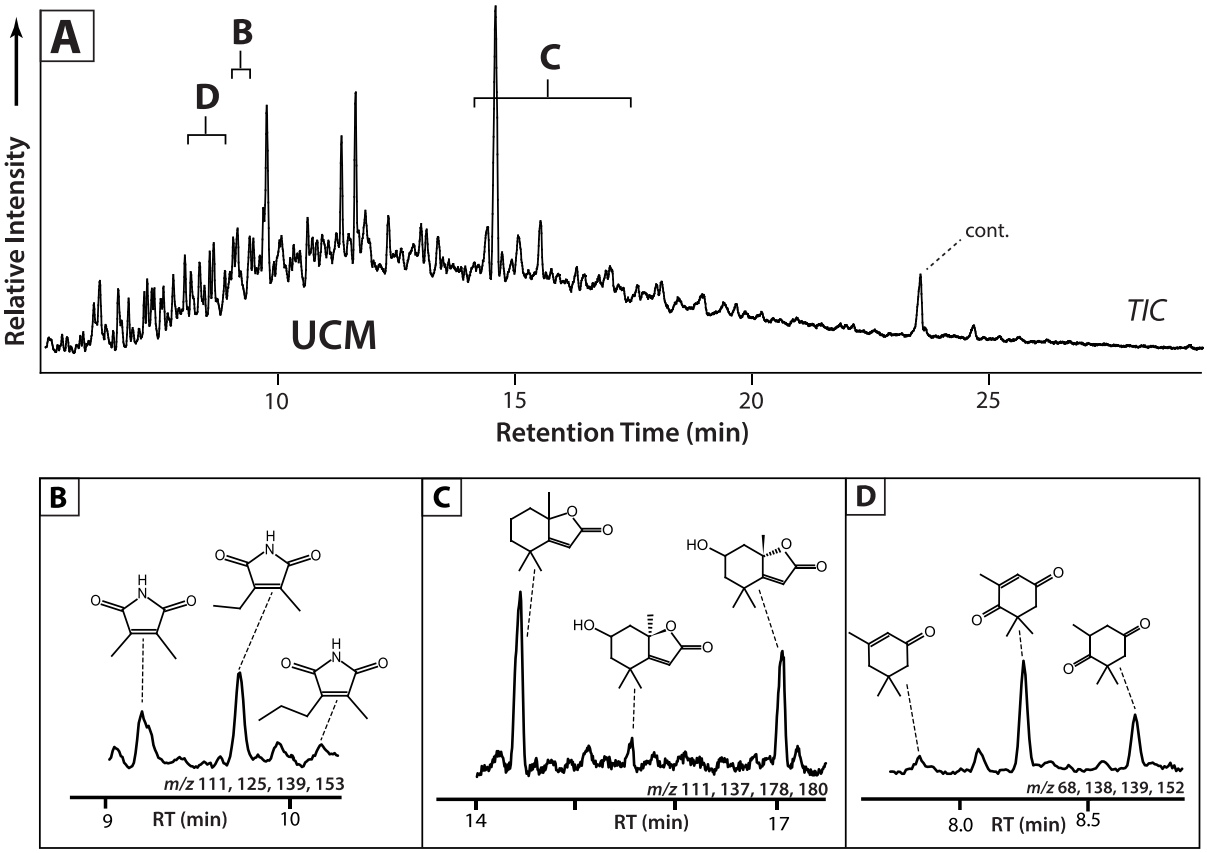

Figure 2: **(A)** GC-MS total ion chromatogram (TIC) of LVBr showing unresolved complex mixture (UCM). "Cont." = common lab contaminant. **(B)** summed ion chromatogram *m/z* 111, 125, 139, 153, major fragmentation and molecular ions for Me,Me, Me,Et and Me,Pr maleimides. **(C)** summed ion chromatogram *m/z* 111, 137, 178, and 180, major fragmentation and molecular ions for loliolide, isololiolide, and dihydroactinidiolide. **(D)** summed ion chromatogram *m/z* 68, 138, 139, and 152, major fragmentation and molecular ions for isophorone, ketoisophorone and dihydroketoisophorone. Note that the summed ion chromatograms are not presented in the order of retention time.

identified based on their mass spectral fragmentation pattern, their relative retention time, and comparison of their spectra with those published in the literature. In addition, utilization of GC×GC-TOF MS allowed for the separation of overlapping peaks in the UCM and, therefore, the tentative identification of additional compounds with structural similarities that belong to the same families (Figure 3). Below, we describe the major molecular families of compounds that comprised the DCM-extractable LVBr environmental metabolome.

2.3.1 Chlorophylls derivatives

A family of maleimides (1*H*-pyrrole-2,5,diones) was tentatively identified upon GC-MS on the basis of mass spectral fragmentation and retention time: 3-methyl-4-ethyl-maleimide (Me,Et), 3,4-dimethyl-maleimide (Me,Me) and 3-methyl-4-propyl-maleimide (Me,Pr; Figure 2B, Martin et al., 1980; Grice et al., 1996, 1997). Figure 2B shows the summed mass chromatogram *m/z* 111, 125, 139, and 153 in GC-MS, representing the major molecular ions for members of the maleimide family. The same summed mass chromatogram with addition of *m/z* 67, the major fragmentation ion for most of the maleimides, as well as *m/z* 97, the molecular ion for maleimide (H,H) obtained upon GC×GC-TOF MS is shown in Figure 4. The UCM formed upon GC-MS masked the presence of some maleimides that were resolved upon GC×GC-TOF MS, allowing for their tentative identification (Figure A1, Appendix II). In particular, maleimide (H,H), present in low abundance (Figure 4A), as well as an additional

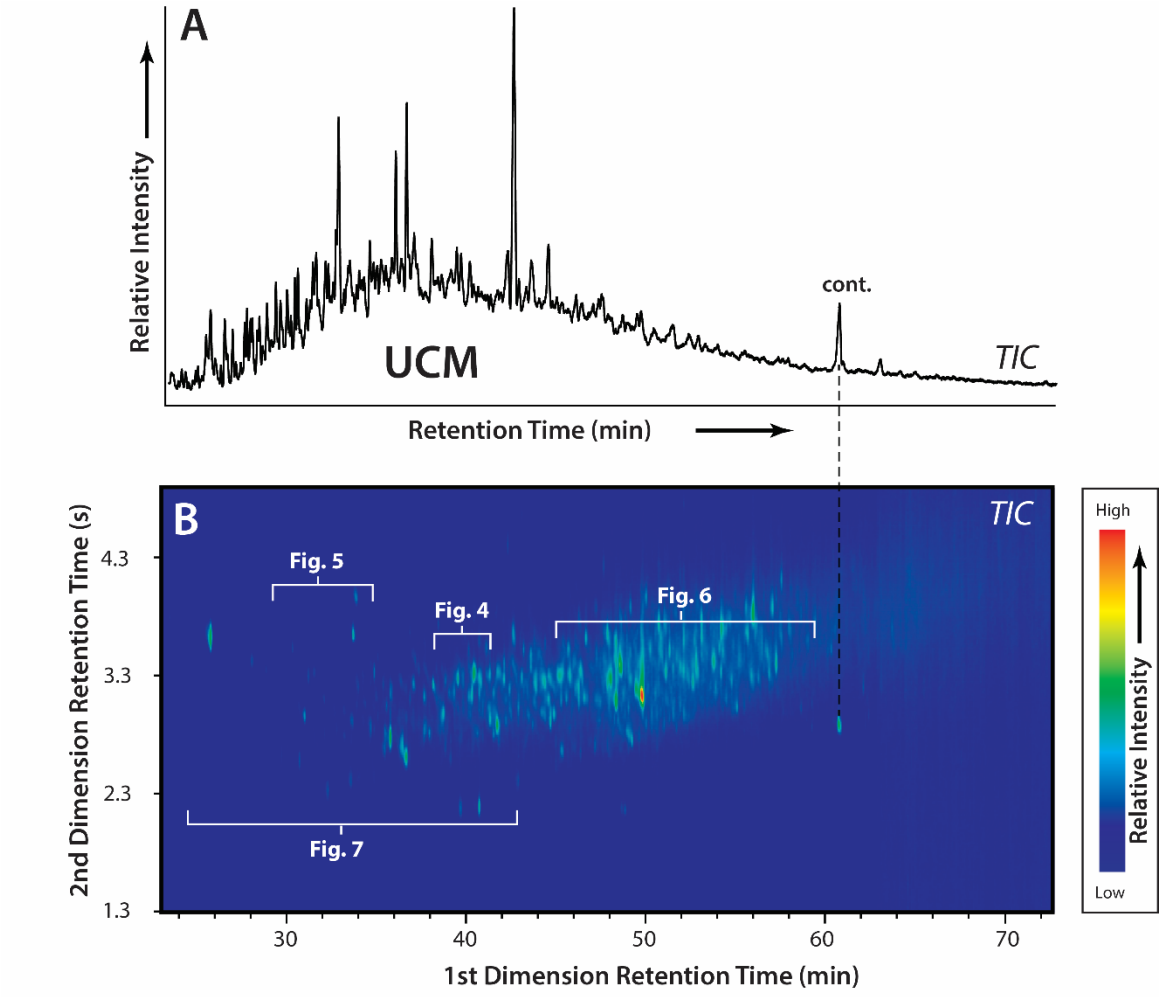

Figure 3: (A) GC-MS TIC of Lake Vida brine total DCM-extractable environmental metabolome containing UCM. "Cont." = common lab contaminant. (B) GC×GC-TOF MS TIC of the same sample.

family of 2,5-pyrrolidinedione (succinimides), the saturated counterparts of maleimide (Figure 5), were tentatively identified (Figure A2, Appendix II). Interestingly, the succinimide corresponding to Me,Pr maleimide, was not observed.

Maleimides are degradation products of tetrapyrrole pigments commonly attributed to chlorophylls and bacteriochlorophylls (Grice et al., 1996, 1997; Pancost et al., 2002; Naeher et al., 2013). The distribution of maleimides in environmental samples is dependent on the original abundance and diversity of their parent chlorophyll molecules (Grice et al., 1996). The formation of Me,Et and Me,Me maleimides is typically a result of the oxidation of the tetrapyrrole ring of chlorophyll *a* (Appendix II, Structure **I**), which has either methyl or ethyl substituents at C_2 (Grice et al. 1996, 1997). In contrast, Me,*n*-Pr and Me,*i*-Bu maleimides are considered to be derived from bacteriochlorophyll *c*, *d*, or *e* (Appendix II, Structure **II**), which contain various alkyl substituents at C_8 (Me, Et, *n*-Pr, *i*-Bu, and neo-Pent), C_{12} (Me, Et), and C_{20} (Grice et al., 1996, 1997; Pancost et al., 2002; Naeher et al., 2013). Me,*n*-Pr maleimide, however, may have a chlorophyll *a* origin if the C_{17} ester undergoes hydrolysis during diagenesis (Verne-Mismer et al., 1986). Bacteriochlorophyll *c*, *d*, and *e* are uniquely synthesized by the green sulfur bacteria Chlorobiaceae (Gloe et al., 1975).

GC×GC-TOF MS allowed for the observation of a family of succinimides (Figure 5), which were originally unresolvable using GC-MS. The 2,5-pyrrolidinedione (H,H), 3,4-dimethyl-2,5-pyrrolidinedione (Me,Me), and 3-methyl-4-ethyl-2,5-pyrrolidinedione (Me,Et) bear structural similarity to maleimides and are suggested here to be the saturated reduction

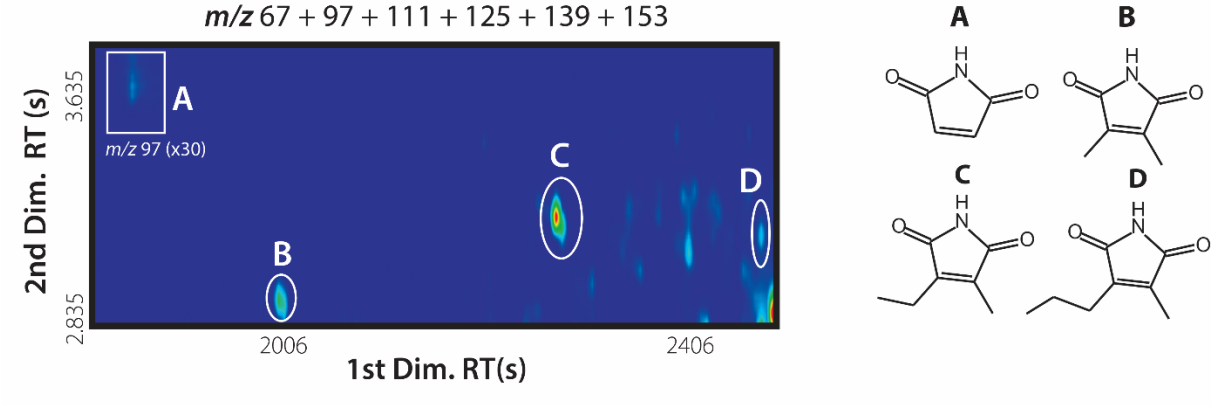

Figure 4: GC×GC-TOF MS of summed mass chromatogram for major fragment (*m/z* 67) and molecular ions (*m/z* 97, 111, 125, 139, and 153) for maleimides.

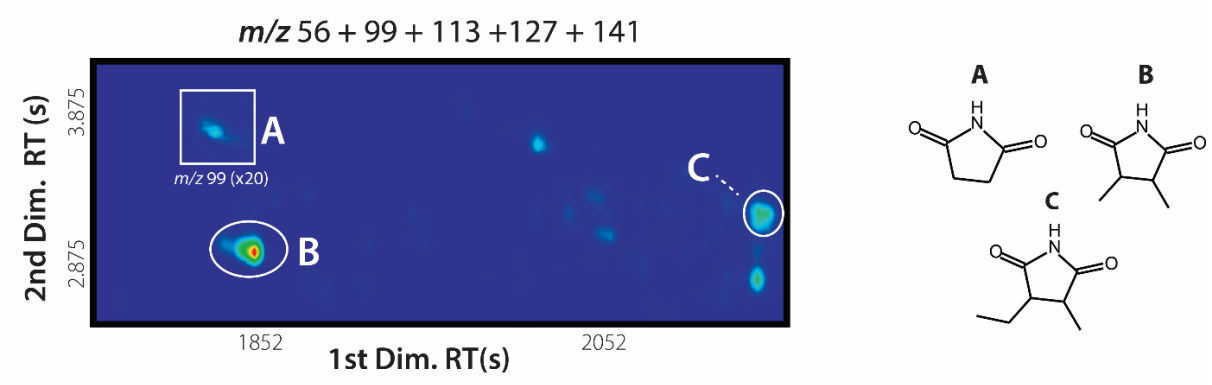

Figure 5: GC×GC-TOF MS of summed mass chromatogram for major fragment (*m/z* 56) and molecular ions (*m/z* 99 (x20 intensity), 113, 127, 141) for succinimides.

products of maleimides. Whether the loss of unsaturation is caused by abiotic diagenetic processes or is an enzymatically driven process remains unclear. To the best of our knowledge, this study is the first to detect succinimides that are associated with maleimides in an environmental sample.

It is likely that the Me,Et and Me,Me maleimides in LVBr are derived from chlorophyll *a* produced by photosynthetic organisms that occupied the Lake Vida basin prior to the evaporation and cryoencapsulation of Lake Vida brine. On the other hand, Me,Pr maleimide in LVBr most likely originated from bacteriochlorophylls *c*, *d*, or *e*, though an origin from chlorophyll *a* cannot be completely ruled out. H,H maleimide was previously detected via GC×GC-TOF MS in the sediment of a monomictic lake with an anoxic hypolimnion, in association with other maleimides observed in this study, as well as Me,*i*-Bu (Naeher et al., 2016). As Me-Pr maleimide in LVBr is derived from Chlorobiaceae (Grice et al., 1996, 1997), it would suggest that the former environmental conditions at Lake Vida had, at some point, a stratified water column with sulfidic bottom waters reaching into the photic zone. Chlorobiaceae have been observed in Ace Lake, a permanently stratified Antarctic lake with anoxic bottom waters (Hopmans et al., 2005; Ng et al., 2010), as well as several other meromictic lakes and permanently stratified fjords in the Vestfold Hills, Antarctica (Burke and Burton, 1988). Alternatively, some of these bacteriochlorophylls may derive as well from benthic mats such as those observed by Jungblut et al. (2016) in Lake Fryxell, another perennially ice-covered lake of the MDVs. Because maleimides are degradation products of

photosynthetic pigments, we suggest that the parent compounds were not synthesized by the current Lake Vida microbial assemblage since the brine is aphotic (Murray et al., 2012).

Two lines of evidence point to an aphotic brine: (1) the ice-cover that encapsulates LVBr contains several layers of thick sediments (>10 cm; Figure 1C), preventing any sunlight from reaching the brine (Dugan et al., 2015b), and (2) the analysis of small ribosomal subunit rRNA of LVBr reveals the presence of bacterial taxa that are known to grow heterotrophically, chemolithoautotrophically, or using fermentation, but not photosynthetically (Murray et al., 2012). Thus, the maleimides detected in the brine must have originated from the chlorophylls produced by the photosynthetic community in the water column or the benthos of the lake during past environmental conditions.

Maleimides can form directly from the photooxidation of chlorophylls in the absence of enzymatic activity (Rontani et al., 1991), though it has been argued by Hendry et al. (1987) that the transformation from pheopigments, macrocyclic rings that result from chlorophylls breakdown via the loss of magnesium, phytol, or modification of the sidechain to smaller N-bearing fragments could be enzymatically driven if light and oxygen are present. Regardless of the mechanism, the transformation from chlorophylls to maleimides seem to only happen in photic and oxic environments (Hendry et al., 1987; Rontani et al., 1991). Therefore, maleimides in LVBr must have formed before the brine got encapsulated and became anoxic and aphotic. Thus, maleimides should be considered paleometabolites, a legacy signature that is not part of the modern environmental metabolome of the LVBr microbial assemblage.

2.3.2 Carotenoid derivatives

A number of compounds observed in the DCM-extractable metabolome of LVBr were derived from the breakdown products of various carotenoids. Three known carotenoid derivatives, (6S,7aR)-6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one (loliolide), (6S,7aS)-6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one (isololiolide), and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (dihydroactinidiolide) were tentatively identified based on their mass fragmentation patterns and retention times in GC-MS (Figure 2C). As expected, dihydroactinidiolide eluted before isololiolide and loliolide (Klok et al., 1984b), respectively, and were present in the same order in the multidimensional GC×GC trace (Figure 6). The GC×GC-TOF MS mass chromatogram for m/z 67, 111, 139, 154, 180, and 194 revealed not only these three compounds, but also two additional carotenoid derivatives (Figure A3, Appendix II), hexahydro-4,4,7a-trimethyl-2(3H)-benzofuranone (tetrahydroactinidiolide), and 4,5,7,7a-tetrahydro-4,4,7a-trimethyl-2,6-benzofurandione (dehydrololiolide).

Loliolide and isololiolide are known degradation products of fucoxanthin (Appendix II, **III**), a major carotenoid pigment that is found in diatoms (Klok et al., 1984a, 1984b; Repeta, 1989). Though fucoxanthin can also be produced by dinoflagellates and haptophyte algae, both of which have been observed in other Antarctic lakes (Coolen et al., 2004; Jaraula et al., 2009), their occurrences have been shown to be proportional to the concentration of biogenic silica

in some lacustrine systems, making them robust proxies for diatom productivity (Castañeda et al., 2009, 2011). Fucoxanthin transformation into loliolide and isololiolide was hypothesized to be a photooxidative process (Klok et al., 1984a, 1984b). The existence of a diagenetic pathway from other carotenoids (i.e. β-carotene, zeaxanthin, or violaxanthin) to loliolide and isololiolide has also been suggested (Isoe et al., 1972; Repeta, 1989). Repeta (1989) speculated that such pathway from β-carotene may be associated to microbial activity, whereas a degradation pathway from fucoxanthin is not microbially mediated.

Dihydroactinidiolide is part of the same structural family as loliolide, but is derived from β-carotene (Appendix II, Structure **IV**; Isoe et al., 1972; Klok et al., 1984b; Kanasawud and Crouzet, 1990; Gloria et al., 1993). Even though carotenes have been suggested as the biogenic precursors to dihydroactinidiolide in anoxic marine sediments, an additional oxidation step mediated by microbial fermentation from β-carotene to carotene epoxides is required before the molecule can further degrade into lower molecular weight derivatives (Repeta, 1990). Dehydrololiolide, a carotenoid-related compound (Uegaki et al., 1979), likely also came from the same source pigment as loliolide, isololiolide, and dihydroactinidiolide, though the mechanism for its formation is unclear. The tetrahydroactinidiolide, a saturated counterpart to dihydroactinidiolide, is also tentatively assigned as a carotenoid-derivative in this study (Figure A3, Appendix II).

Additionally, volatile carotenoid-derived compounds, 2,6,6-trimethyl-2-cyclohexene-1-4-dione (ketoisophorone), 3,5,5-trimethyl-2-cyclohexenone (isophorone) and 2,2,6-tri

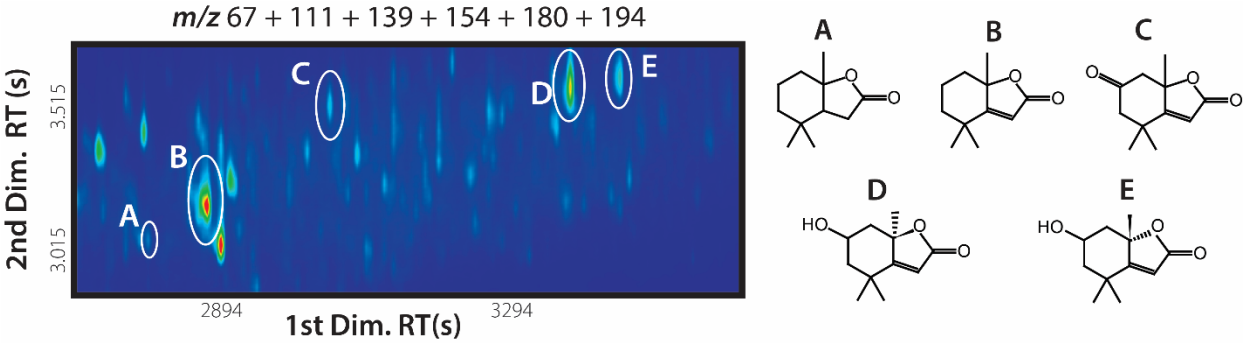

Figure 6: GC×GC-TOF MS of summed mass chromatogram for major fragment ions of tetrahydroactinidiolide, dihydroactinidiolide, dehydrololiolide, isololiolide, and loliolide.

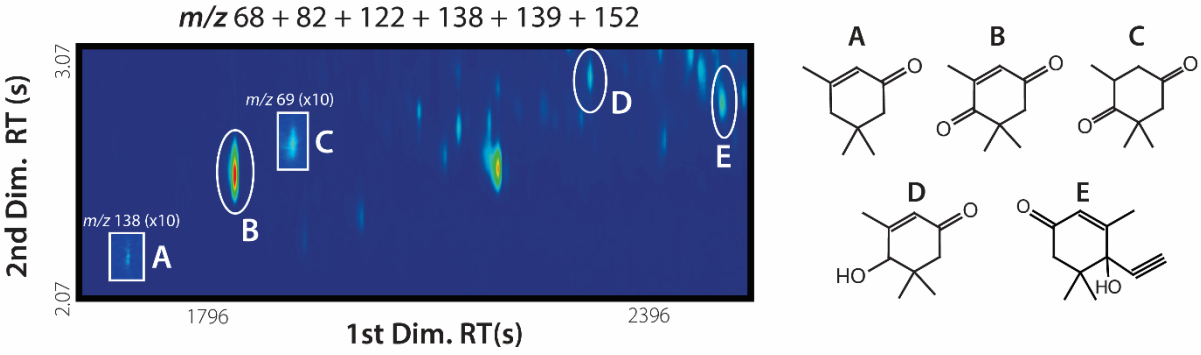

Figure 7: GC×GC-TOF MS of summed mass chromatogram for major fragment ions of volatile carotenoid-derivatives.

methyl-1,4-cyclohexanedione (dihydrooxophorone) observed in the UCM of the total DCM-extractable environmental metabolome of LVBr (Figure 2D), and 4-hydroxy-3,5,5-trimethyl-cyclohexenone (4-hydroxyisophorone) detected via GC×GC-TOF MS (Figure A4, Appendix II) have also been previously attributed to the degradation products of β-carotene (Kanasawud and Crouzet, 1990). The tentative identification of 4-ethynyl-4-hydroxy-3,5,5-trimethyl-2-cyclohexenone (Figure A4, Appendix II) raises the possibility that some of these carotenoid derivatives may have arisen from diadinoxanthin (Appendix II, Structure **V**), bearing structural similarity to its side ring and functionality. Diadinoxanthin is another phytoplankton carotenoid that have been used as a proxy for diatom production in the lacustrine and marine habitats of East Antarctica (Verleyen et al., 2004).

Carotenoids have been extensively documented in the MDVs lakes of Antarctica, and are used as a proxy for phytoplankton populations and diversity (Lizotte and Priscu, 1992, 1994; Fritsen and Priscu, 1998; Squier et al., 2005). Most perennially ice-covered lakes are comprised of microorganisms, dominated mostly by bacteria, algae, and heterotrophic protists (Fritsen and Priscu, 1998; Morgan-Kiss et al., 2006; Bielewicz et al., 2011). Given the low temperature and slow metabolic rates in LVBr and the fact that modern microbes are not photosynthetic, the carotenoid derived compounds in the DCM-extractable fraction of LVBr environmental metabolome are most likely the degradation products of legacy pigments that were produced in the lake under previous environmental conditions, prior to the evaporation and encapsulation of the residual brine. Whether the carotenoid derivatives observed in LVBr

are exclusively formed in a past ecosystem (as a result of photooxidation or autooxidation) or if they also represent, in part, the metabolic breakdown of carotenoids by modern LVBr microbes, remains unclear.

2.3.3 Implications for environmental metabolomics of Antarctic ecosystems

For now, the presence of legacy compounds in the DCM-extractable environmental metabolome of LVBr has challenged our ability to obtain an unambiguous signal of metabolites from which we can infer the current metabolic activities. This challenge arises from the need to assign compounds as being derived from the modern ecosystem, from legacy, or from both. The examples of compounds shown here (chlorophyll and carotenoid derivatives) are easily assigned to legacy in LVBr. Such a determination is not trivial for the hundreds of other compounds of the brine, many of which remain to be identified. However, we have yet to analyze the particulate microbial biomass material collected by filtration to look at metabolites associated with the modern brine microbes.

In lacustrine sediments, organic carbon can be sourced autochthonously or allochthonously (see review by Meyers and Ishiwatari, 1993). Source-specific refractory organic compounds (biomarkers) can contain valuable information on the characteristics of past or present environmental conditions as well as influences from detrital organic carbon input. The contemporaneous contribution of organic material from different ages can usually

be resolved by compound-specific isotope analyses (Eglinton et al., 1996). Radiocarbon measurements of aquatic organic carbon are calibrated based on the reservoir age, which is reflected by the mixing of deep waters with surface layers that are in contact with the atmosphere. This conversion is not trivial in systems like LVBr (past or present) since the "reservoir effect" are known to be highly variable in the MDV lakes (Doran et al., 1999, 2014). Though the radiocarbon assessment of the LVBr DOC provided constraints on the ages of the various fractions of LVBr DOC (Cawley et al., 2016), paleometabolites such as those discussed in this study may serve as a practical indicator for the legacy contribution of previous ecosystems into the total organic carbon pool without using radiocarbon dating. Thus, compounds such as monopyrolles (deriving from chlorophylls; Suzuki and Shioi, 1999) or norisoprenoids (deriving from carotenoids; Winterhalter and Rouseff, 2002) are not only valuable in elucidating their source organism, but may also retain information on the past environmental conditions that accompanied their transformation in the evolving ecosystem.

Why is the legacy effect so prominent in LVBr? The presence of a legacy component in the metabolites of LVBr is concordant with the high DOC level and low temperature limited microbial community. Significant alteration of the DOC reservoir is unlikely because of the temperature-limited microbial growth rates, with an estimated generation time of ~120 years (estimated by leucine incorporation into proteins; Murray et al., 2012). This slow metabolism is reflected in the very abundant DOC of LVBr (580 mg-C·L^{-1}; Cawley et al. 2016) compared to e.g. the Blood Falls' subglacial brine in Taylor Valley, Antarctica, with a DOC of 9.25 mg-C·L^{-}

[1] for a generation time of about ~300 days (estimated by thymidine incorporation into DNA; Mikucki et al., 2004, 2009). Characterization of LVBr dissolved organic matter demonstrated that the brine's DOC inventory is composed of a combination of compounds deriving from modern microbial processes as well as a dominant, altered legacy component (Cawley et al., 2016).

Ecological legacies in Antarctica have been extensively described as a functional link between past and present ecosystems, which ultimately influences the variations in biological activities overtime (McKnight et al., 1999; Moorhead et al., 1999; Knoepfle et al., 2009). In perennially ice-covered lakes, stratification can persist over long periods of time and pools of nutrients can collect in significant concentrations in deeper waters (Priscu et al., 1999). For example, the bottom waters of the west lobe of Lake Bonney (Taylor Valley, MDVs) have an apparent radiocarbon age of 22,950 ± 250 years BP, totally disconnected from the modern processes occurring in the upper water column (Doran et al., 2014). The dry valley lakes appear to contain legacies from past ecosystem conditions that have captured a record of evolving limnology. In LVBr, the legacy component of metabolites are dominated by compounds derived from photosynthetic algae and bacteria, even though the current ecosystem is entirely dominated by non-photosynthetic bacteria.

Some significant information on the past ecosystem is retained in paleometabolites and may provide constraints on our understanding of Lake Vida's past biogeochemical processes. The presence of maleimides derived from chlorophyll *a* suggests that the previous lake's

ecosystem was supported by primary productivity, whereas the occurrence of maleimides that originated from bacteriochlorophyll *c*, *d*, or *e*, biomarkers for green sulfur bacteria, suggests that at one point, the lower water column of Lake Vida's was euxinic (anoxic and sulfidic) and that the euxinic water penetrated into the photic zone, or the conditions permitted light to penetrate to the benthos which was supported with euxinic conditions.

The occurrence of carotenoid derivatives in LVBr is an effect of legacy, as the carotenoids themselves were likely synthesized during previous environmental conditions and were photooxidized or autooxidized prior to the brine encapsulation. Though it is likely that the catabolism of these compounds occurred in the prior ecosystem as well, we cannot discount the possibility that the modern LVBr microbes are actually degrading or have degraded those carotenoids.

2.3.4 Paleometabolites in other slow-growing ecosystems

Our research points to a question of whether legacy significantly affects the interpretation of data obtained on organic material in other slow-growing ecosystems. In a habitat that has very low metabolic activities, and consequently slow turnover rates, the legacy metabolites would be very slowly, or not at all, degraded.

The conditions of permafrost cryopegs such as temperature and salinity as well as cell counts are comparable to those of LVBr (Gilichinsky et al., 2003; Murray et al., 2012;

Colangelo-Lillis et al., 2016). Biological activities of cryopeg communitites inferred from reazurin reduction rate (Bakermans et al., 2003) and ^{14}C-labelled glucose uptake (Gilichinsky et al., 2003) have been demonstrated in the laboratory at temperatures as low as -10°C and -15°C, respectively (Gilichinsky et al., 2003). Considering the low temperatures of the cryopegs, extremely slow *in situ* metabolic rates are likely, thereby limiting biological activities to maintenance of vital cell functions for survival (i.e. DNA, amino acid racemization, and cell membrane repair) as suggested by Gilichinsky et al. (2003). Thus, it is likely that cryopeg brines contain legacy metabolites. To the best of our knowledge, no data on the constituents or age of organic matter of cryopeg communities have been reported. Obtaining such information may help reveal the extent at which legacy metabolites co-occur with modern metabolites from extant communities with slow metabolic rates.

The global deep subsurface contains a microbial biosphere with cell numbers estimated to reach up to ~10^{30}, with cell densities averaging ~5×10^5 cells/mL in the subsurface marine sediments (below 1 km) compared to ~5×10^8 cells/mL in near-surface (0-10 cm) marine sediments (Whitman et al., 1998). In the deep subsurface (continental and marine), cells have extremely slow generation times due to energy-limited conditions (Chapelle et al., 1988; D'Hondt et al., 2002; Jørgensen and Boetius, 2007). Previous attempts to estimate the extant microbial biomass and to characterize the diversity of microorganisms in the deep subsurface utilize a wide array of methods such as viable cell count (Cragg et al., 1992; Chapelle et al., 2002), direct cell staining (Cragg et al., 1992; Chapelle et al., 2002; Schippers et al., 2005), as

well as genomic-based (Chapelle et al., 2002; Schippers et al., 2005) and lipid-based analyses (Harvey et al., 1986; Sturt et al., 2004; Lipp et al., 2008). Many of these analyses rely on the detection and quantification of organic material, which abundance depends on the fluxes of metabolites being synthesized or degraded by the extant community. In the deep subsurface, the energy limitation has a significant control on the speed of metabolic activities (Hoehler and Jørgensen, 2013). We suggest that the legacy components of these deep subsurface environments can persist for long periods of time and may significantly convolute data interpretation of *in situ* organic material obtained from the deep subsurface.

2.4. Conclusions

Many of the compounds discovered in the DCM-extractable environmental metabolome of LVBr can be traced to degraded photosynthetic pigments (chlorophylls and carotenoids). The paleometabolites in the DCM-extractable metabolome of LVBr are derived from previous environmental conditions and do not represent biological activities of the modern bacterial community. This dominance in legacy components is concordant with the subzero environmental temperatures and slow metabolisms of LVBr microbes. Consequently, the information on the extant community carried by the total dissolved organic carbon pool can be mixed with legacy signals, and in the case of LVBr, can be difficult to detect due to low signal amongst a large legacy "noise". As a result, interpretation of exogenous materials

contemporaneous to ecosystem production with low metabolic rates is difficult, as the large pool of legacy compounds may mask the biological signals of the extant microbial community. However, the presence of legacy materials is an opportunity to observe paleometabolites that represent past biogeochemical processes. Ultimately, the presence of a legacy component in slow and isolated microbial ecosystems is a caveat that must first be addressed in order to prevent the misinterpretation of the current metabolite collection in the total dissolved organic carbon pool. Furthermore, in order to characterize the current metabolites, extant ecosystem biomass also needs to be assessed.

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Work Cited

Amend, J.P., Teske, A., 2005. Expanding frontiers in deep subsurface microbiology. Palaeogeography, Palaeoclimatology, Palaeoecology 219, 131–155.

Bakermans, C., Tsapin, A.I., Souza-Egipsy, V., Gilichinsky, D.A., Nealson, K.H., 2003. Reproduction and metabolism at -10°C of bacteria isolated from Siberian permafrost. Environmental Microbiology 5, 321–326.

Bielewicz, S., Bell, E., Kong, W., Friedberg, I., Priscu, J.C., Morgan-Kiss, R.M., 2011. Protist diversity in a permanently ice-covered Antarctic Lake during the polar night transition. The ISME Journal 5, 1559–1564.

Bundy, J.G., Davey, M.P., Viant, M.R., 2009. Environmental metabolomics: A critical review and future perspectives. Metabolomics.

Burke, C.M., Burton, H.R., 1988. Photosynthetic bacteria in meromictic lakes and stratified fjords of the Vestfold Hills, Antarctica. Hydrobiologia 165, 13–23.

Castañeda, I.S., Werne, J.F., Johnson, T.C., 2009. Influence of climate change on algal community structure and primary productivity of Lake Malawi (East Africa) from the Last Glacial Maximum to present. Limnology and Oceanography 54, 2431–2447.

Castañeda, I.S., Werne, J.P., Johnson, T.C., Powers, L.A., 2011. Organic geochemical records from Lake Malawi (East Africa) of the last 700 years, part II: Biomarker evidence for recent changes in primary productivity. Palaeogeography, Palaeoclimatology, Palaeoecology 303, 140–154.

Cawley, K.M., Murray, A.E., Doran, P.T., Kenig, F., Stubbins, A., Chen, H., Hatcher, P.G., Mcknight, D.M., Hatcher, P.G., Mcknight, D.M., 2016. Characterization of dissolved organic material in the interstitial brine of Lake Vida, Antarctica. Geochimica et Cosmochimica Acta 183, 63–78.

Chapelle, F.H., Lovley, D.R., 1990. Rates of microbial metabolism in deep subsurface environments. Applied and Environmental Microbiology 56, 1865–1874.

Chapelle, F.H., Morris, J.T., McMahon, P.B., Zelibor, J.L., 1988. Bacterial metabolism and the d13C composition of ground water, Floridan aquifer system, South Carolina. Geology 16, 117–121.

Chapelle, F.H., O'Neill, K., Bradley, P.M., Methe, B.A., Clufo, S.A., Knobel, L.L., Lovley, D.R., 2002. A hydrogen-based subsurface microbial community dominated by methanogens. Nature 415.

Colangelo-Lillis, J., Eicken, H., Carpenter, S.D., Deming, J.W., 2016. Evidence for marine origin and microbial-viral habitability of sub-zero hypersaline aqueous inclusions within permafrost near Barrow, Alaska. FEMS Microbiology Ecology 92.

Coolen, M.J.. L., Muyzer, G., Rijpstra, W.I.C., Schouten, S., Volkman, J.K., Sinninghe Damsté, J.S., 2004. Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake. Earth and Planetary Science Letters 223, 225–239.

Cragg, B.A., Harvey, S.M., Fry, J.C., Herbert, R.A., Parkes, R.J., 1992. Bacterial Biomass and Activity in the Deep Sediment Layers of the Japan Sea, hole 798B. Proceedings of the Ocean Drilling Program, Scientific Results 127.

D'Hondt, S., Rutherford, S., Spivack, A.J., D 'Hondt, S., Rutherford, S., Spivack, A.J., 2002. Metabolic Activity of Subsurface Life in Deep-Sea Sediments. Science 295, 2067–2070.

Doran, P., Kenig, F., Knoepfle, L.J., Mikucki, A.J., Lyons, W.B., Doran, P.T., Kenig, F., Knoepfle, J.L., Mikucki, J.A., Lyons, W.B., 2014. Radiocarbon distribution and the effect of legacy in lakes of the McMurdo Dry. Limnology and Oceanography 59, 811–826.

Doran, P.T., Berger, G.W., Lyons, W.B., Wharton, R.A., Davisson, M.L., Southon, J., Dibb, J.E., 1999. Dating Quaternary lacustrine sediments in the McMurdo Dry Valleys, Antarctica. Palaeogeography, Palaeoclimatology, Palaeoecology 147, 223–239.

Doran, P.T., Fritsen, C.H., McKay, C.P., Priscu, J.C., Adams, E.E., 2003. Formation and character of an ancient 19-m ice cover and underlying trapped brine in an ice-sealed" east Antarctic lake. Proceedings of the National Academy of Sciences 100, 26–31.

Doran, T.P., Fritsen, C.H., Murray, A.E., Kenig, F., Mckay, C.P., Kyne, J.D., 2008. Entry approach into pristine ice-sealed lakes - Lake Vida , East Antarctica , a model ecosystem. Limnology and Oceanography: Methods 6, 542–547.

Dugan, H.A., Doran, P.T., Tulaczyk, S., Mikucki, J.A., Arcone, S.A., Auken, E., Schamper, C., Virginia, R.A., 2015a. Subsurface imaging reveals a confined aquifer beneath an ice-sealed Antarctic lake. Geophysical Research Letters 42, 96–103.

Dugan, H.A., Doran, P.T., Wagner, B., Kenig, F., Fritsen, C.H., Arcone, S.A., Kuhn, E., Ostrom, N.E., Warnock, J.P., Murray, A.E., 2015b. Stratigraphy of Lake Vida, Antarctica: hydrologic implications of 27 m of ice. The Cryosphere 9, 439–450.

Edwards, K.J., Becker, K., Colwell, F., 2012. The Deep, Dark Energy Biosphere: Intraterrestrial Life on Earth. Annu. Rev. Earth Planet. Sci 40, 551–68.

Eglinton, T.I., Benitez-Nelson, B.C., Pearson, A., McNichol, A.P., Bauer, J.E., Druffel, E.R.M., 1996. Variability in Radiocarbon Ages of Individual Organic Compounds from Marine Sediments. Science 24, 1115–1304.

Fritsen, C.H., Priscu, J.C., 1998. Cyanobacterial assemblages in permanent ice covers on Antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. Journal of Phycology 34, 587–597.

Gilichinsky, D., Rivkina, E., Bakermans, C., Shcherbakova, V., Petrovskaya, L., Ozerskaya, S., Ivanushkina, N., Kochkina, G., Laurinavichuis, K., Pecheritsina, S., Fattakhova, R., Tiedje, J.M., 2005. Biodiversity of cryopegs in permafrost. FEMS Microbiology Ecology 53, 117–128.

Gilichinsky, D., Rivkina, E., Shcherbakova, V., Laurinavichuis, K., Tiedje, J., 2003. Supercooled Water Brines Within Permafrost—An Unknown Ecological Niche for Microorganisms: A Model for Astrobiology. Astrobiology 3, 331–341.

Gloe, A., Pfennig, N., Brockmann, H., Trowitzsch, W., 1975. A New Bacteriochlorophyll from Brown-Colored Chlorobiaceae*. Archives of Microbiology 102, 103–109.

Gloria, M.A.B., Grulke, A.E., Gray, J.I., 1993. Effect of type of oxidation on beta-carotene loss and volatile products formation in model system. Food Chemistry 46, 401–406.

Grice, K., Gibbison, R., Atkinson, J.E., Schwark, L., Eckardt, C.B., Maxwell, J.R., 1996. Maleimides (1H-pyrrole-2,5-diones) as molecular indicators of anoxygenic photosynthesis in ancient water columns. Geochimica et Cosmochimica Acta 60, 3913–3924.

Grice, K., Schaeffert, P., Schwark, L., Maxwelu, J.R., 1997. Changes in palaeoenvironmental conditions during deposition of the Permian Kupferschiefer (Lower Rhine Basin, northwest Germany) inferred from molecular and isotopic compositions of biomarker components. Organic Geochemistry 26, 11–12.

Harvey, H.R., Fallon, R.D., Patton, J.S., 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta 50, 795–804.

Hendry, G.A.F., Houghton, J.D., Brown, S.B., 1987. The degradation of chlorophyll---a biological enigma. New Phytologist 107, 255–302.

Hoehler, T.M., Jørgensen, B.B., 2013. Microbial life under extreme energy limitation. Nature Reviews Microbiology 11, 83–94.

Hopmans, E.C., Schouten, S., Rijpstra, W.I.C., Sinninghe Damst??, J.S., 2005. Identification of carotenals in sediments. Organic Geochemistry 36, 485–495.

Isoe, S., Iiyeon, S.B., Ratsumura, S., Sakan, T., 1972. Photo-oxygenation of Carotenoids. II. The Absolute Configuration of Loliolide and Dihydroactinidiolide. Tetrahedron Letters 25, 2517–252.

Jansson, J.K., Taş, N., 2014. The microbial ecology of permafrost. Nature Reviews Microbiology 12, 414–425.

Jaraula, C.M.B., Brassell, S.C., Morgan-Kiss, R.M., Doran, P.T., Kenig, F., 2009. Origin and tentative identification of tri to pentaunsaturated ketones in sediments from Lake Fryxell, East Antarctica. Organic Geochemistry 41, 386–397.

Jørgensen, B.B., Boetius, A., 2007. Feast and famine — microbial life in the deep-sea bed. Nature Reviews Microbiology 5, 770–781.

Jungblut, A.D., Hawes, I., Mackey, T.J., Krusor, M., Doran, P.T., Sumner, D.Y., Eisen, J.A., Hillman, C., Goroncy, A.K., 2016. Microbial mat communities along an oxygen gradient in a perennially ice-covered Antarctic lake. Applied and Environmental Microbiology 82, 620–630.

Kanasawud, P., Crouzet, J.C., 1990. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 1. beta-Carotene degradation. Journal of Agricultural and Food Chemistry 38, 237–243.

Kido Soule, M.C., Longnecker, K., Johnson, W.M., Kujawinski, E.B., 2015. Environmental metabolomics: Analytical strategies. Marine Chemistry 177, 374–387.

Klok, J., Baas, M., Cox, H.C., de Leeuw, J.W., Rijpstra, W.I.C., Schenck, P.A., 1984a. Qualitative and quantitative characterization of the total organic matter in a recent marine sediment (Part II). Organic Geochemistry 6, 265–278.

Klok, J., Baas, M., Cox, H.C., de Leeuw, J.W., Schenck, P.A., 1984b. Loliolides and dihydroactinidiolide in a recent sediment probably indicate a major transformation pathway of carotenoids. Tetrahedron Letters 25, 5577–5580.

Knoepfle, L.J., Doran, T.P., Kenig, F., Lyons, W.B., Galchenko, V.F., 2009. Particulate organic and dissolved inorganic carbon stable isotopic compositions in Taylor Valley lakes , Antarctica : the effect of legacy. Hydrobiologia 632, 139–156.

Kozono, M., Nomoto, S., Mita, H., Shimoyama, A., 2001. Detection of maleimides and their characteristics in Neogene sediments of the Shinjo basin , Japan. Geochemical Journal 35, 225–236.

Lipp, J.S., Morono, Y., Inagaki, F., Hinrichs, K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454, 991–994.

Lizotte, M.P., Priscu, J.C., 1994. Natural Fluorescence and Quantum Yields in Vertically Stationary Phytoplankton from Perennially Ice-Covered Lakes. Limnology and Oceanography 39, 1399–1410.

Lizotte, P.M., Priscu, J.C., 1992. Photosynthesis-irradiance relationships in phytoplankton from the physically stable water column of the perennially ice-covered lake (Lake Bonney, Antarctica). Journal of Phycology 28, 179–185.

Lyons, W.B., Fountain, A., Doran, T.P., Priscu, J.C., Neumann, K., 2000. Importance of landscape position and legacy : the evolution of the lakes in Taylor Valley , Antarctica. Freshwater Biology 43, 355–367.

Martin, J., Quirke, E., Shaw, G.J., Super, P.D., Maxwell, J.R., 1980. The Presence of Porphyrins with Extended Alkyl Substituents. Tetrahedron 36, 3261–3267.

McKnight, D.M., Niyogi, D.K., Alger, A.S., Bomblies, A., Conovitz, P. a., Tate, C.M., 1999. Dry Valley Streams in Antarctica: Ecosystems Waiting for Water. BioScience 49, 985.

Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry--an overview of indicators of organic matter sources and diagenesis in lake sediments. Organic Geochemistry 20, 867–900.

Mikucki, J.A., Foreman, C.M., Sattler, B., Berry Lyons, W., Priscu, J.C., 2004. Geomicrobiology of Blood Falls: An Iron-Rich Saline Discharge at the Terminus of the Taylor Glacier, Antarctica. Aquatic Geochemistry 10, 199–220.

Mikucki, J.A., Pearson, A., Johnston, D.T., Turchyn, A. V., Farquhar, J., Shrag, P.D., Anbar, A.D., Priscu, J.C., Lee, P.A., 2009. A Contemporary Microbially Maintained Subglacial Ferrous "Ocean." Science 324, 397–400.

Moorhead, L.D., Doran, T.P., Fountain, G.A., Lyons, W.B., McKnight, M.D., Priscu, J.C., Virginia, A.R., Wall, H.D., 1999. Ecological Legacies: Impacts on Ecosystems of the McMurdo Dry Valleys. BioScience 49, 1009–1019.

Morgan-Kiss, R., Priscu, J., Pocock, T., Gudynaite-Savitch, L., Huner, N., 2006. Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently Cold Environments. Microbiology and Molecular Biology Reviews 70, 222–252.

Murray, A.E., Kenig, F., Fritsen, C.H., Mckay, C.P., Cawley, K.M., Edwards, R., Kuhn, E., Mcknight, D.M., Ostrom, N.E., Peng, V., Ponce, A., Priscu, J.C., Samarkin, V., Townsend, A.T., Wagh, P., Young, S.A., To, P., Doran, T.P., 2012. Microbial life at − 13 ° C in the brine of an ice-sealed Antarctic lake. Proceedings of the National Academy of Sciences 109, 20626–20631.

Naeher, S., Lengger, S.K., Grice, K., 2016. A new method for the rapid analysis of 1H-Pyrrole-2,5-diones (maleimides) in environmental samples by two-dimensional gas chromatography time-of-flight mass spectrometry. Journal of Chromatography A 1435, 125–135.

Naeher, S., Schaeffer, P., Adam, P., Schubert, C.J., 2013. Maleimides in recent sediments – Using chlorophyll degradation products for palaeoenvironmental reconstructions. Geochimica et Cosmochimica Acta 119, 248–263.

Ng, C., DeMaere, M.Z., Williams, T.J., Lauro, F.M., Raftery, M., Gibson, J. a E., Andrews-Pfannkoch, C., Lewis, M., Hoffman, J.M., Thomas, T., Cavicchioli, R., 2010. Metaproteogenomic analysis of a dominant green sulfur bacterium from Ace Lake, Antarctica. The ISME journal 4, 1002–1019.

Pancost, R.D., Crawford, N., Maxwell, J.R., 2002. Molecular evidence for basin-scale photic zone euxinia in the Permian Zechstein Sea. Chemical Geology 188, 217–227.

Pedersen, K., 2000. Exploration of deep interterrestrial microbial life: Current perspectives. FEMS Microbiology Letters 185, 9–16.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proceedings of the National Academy of Sciences 101, 4631–4636.

Priscu, J.C., Wolf, C.F., Takacs, C.D., Fritsen, C.H., Laybourn-Parry, J., Roberts, E.C., Sattler, B., Lyons, W.B., 1999. Carbon Transformations in a Perennially Ice-Covered Antarctic Lake. BioScience 49, 997–1008.

Proemse, B.C., Murray, A.E., Schallenberg, C., McKiernan, B., Glazer, B.T., Young, S.A., Ostrom, N.E., Bowie, A.R., Wieser, M.E., Kenig, F., Doran, P.T., Edwards, R., 2017. Iron cycling in the anoxic cryo-ecosystem of Antarctic Lake Vida. Biogeochemistry 1–11.

Repeta, D.J., 1989. Carotenoid diagenesis in recent marine sediments: II. Degradation of fucoxanthin to loliolide. Geochimica et. Cosmochimica Acta 53, 699–707.

Repeta, D.J., 1990. 37. Carotenoid diagenesis in Pleistocene to Miocene sediments from the Peru Margin. Proceedings of the Ocean Drilling Program, Scientific Results 112.

Rontani, J.F., Baillet, G., Aubert, C., 1991. Production of acyclic isoprenoid compounds during the photodegradation of chlorophyll-a in seawater. Journal of Photochemistry and Photobiology, A: Chemistry 59, 369–377.

Schippers, A., Neretin, L.N., Kallmeyer, J., Ferdelman, T.G., Cragg, B.A., Parkes, J.R., Jorgensen, B.B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bateria. Nature 433, 861–864.

Shimoyama, A., Kozono, M., Mita, H., Nomoto, S., 2001. Maleimides in the Cretaceous/Tertiary boundary sediments at Kawaruppu, Hokkaido, Japan. Geochemical Journal 35, 365–375.

Squier, A.H., Hodgson, D.A., Keely, B.J., 2005. Evidence of late Quaternary environmental change in a continental east Antarctic lake from lacustrine sedimentary pigment distributions. Antarctic Science 17, 361–376.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry--new biomarkers for biogeochemistry and microbial ecology. Rapid communications in mass spectrometry 18, 617–628.

Suzuki, Y., Shioi, Y., 1999. Detection of Chlorophyll Breakdown Products in the Senescent Leaves of Higher Plants. Plant Cell Physiology 40, 909–915.

Tison, J.-L., Souchez, R., Wolff, W.E., Moore, C.J., Legrand, R.M., Angelis, M. de, 1998. Is a periglacial biota responsible for enhanced dielectric response in basal ice from the Greenland Ice Core Project ice core? Journal of Geophysical Research 103, 18885–18894.

Uegaki, R., Fuiimori, T., Kaneko, H., Kato, K., Noguchi, M., 1979. Isolation of Dehydrololiolide and 3-Oxo-actinidol from Nicotiana tabacum. Agric. Biol. Chem 43, 1149–1150.

Verleyen, E., Hodgson, D.A., Leavitt, P.R., Sabbe, K., Vyverman, W., 2004. Quantifying habitat-specific diatom production: A critical assessment using morphological and biogeochemical markers in Antarctic marine and lake sediments. Limnology and Oceanography 49, 1528–1539.

Verne-Mismer, J., Ocampo, R., Callot, H.J., Albrecht, P., 1986. Identification of a Novel C33 DPEP Petroporphyrin from Boscan Crude Oil: Evidence for Geochemical Reduction of Carboxylic Acids. Tetrahedron Letters 27, 5257–5260.

Viant, R.M., 2007. Metabolomics of aquatic organisms: the new "omics" on the block. Marine Ecology Progress Series 332, 301–306.

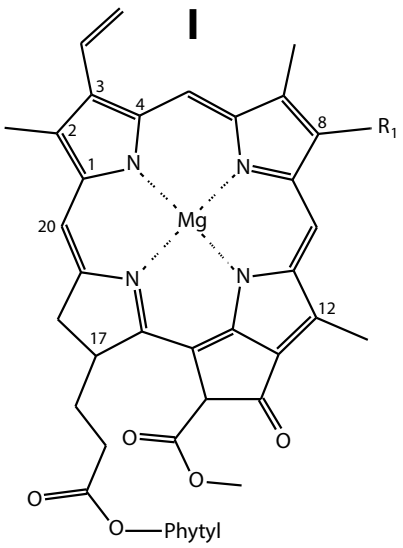
Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Perspective Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences 95, 6578–6583.

Winterhalter, P., Rouseff, R., 2002. Carotenoid-Derived Aroma Compounds: An Introduction, in: Carotenoid-Derived Aroma Compounds. American Chemical Society, pp. 1–17.

APPENDIX II

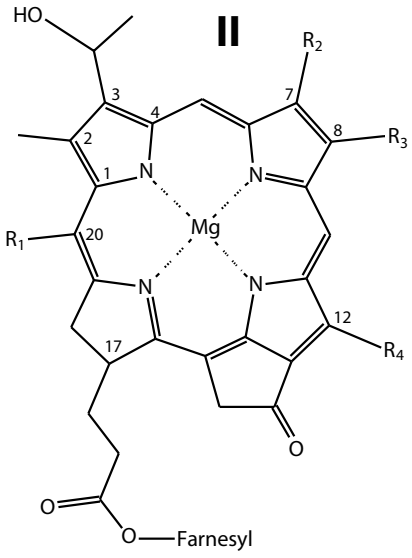
Chlorophyll *a*

R_1 = Me, Et

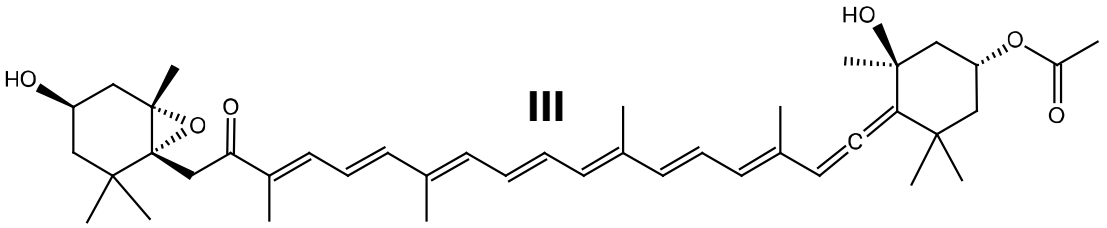


Bacteriochlorophyll

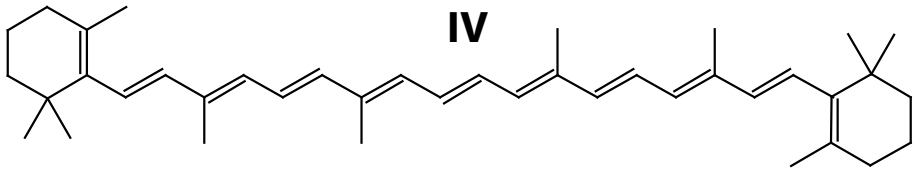
(a) **Bchl *c***	(a) **Bchl *d***	(a) **Bchl *e***
R_1 = Me	R_1 = H	R_1 = Me
R_2 = Me	R_2 = Me	R_2 = CH_2O
R_3 = Et, n-Pr, i-Bu	R_3 = Et, n-Pr, i-Bu, neo-Pent	R_3 = Et, n-Pr, i-Bu
R_4 = Me, Et	R_4 = Me, Et	R_4 = Et



Fucoxanthin



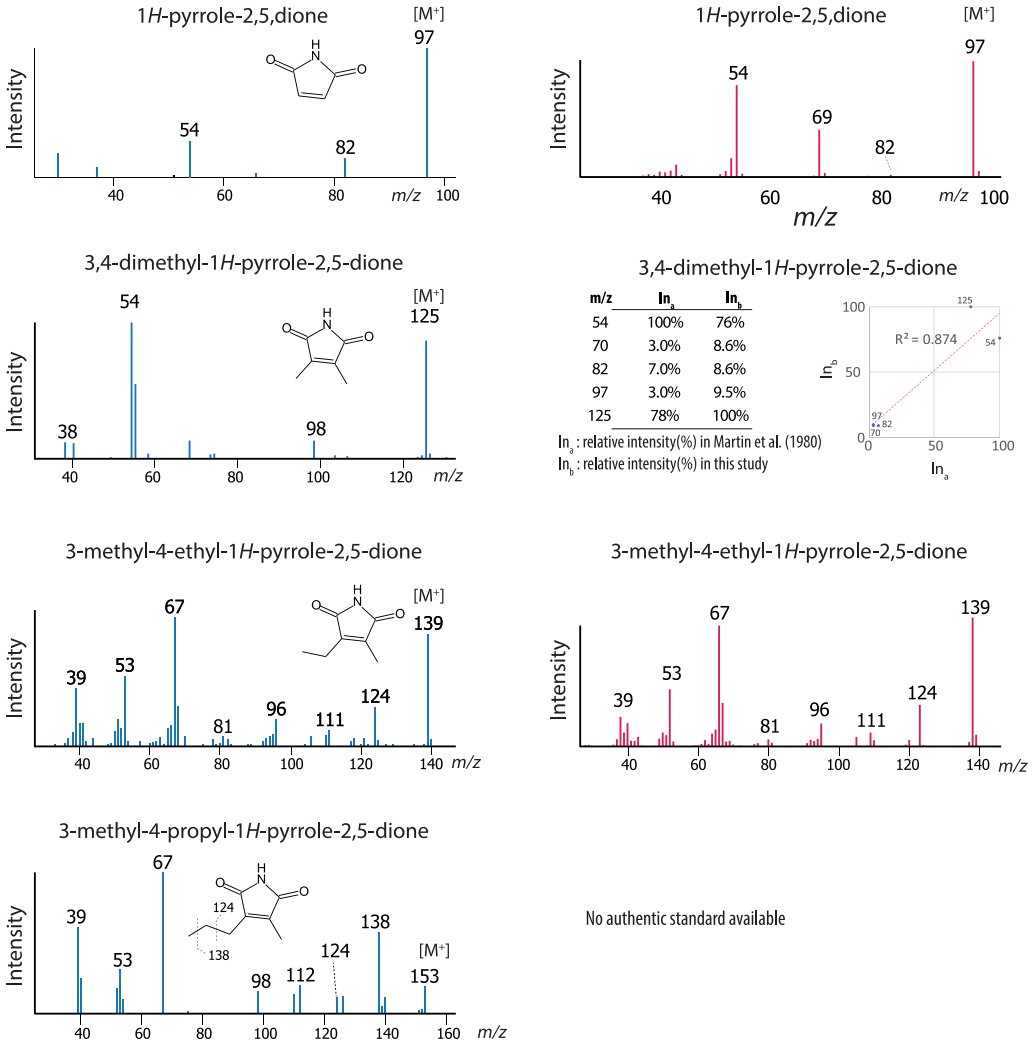
β-carotene



APPENDIX II (cont.)

Mass spectra of compounds discussed in this study (Chapter II) is presented in the **left column** of Figure A1 to A4. All mass spectra of paleometabolites were retrieved from the ChromaTOF software, a mass spectral processing tool of the Leco's software package, which utilizes the "Peak True" deconvolution algorithm to subtract peaks from background noise.

Comparisons were made to mass spectra of compounds obtained from the NIST Chemistry WebBook, the Wiley Database, and various literature shown in the **right column** of Figure A1 to A4. For spectra that were obtained from the literature, we plotted the relative abundances of fragment ions to the base peak ions (only those that are reported in the studies) against those relative abundances in the mass spectra of our study. R^2 values were generated to show how well the compounds and their fragmentation patterns are correlated.



m/z	In_a	In_b
54	100%	76%
70	3.0%	8.6%
82	7.0%	8.6%
97	3.0%	9.5%
125	78%	100%

Figure A1: Mass spectra of maleimides (1H-pyrrole-2,5,diones) from this study (left) compared to library spectra of authentic standards (right).

APPENDIX II (cont.)

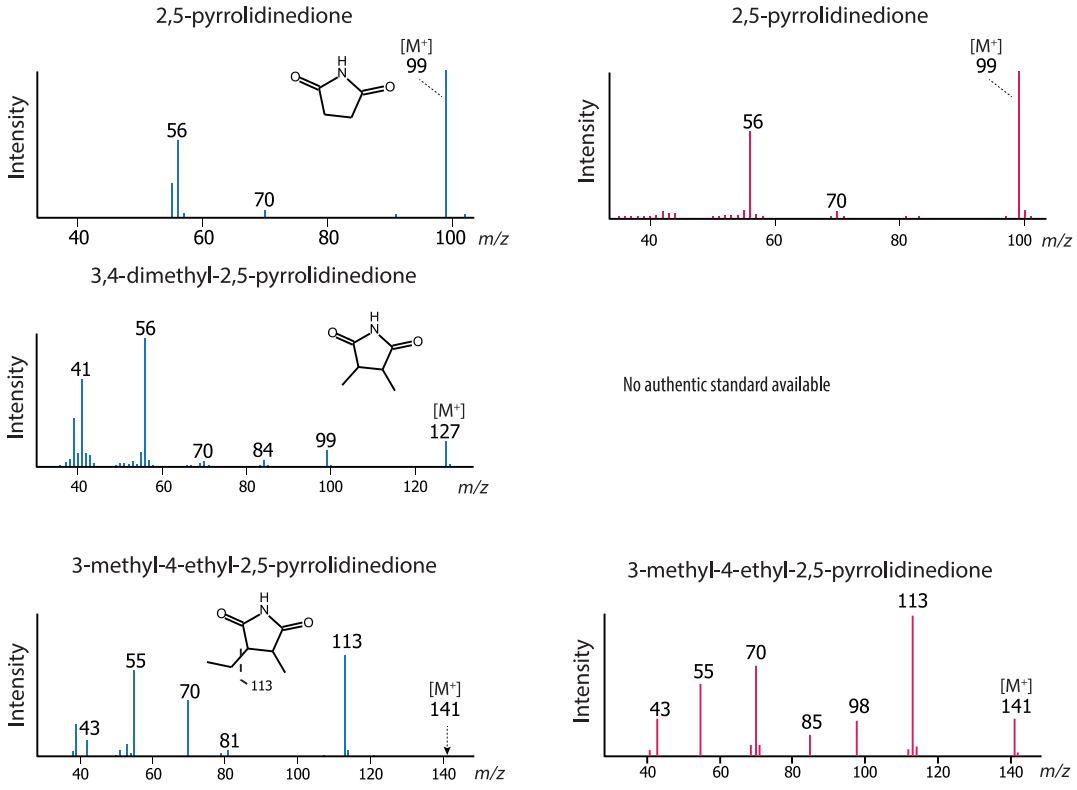


Figure A2: Mass spectra of succinimides (2,5-pyrrolidinedione) from this study (left) compared to library spectra of authentic standards (right).

APPENDIX II (cont.)

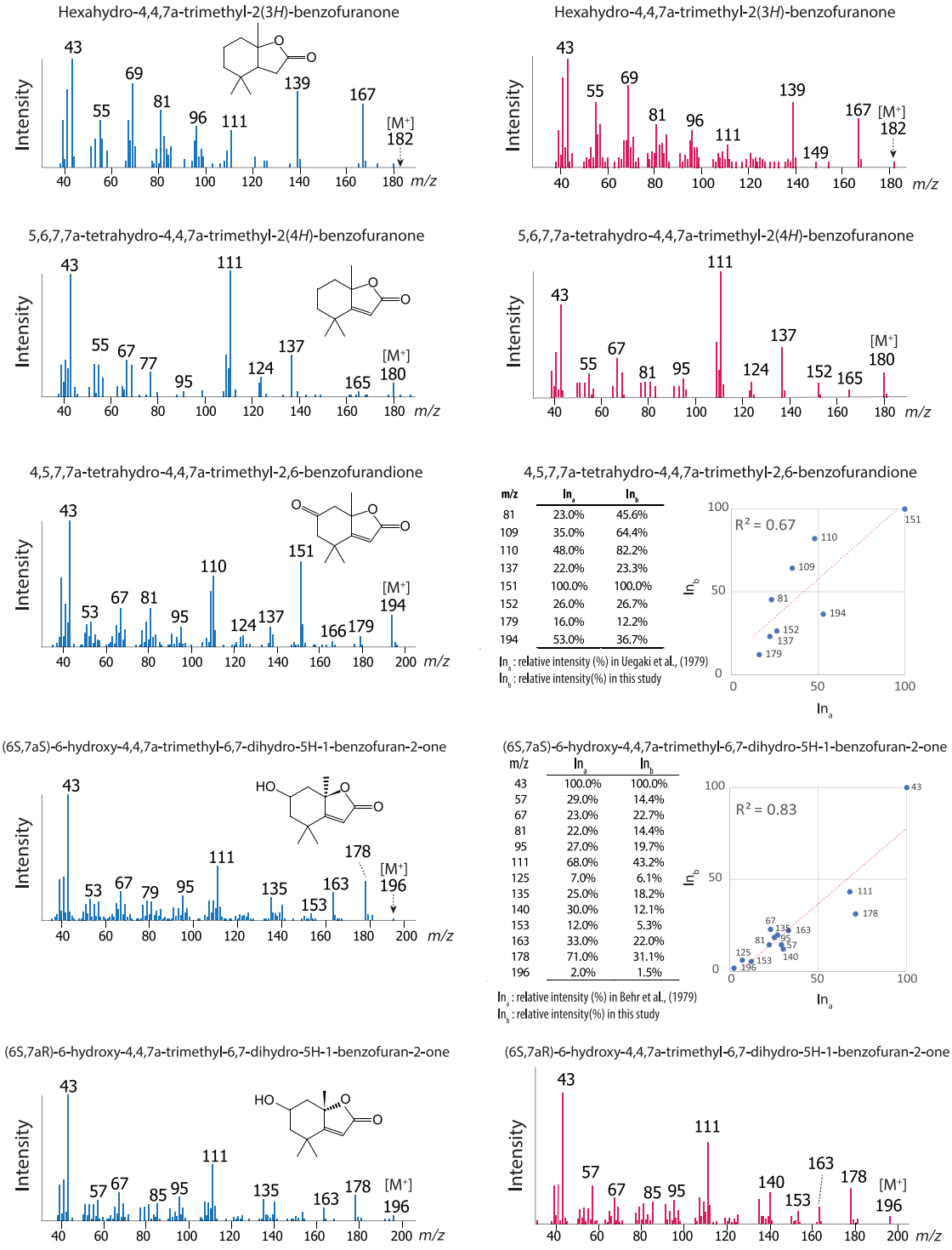


Figure A3: Mass spectra of carotenoid derivatives from this study (left) compared to library spectra of authentic standards (right).

APPENDIX II (cont.)

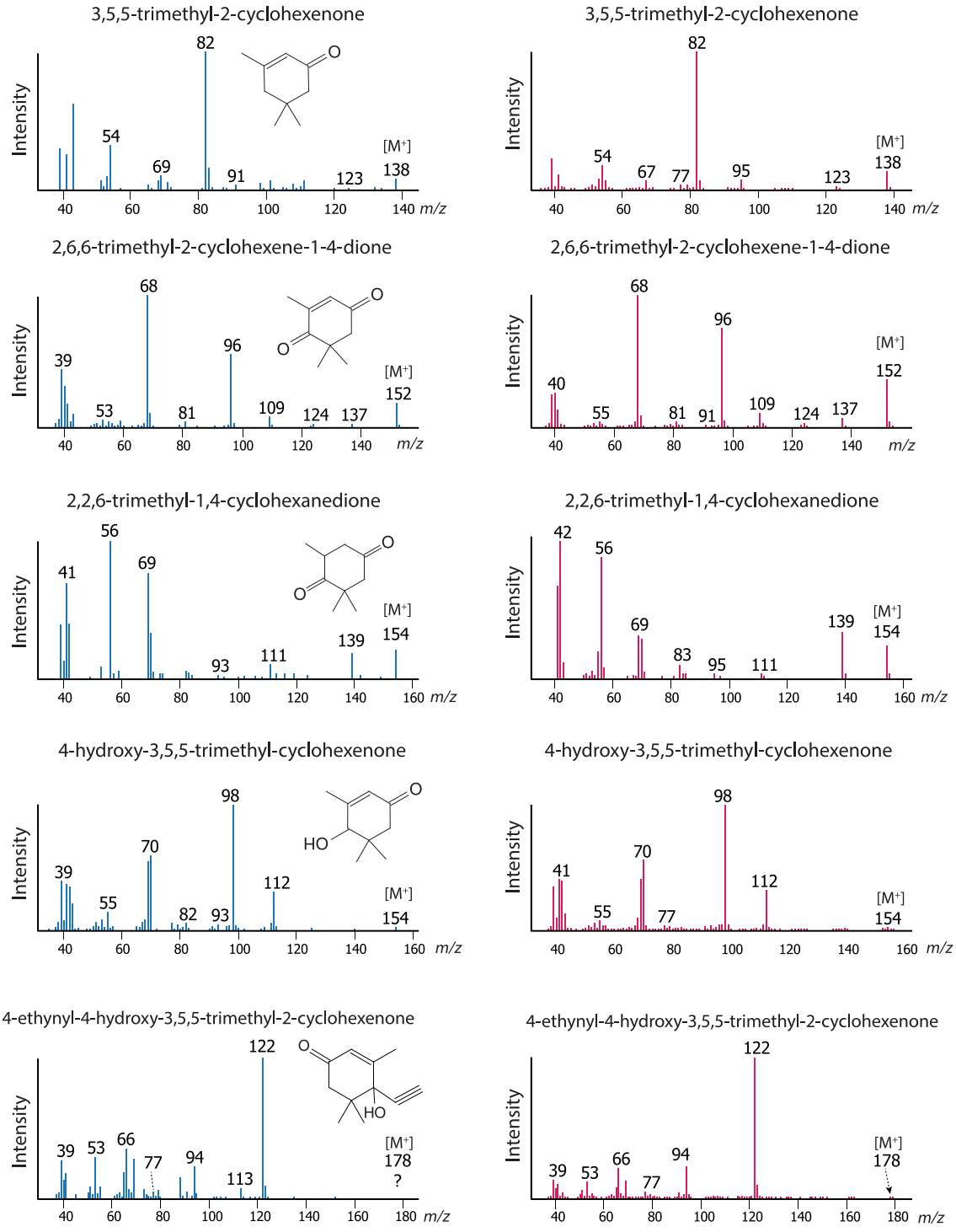


Figure A4: Mass spectra of carotenoid derivatives from this study (left) compared to library spectra of authentic standards (right).

APPENDIX II (cont.)

Work Cited

Behr, D., Wahlberg, I., Nishida, T., Enzell, C.R., 1979. Tobacco chemistry, 50:(3S, 5R, 8S, 9x)-5, 8-epoxy-6-megastigmene-3, 9-diol and (3S, 5R, 6R, 7E, 9x)-3, 6-epoxy-7-megastigmene-5, 9-diol: Two new nor-carotenoids of Greek tobacco. Acta Chemica Scandinavica Series B, 701–704.

John Wiley & Sons, Inc. "Wiley Scientific, Technical, and Medical Databases" 11th edition. Hoboken NJ, 07030.

Martin, J., Quirke, E., Shaw, G.J., Super, P.D., Maxwell, J.R., 1980. The Presence of Porphyrins with Extended Alkyl Substituents. Tetrahedron 36, 3261–3267.

S.E. Stein, "Mass Spectra" in NIST Chemistry WebBook, NIST Mass Spec Data Center, Eds. P.J. Linstrom and W.G. Mallard, National Institute of Standards and Technology, Gaithersburg MD, 20899

Uegaki, R., Fuiimori, T., Kaneko, H., Kato, K., Noguchi, M., 1979. Isolation of Dehydrololiolide and 3-Oxo-actinidol from Nicotiana tabacum. Agric. Biol. Chem 43, 1149–1150.

CHAPTER III

ORGANIC SULFONES IN THE BRINE OF LAKE VIDA (EAST ANTARCTICA)

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The contents of Chapter III will be reformatted for submission to *Geochimica et Cosmochimica Acta*

Abstract

Located in a closed basin in the McMurdo Dry Valleys, East Antarctica, Lake Vida brine is a cold (-13°C), interstitial, anoxic, and aphotic ecosystem trapped within 27+ m of ice. This brine is not in contact with the atmosphere, and currently hosts a slow-growing, cold-limited bacterial ecosystem that has persisted for at least ~2800 years. The dichloromethane extractable fraction of dissolved organic matter of Lake Vida brine revealed the presence of 11 novel organic sulfones, tentatively identified on the basis of mass spectral fragmentation. A total of 19 sulfones were observed, none of which were previously described in any other natural ecosystems. Our results suggest that these compounds may be legacy of a previous lake system in which a dissolved organic carbon pool was oxidized in lake waters in contact with the atmosphere. Alternative hypotheses on the origin of these sulfones, involving potential abiotic alterations of dissolved organic sulfur as a consequence of long-term brine-rock reactions that generated reactive oxygen species, are discussed as well.

Keywords: sulfones, crysophere, organic sulfur compounds, Antarctica, brine

3.1. Introduction

Dissolved organic sulfur (DOS) plays an important role in the sulfur cycle and can be the main source of sulfur for biological growth in aquatic environments (Kertesz, 1999; Tripp et al., 2008; Ksionzek et al., 2016). Not only is sulfur an essential element in the amino acids cysteine and methionine, it is also found in sulfolipids, vital enzyme cofactors such as biotin, coenzyme A, coenzyme M, lipoic acid, S-adenosyl methionine, and iron-sulfur. The reduction of oxidized inorganic sulfur such as sulfate (SO_4^{2-}) and sulfite (SO_3^{2-}) can be either assimilatory, in which organic sulfur compounds (OSCs) such as cysteine and methionine are synthesized by organisms using energy, or dissimilatory, in which electron acceptors like sulfate are reduced to yield energy while forming reduced byproducts such as H_2S. Aside from being incorporated in metal sulfides such as pyrite, H_2S can also be methylated to form methanethiol (MeSH), or dimethylsulfide (DMS), through biological and abiotic processes (Drotar et al., 1987; Schulz and Dickschat, 2007; Sela-Adler et al., 2015).

Though our understanding of the sulfur cycle has increased significantly in the last few decades, partially due to the recognized role of DMS in the global sulfur cycle, little is known about chemical pathways between biosynthesized OSCs and recalcitrant dissolved organic sulfur (DOS) that may persist in aquatic systems or eventually be deposited in sedimentary environments. This DOS pool is linked to the sulfur biogeochemical cycle. The DOS chemical inventory may hold critical information on the complex interaction between organic sulfur compounds that support biological activities and volatile sulfur compounds that are released

into the atmosphere and are critical for climate regulation. In phytoplankton biomass, sulfur is first assimilated via the proteinogenic synthesis of methionine and cysteine. The amino acids can subsequently be used as precursors for other important sulfur-containing compounds such as S-adenosyl-methionine (SAM), a cofactor that typically facilitates the catalysis of methyl group transfers in biochemical pathways, or dimethylsulfoniopropionate (DMSP), an osmolyte, cryoprotectant, and antioxidant agent utilized by a variety of marine microorganisms (Stefels, 2000; Sunda et al., 2002; Asher et al., 2011).

DMSP is one of the most abundant OSCs on the Earth's surface (Kirst et al., 1991; Yoch, 2002; Dickschat et al., 2015). Though DMSP production is commonly attributed to phytoplankton, micro and macroalgae, as well as other photosynthetic eukaryotes (Blunden et al., 1982; Van Diggelen et al., 1986; Kasamatsu et al., 2004), it has been recently shown that marine heterotrophic bacteria also have the genetic potential to synthesize DMSP, likely via the same methionine transamination pathway found in microalgae and phytoplankton (Curson et al., 2017). This discovery potentially broadens the range of environments where DMSP production may occur. DMSP is released into the water column upon cell lysis (via grazing, or due to viral infections) and can be taken up by marine bacteria and subsequently degraded into volatile OSCs (VOSC's) such as DMS or MeSH. There are two different pathways for which marine bacteria can catabolize DMSP: (1) the cleavage of DMSP to DMS and acrylate and (2) the demethylation of DMSP into MeSH and 3-(methylmercapto) propionate, and further into 3-mercaptopropionate (Taylor and Gilchrist, 1991). In addition,

bacterial methylation to form MeSH occurs in anoxic fresh water systems via dissimilatory sulfate reduction (Schulz and Dickschat, 2007; Sela-Adler et al., 2015). Downstream alteration (i.e. microbial degradation, photodegradation, photooxidation) of VOSC's or other non-volatile byproducts produced by DMSP catabolism may contribute significantly to the total DOS pool in marine environments.

In the context of organic matter cycling, biogenic OSCs synthesized by primary producers are often referred to as "primary organic sulfur". Inorganic reduced sulfur can also abiotically react with organic matter in sulfidic water columns and sediments as part of early diagenetic processes (Sinninghe Damsté et al., 1989; Kenig and Huc, 1990; Vairavamurthy et al., 1994; Werne et al., 2003; Sleighter et al., 2014). These OSCs are termed "secondary organic sulfur". Processes that promote the incorporation of reduced sulfur into organic matter persist mainly in sulfidic environments where high concentrations of sulfides such as H_2S (Sinninghe Damsté et al., 1989), or reactive intermediate sulfur such as polysulfides (Kohnen et al., 1989), elemental sulfur or thiosulfate ($S_2O_3^{2-}$) are present (Werne et al., 2003). Sulfur may also be incorporated into dissolved organic matter (DOM; Heitmann and Blodau, 2006). In marine environments, this has been demonstrated through laboratory simulations of abiotic sulfurization of DOM in sulfidic conditions (Pohlabeln et al., 2017) and is evidenced by the high levels of DOS that have been in contact with recirculating reduced (H_2S-rich) marine hydrothermal fluids (Gomez-Saez et al., 2016). Though attempts have been made to elucidate the chemical diversity of DOS to constrain its origin and distribution in aquatic systems

(Pohlabeln and Dittmar, 2015), the mechanisms by which OSCs are transformed to recalcitrant DOS remain speculative.

Our understanding of the chemical complexity of DOS mainly came from studies of marine DOM. A majority of the analytical methods used to characterize all the organic constituents within a complex DOM sample involve applications such as nuclear magnetic resonance (NMR), fluorescent spectroscopy, or more recently, electrospray ionization Fourier transform-ion cyclotron resonance-mass spectrometry (ESI FT-ICR-MS), each of which have different advantages and disadvantages. For elucidating chemical differences in DOM chemistry, ESI FT-ICR-MS has been shown to be a powerful and sensitive technique that can provide accurate molecular formulas for a number of organic compounds found in DOM (Dittmar, 2015; Cawley et al., 2016), including S-bearing compounds (Ksionzek et al., 2016; Poulin et al., 2017). However, ESI FT-ICR-MS may only provide chemical formula of S-bearing organic compounds in DOM, not their structures. Collision induced tandem mass spectrometry may be useful in determining S-bearing functional groups by observing neutral losses when compounds are introduced into a collision cell (Pohlabeln and Dittmar, 2015). However, reconstructing the exact molecular structure from this method is particularly challenging as tandem mass spectral libraries are not as comprehensive as electron ionization mass spectral databases. Similarly, other approaches have been used to attempt quantification of sulfur functional groups and oxidation states in organic matter using stable isotope probing

or X-ray absorption near edge structure (XANES) spectroscopy (Vairavamurthy, 1998; Jokic et al., 2003; Manceau and Nagy, 2012; Zhu et al., 2014).

Here, we report on organic sulfur compounds (sulfones) and their tentative structures as determined on the basis of mass spectrometry analysis. These compounds were extracted from a subsurface interstitial hypersaline brine located in the icy matrix of frozen Lake Vida, in East Antarctica. We performed conventional liquid-liquid extraction of the dissolved organic matter in Lake Vida brine (LVBr) using dichloromethane (DCM). The extract was analyzed using comprehensive multidimensional gas chromatography-time of flight-mass spectrometry (GC×GC-TOF MS), which provides a high resolving power and detection sensitivity capable of elucidating small molecules (low molecular weight analytes; 5 to 1000 Daltons) in a complex environmental sample. We tentatively identify a suite of oxidized OSCs bearing sulfones functional groups using mass spectral fragmentation and by comparisons to spectra published in the literature (Bowie et al., 1966; Truce et al., 1968; Smakman and de Boer, 1970; Kingston et al., 1974; Dickschat et al., 2010). Based on our understanding of the Lake Vida hydrological history (Dugan et al., 2015b, 2015a), microbiology and geochemistry (Murray et al., 2012; Kuhn et al., 2014; Ostrom et al., 2016; Proemse et al., 2017), DOM chemistry (Cawley et al., 2016), and legacy effects on environmental metabolomics (Chou et al., 2018), we propose potential sources for the OSCs identified in this study and the mechanisms by which they may be produced, transformed, and preserved in LVBr.

3.2. Sampling Site Description

Lake Vida (77°23′S 161°56′E) is a permanently frozen lake located in Victoria Valley, the northernmost valley of the McMurdo Dry Valleys, East Antarctica. The icy lake body encapsulates an aphotic, anoxic, very cold (-13.4°C), interstitial hypersaline brine (salinity 188; Murray et al., 2012) within >27 m of ice (Dugan et al., 2015b), which may extend down to a depth of 100 m below the bottom of the lake ice (Dugan et al., 2015a). The present-day brine network is not in contact with the atmosphere, as demonstrated by the pressurized behavior of the subsurface fluids during sampling (Dugan et al., 2015a), and supported by noble gas analyses (Malone et al., 2010). Various radiocarbon dating of organic matter embedded within the ice cover (at 12 m beneath the surface of the lake) and dissolved organic matter from LVBr suggests that the brine has been isolated from the surface environment for at least ~2800 ^{14}C years (Doran et al., 2003). The dissolved organic carbon in LVBr is old (radiocarbon age of 2955 to 4150 ^{14}C years BP ; Cawley et al., 2016). The dissolved organic carbon (DOC) concentration in LVBr is unusually high (580 mg $C{\cdot}L^{-1}$) by comparison to the subglacial brine in Taylor Valley, Blood Falls (9.25 mg $C{\cdot}L^{-1}$; Mikucki et al., 2004), other lakes of the dry valleys such as the bottom waters of Lake Bonney, 20 mg $C{\cdot}L^{-1}$ (Takacs and Priscu, 1998), or the bottom of Lake Fryxell, 24 mg $C{\cdot}L^{-1}$; (Karr et al., 2005), as well as other saline lakes (e.g., Mono Lake, 80 $mg{\cdot}L^{-1}$; Hollibaugh et al., 2001). LVBr hosts a temperature-limited, exclusively bacterial community with an average estimated generation time of 120 years (Murray et al.,

2012). The metabolic lifestyles extrapolated from the environmental representatives of the Lake Vida brine microorganisms include facultative chemoheterotrophy, fermentation, and chemolithoautotrophy, though it is suggested that their present day contributions, through production and consumption processes, to the total organic carbon pool is minute due to a large reservoir of paleometabolites (Chou et al., 2018).

Several lines of evidence suggest that Lake Vida brine is, in part, derived from an older lacustrine system that previously occupied the Lake Vida basin a few thousand years ago years BP, including paleometabolites that derived from photosynthetic communities. This particular ancient lake system was thus supported by primary productivity, in contrast to the current Lake Vida brine, which is totally aphotic, anoxic, and is solely supported by heterotrophy and chemolithoautotrophy. Evidence of photic zone euxinia in the previous lake was obtained from the detection of biomarkers of green sulfur bacteria, Chlorobiaceae, suggesting that the ancient Lake Vida contained, at least temporarily, a stratified water column (Chou et al., 2018). The effects of legacy on the organic constituents of current ecosystems is not only limited to Lake Vida brine, but have been suggested for many other systems in the McMurdo Dry Valleys (Burkins et al., 2000; Lyons et al., 2000; Knoepfle et al., 2009; Doran et al., 2014).

The concentration of SO_4^{2-} in LVBr is 58.4 $mmol \cdot L^{-1}$, whereas, H_2S and sulfur intermediates such as thiosulfate ($S_2O_3^{2-}$) were not detected (Murray et al., 2012). The levels of SO_4^{2-} in LVBr is high compared to other subglacial Antarctic lakes, such as the bottom of Lake Fryxell (1.58 $mmol \cdot L^{-1}$ and ; Green et al., 1989) or the bottom of Lake Vanda (0.31 $mmol \cdot L^{-1}$;

Webster, 1994). On the other hand, levels of H_2S in Lake Fryxell and Lake Vanda (1.2 mmol·L^{-1} and 0.33 mmol·L^{-1}, respectively) are high compared to LVBr, in which H_2S was not detectable (Green et al., 1989; Webster, 1994; Murray et al., 2012). However, the concentration of SO_4^{2-} in LVbr is comparable to that of Blood Falls brine discharge (50 mmol·L^{-1}; Mikucki et al., 2009), though levels of H_2S in Blood Falls was not measured. Previous detection of sulfur-bearing compounds in LVBr, by Murray et al. (2012), dimethylsulfoxide (DMSO; 25 μmol·L^{-1}), methanethiol (CH_3SH at 0.2 μmol·L^{-1}), dimethylsulfide (DMS; 0.1 μmol·L^{-1}) , as well as by Kenig et al. (2016), dimethyldisulfide (DMDS), trace levels of dimethyltrisulfide (DMTS), and carbon disulfide (CS_2) led us to speculate that the brine of Lake Vida may also contain other OSCs.

3.3. Materials and Methods

3.3.1. Sample collection and extraction

The brine sample analyzed here was collected during a 2010 expedition to Lake Vida. Clean sampling strategy was implemented to collect the brine with minimized forward contamination and is fully described in Doran et al. (2008). The lake ice was cored until the brine was reached. The brine infiltrated the borehole to a depth of 16 m below the surface of the 27+ m lake ice and rose to 11 m. The sample was collected at 18.5 m. A stainless steel submersible pump with PTFE tubing was used to collect brine samples, which were then stored

in 1L sterile and solvent-cleaned (DCM:MeOH 1:1) PTFE bottles. Samples were spiked with 4 mM $HgCl_2$ to prevent further biological activities. Brines were stored at 4°C until extraction.

A 200 mL sample of Lake Vida brine was extracted using conventional liquid-liquid extraction with dichloromethane (DCM). Briefly, organic-free water was obtained by extracting Milli-Q® water three times with DCM. The Lake Vida brine was amended with 100 mL of this clean Milli-Q® to increase the density contrast between the briny aqueous phase and the denser DCM phase. The brine was extracted three times using 100 mL of DCM per extraction. The total extract was then rotary-evaporated to near dryness, further dried under low flow N_2, and redissolved into cyclohexane (1 $mg \cdot mL^{-1}$ of extract). The sample was then injected into the multidimensional GC×GC.

3.3.2. Multidimensional Comprehensive Gas Chromatography-Time of Flight-Mass Spectrometry (GC×GC-TOF MS)

For GC×GC-TOF MS, the sample was injected in a Leco Pegasus 4D, which consists of an Agilent 7890 gas chromatograph with a split/splitless injector, two GC capillary columns, a pulsed jet modulator cooled by liquid nitrogen, coupled to a TOF mass spectrometer. The first capillary column was a 5% phenyl polydimethylsiloxane phase (SGE BPX-5, 24.47 m; 0.25 mm ID; 0.25 μm film thickness) and the secondary capillary column has a more polar 50% phenyl/50% polydimethylsiloxane phase (SGE BPX-50; 1.65 m; 0.1 mm ID; 0.1 μm film thickness). The sample was injected in pulsed splitless mode at 250°C (with a purge time of 60

s). The first column was kept at 40°C for 1 min, then ramped at 3°C·min^{-1} to 300°C and held isothermal for 10 mins. The secondary column was held 10°C hotter than the first column. A transfer line to the TOF detector comprise of the same capillary column as the secondary GC column (SGE BPX-50, 0.21 m, 0.1 mm ID). Helium (>99.999% ultra-high purity, Praxair®) was used as the carrier gas in constant flow of 1 mL·min^{-1}. The modulation period for the GC×GC was 6 s with a hot pulse time of 0.5 s and a cool time of 2.5 s. The mass spectrometer operates in electron ionization mode at 70 eV with an ion source temperature of 200°C. There was a 660 s solvent delay. Spectra were collected from *m/z* 30 to *m/z* 500 at the rate of 200 spectra per second. The Leco software package, ChromaTOF, was used to process the data. The mass spectra in this study are derived from ChromaTOF's "Peak True" deconvolution algorithm used to subtract background noise from peaks, delivering a robust baseline correction method for data processing.

3.3.3. Mass spectral interpretation

We based the tentative identification of unknown compounds reported in this investigation on interpretation of mass spectral fragmentation and comparison with previous mass spectral studies of other authentic sulfones, sulfoxides, alkyl sulfonates, cyclic sulfones, and organic sulfur-bearing compounds (McLafferty, 1959; Bowie et al., 1966; Truce et al., 1968; Smakman and de Boer, 1970; Kingston et al., 1974; Dickschat et al., 2010).

To compare the mass spectra of compounds observed in LVBr that have existing mass spectra of authentic standards in the literature, or in the NIST database, we employed least square regression statistics and calculated r^2 and p values. We only include peaks if they are observed in both spectra, though none omitted were more than ~2% abundant relative to the base peak. To avoid artificial forcing of the regression between authentic standards and observed spectra, we included neither the base peak nor a 0,0 intercept.

The mass spectra of the OSCs observed here all show a positive correlation to the corresponding mass spectra of their authentic standards ($r^2 > 0.81$ and $p < 0.006$). The variability in mass abundances in each comparison can be attributed to several factors including differences in mass spectrometers used, as well as the fact that authentic standards are typically analyzed as pure compounds, which would provide cleaner mass spectra than would our environmental samples.

3.4. Results

The OSCs reported in this study are some of the most polar compounds present in the total DCM-extractable organic matter fraction of LVBr, with elution times in the secondary GC dimension above 2.8 s (Fig. 1a). The OSCs observed in the brine extract form five groups: (1) aliphatic sulfones, (2) amino sulfones, (3) cyclic sulfones, (4) aromatic sulfones and (5) a group of miscellaneous sulfones and sulfur bearing compounds. Most of the compounds

tentatively identified here contain a methylated sulfone moiety, with a sulfur oxidation state of +2. The base fragment ions in the mass spectra of methyl sulfones is *m/z* 79 $[CH_3SO_2]^+$ (Fig. 1b). In addition, electron ionization of methylsulfone-bearing compounds that contain various aliphatic, cyclized, or aromatic constituents can induce rearrangements, leading to specific, diagnostic fragmentation patterns.

3.4.1. Acyclic aliphatic sulfones

The simplest oxidized OSCs in LVBr are DMSO and $DMSO_2$. Upon GC×GC-TOF MS, peak b (Fig. 1a) was tentatively identified as a dimethylsulfone ($DMSO_2$) with its typical fragment ion *m/z* 79 and molecular ion *m/z* 94 (Fig. 1a and b) and by comparison of its mass spectra with that published by Bowie et al. (1966; $p = 8.5\times10^{-13}$; Fig. 2a). In addition, the mass spectra of $DMSO_2$ displays a fragment ion at *m/z* 63 for $[M\text{-}OCH_3]^+$ (Fig. 1b). The mass spectra of $DMSO_2$ also displays the characteristic isotopic peak of $[M+2]^+$, ~4% of $[M]^+$, due to the natural ratio of ^{34}S to ^{32}S. Note that we did not observe DMSO in LVBr upon GC×GC-TOF MS analysis, potentially due to its high vapor pressure, making it generally more volatile than $DMSO_2$. DMSO may have been lost during sample preparation. DMSO, however, was detected at high concentrations (25 μmol·L^{-1}) in LVBr compared to DMS (0.1 μmol·L^{-1}; Murray et al., 2012).

We tentatively identified the structures of three novel aliphatic methyl sulfones in the summed chromatogram of mass fragments common in small methyl sulfones (Fig. 1c). We assign the structures of 1-(methylsulfonyl)-*i*-propane (Fig. 1d), 1-(methylsulfonyl)-*n*-propane (Fig. 1e), and 1-(methylsulfonyl)-*i*-butane (Fig. 1f) on the basis of mass spectral characteristics, relative retention time, and comparison of published fragmentation patterns observed on other sulfones (Bowie et al., 1966; Truce et al., 1968; Smakman and de Boer, 1970; Kingston et al., 1974). We also tentatively identified 1-(methylsulfonyl)-*n*-butane and as we could compare its mass spectra (Fig. 1g) to that described in detail by Smakman and de Boer (1970; $p = 1.8 \times 10^{-15}$; Fig. 2b).

The mass spectra of aliphatic sulfones are dominated by hydrocarbon fragment ions (e.g., *m/z* 43 and *m/z* 57) corresponding to the loss of the biggest *n*-alkyl fragment by the cleavage of the bond α to the sulfone (Fig. 1 d, e, f, g). Smakman and de Boer (1970) noted that when a sulfone has an α-branched alkyl substituent and an *n*-alkyl substituent, the α-branched alkyl provides a more abundant fragment ion than for an *n*-alkyl substituent. Furthermore, upon EI, up to two hydrogen atoms can migrate from the hydrocarbon chain to the oxygen atoms of the sulfone (Smakman and de Boer, 1970) if there are one or more hydrogens available at the β and/or γ positions (Fig. 1f and Fig 1g), more rarely if the hydrogen is at the α position (Bowie et al., 1966). The relative intensities of the fragment ions *m/z* 79 for $[CH_3SO_2]^+$, and *m/z* 80, and *m/z* 81 for, $[CH_3SO_2H]^+$, and $[CH_3SO_2H_2]^+$ with the hydrogen(s) transfer, respectively, thus vary as a function of the size of the alkyl side chain with a higher propensity for hydrogen

transfer with increasing size of the alkyl groups (Bowie et al., 1966). This influence of the chain length is visible in the higher abundance of fragment ion *m/z* 81 relative to *m/z* 79 and *m/z* 80 in the mass spectra of the sulfones with a butane substituent (Fig. 1f and g) versus sulfones with a propane substituent (Fig. 1d and e).

We tentatively assigned the structure of two compounds as aliphatic sulfones with α branched alkyl sidechains, because of the lack of stable, large $[M\text{-}CH_3]^+$ fragment ions such as *m/z* 107 for 1-(methylsulfonyl)-*i*-propane (Fig. 1 d) and *m/z* 121 in 1-(methylsulfonyl)-*i*-butane) (Fig. 1f) due to the preferential cleavage α to the sulfur of the α-branched alkyl substituents (Smakman and de Boer, 1970). In contrast, the fragment ions $[M\text{-}CH_3]^+$ are observed in the mass spectra of 1-(methylsulfonyl)-*n*-propane (Fig. 1e) and 1-(methylsulfonyl)-*n*-butane (Fig. 1g). It is notable that we did not observe molecular ions for the sulfones containing the α-branched alkyl substituent. It is also important to note that the methyl sulfones with α-branched alkyl precede their *n*-alkyl counterparts in both dimensions of the GC×GC trace (Fig. 1c).

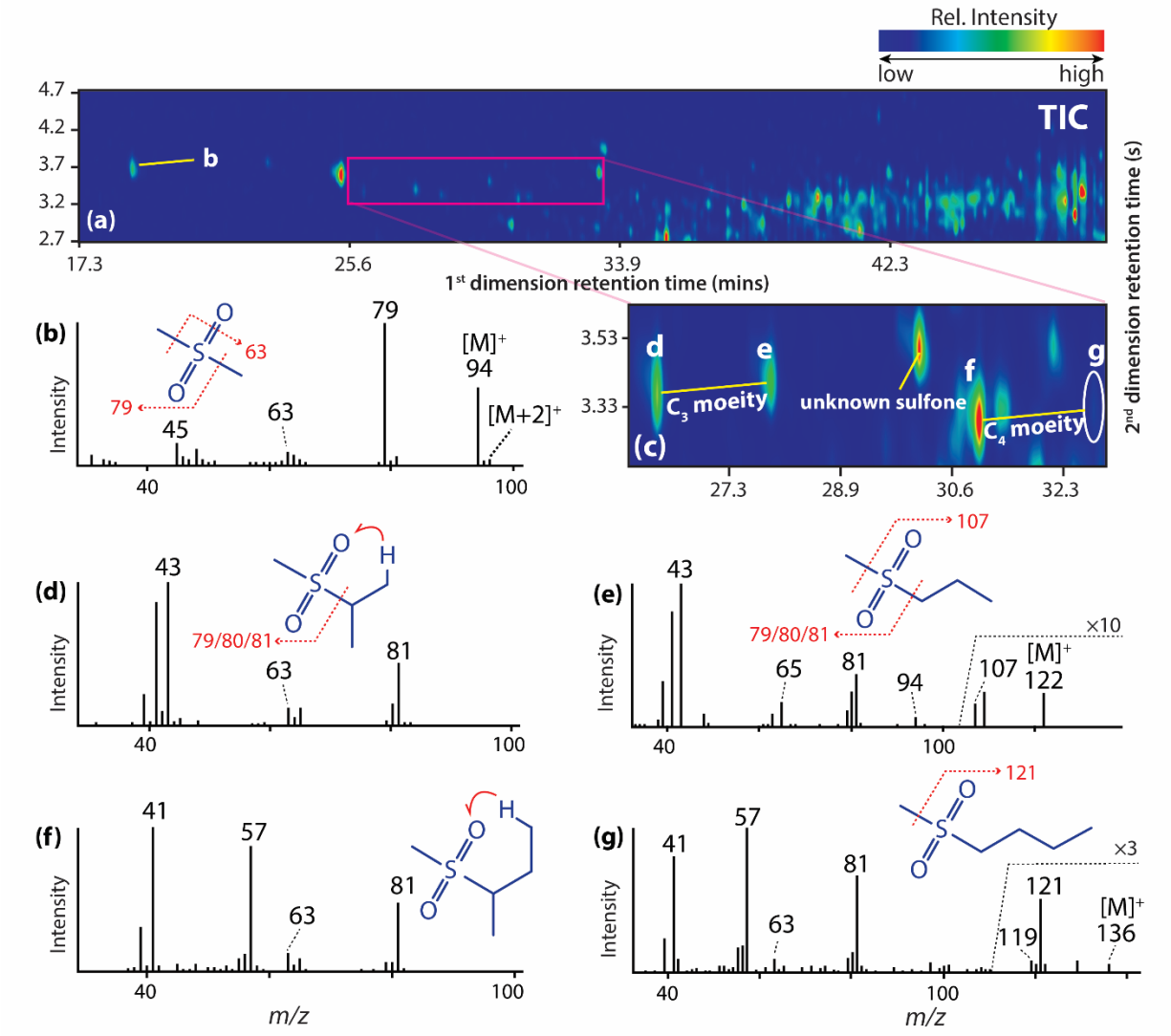

Figure 1: (a) GC×GC-TOF-MS total ion current (TIC) chromatogram of the total DCM-extractable environmental metabolome of LVBr. **(b)** Mass spectra of peak b, dimethylsulfone. **(c)** GC×GC-TOF-MS partial summed mass ion chromatogram for *m/z* 41 + *m/z* 43 + *m/z* 57 + *m/z* 81 + *m/z* 121 (x20 intensity) expanded from the box in (a). **(d), (e), (f), (g)** mass spectra of peaks d, e, f, g, respectively, and their tentative structures.

Two types of rearrangements occur in association with a γ hydrogen that is transferred to the oxygen of the sulfone. The first rearrangement is a McLafferty-type leading to fragment ion *m/z* 94 (McLafferty, 1959; Smakman and de Boer, 1970; Kingston et al., 1974), as visible in the mass spectra of 1-(methylsulfonyl)-*n*-butane and -*n*-propane (Fig. 1e and 1g). This McLafferty-type rearrangement has been previously described in the mass spectral studies of sulfonate esters (Truce et al., 1968). The second rearrangement is the loss of an hydroxyl $[OH]^{\bullet}$ radical and the formation of a stable five-membered ring system, involving the second oxygen and the γ carbon (Smakman and de Boer, 1970). This hydroxyl loss is observable in the mass spectra of 1-(methylsulfonyl)-*n*-propane, as fragment ion *m/z* 119, $[M\text{-}OH]^{+}$ (Fig. 1e).

3.4.2. Amino sulfones

The mass spectral fragmentation patterns of nitrogen-bearing sulfones are less studied compared to those of aliphatic sulfones. Here, we tentatively identified the structures of four novel methyl amino sulfones (Fig. 3a and 3b) on the basis of mass spectral fragmentation and application of the nitrogen rule in presence of one nitrogen: odd molecular ions and even-numbered fragment ions. In addition, we identified methane sulfonamide and *N*,*N*-dimethyl methanesulfonamide by comparison of its mass spectra with that of the NIST library and comparison of its fragmentation pattern with that of an authentic standard described in Truce et al. (1968) (p =8.3×10^{-12} and 1.9×10^{-11} respectively; Fig. 2c and 2d).

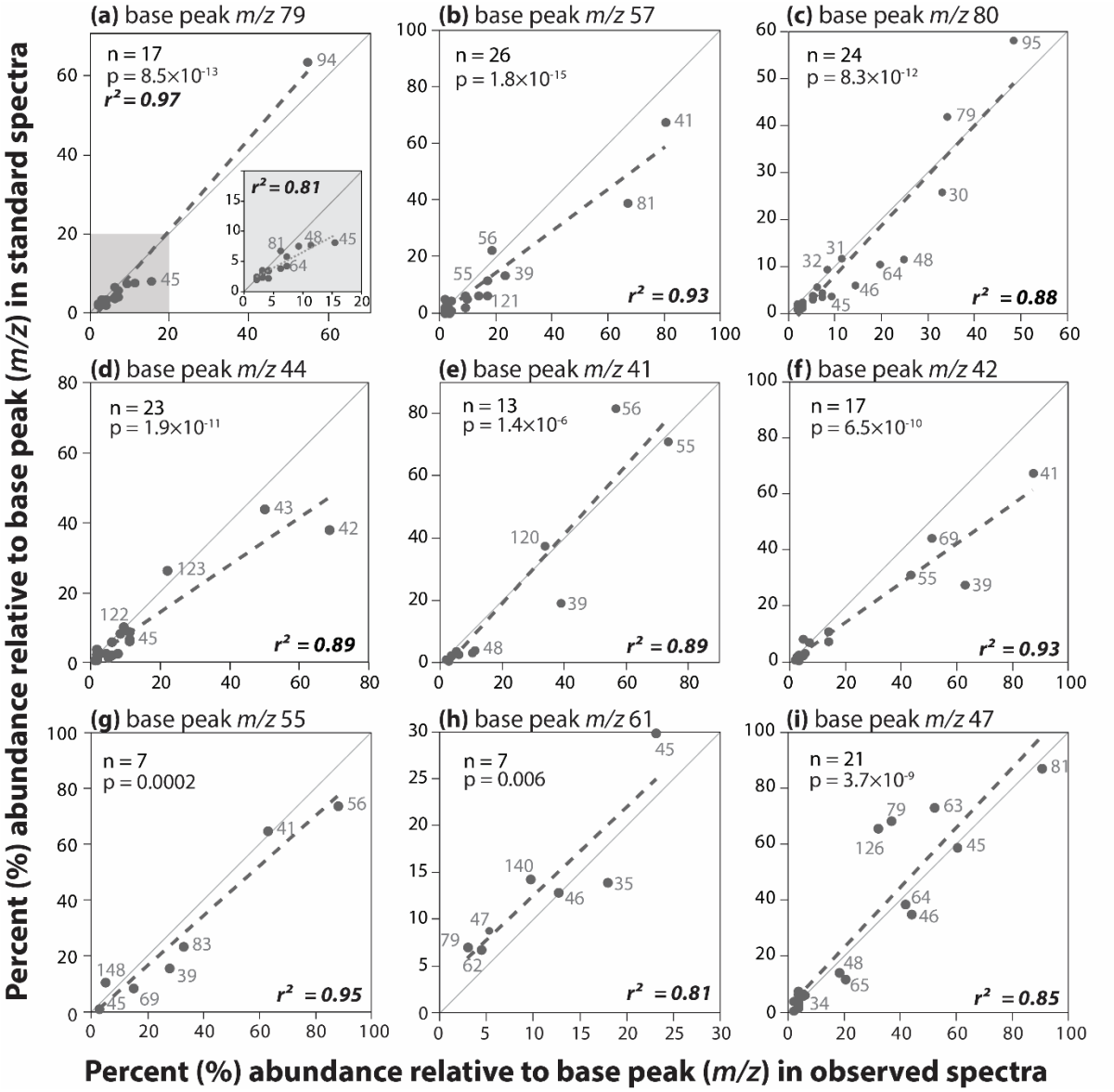

Figure 2: Plot of the percent abundance of fragment ions to base peak (100%) in the mass spectra of authentic standards obtained from the literature or from the National Institute of Standards and Technology (NIST) Database versus % abundance relative to base peak in the observed spectra. The grey dashed line shows the least squares regression line of sample, and the orange line is the least squares regression line with r^2=1; **(a)** dimethylsulfone, **(b)** 1-(methylsulfonyl)-n-butane, **(c)** methane sulfonamide, **(d)** N,N-dimethyl methanesulfonamide, **(e)** tetrahydro-1,1-dioxide thiophene, **(f)** tetrahydro-1,1-dioxide thiopyran, **(g)** 1,1-dioxide thiepane, **(h)** methyl (methylthio)methyl disulfide, and **(i)** S-methyl methanethiosulfonate.

The simplest of the new tentatively identified amino sulfone, methane sulfonamide (Fig. 3c), is structurally analogous to $DMSO_2$ and their fragmentation patterns are similar, with a molecular ion at *m/z* 95 (~50% of the base peak), a characteristic $[M+2]^+$ ion for the ^{34}S, and a base fragment ion at *m/z* 80, which like for $DMSO_2$ corresponds to the loss of a methyl group, $[M-CH_3]^+$. The fragment ion *m/z* 79 correspond to the loss of the amine group $[M-NH_2]^+$. The fragment ion *m/z* 64 $[M-OCH_3]^+$ in the mass spectra of the tentatively identified methane sulfonamide (Fig. 2c) is analogous to the fragment ion *m/z* 63 for $[M-OCH_3]^+$ of $DMSO_2$.

The mass spectrum of *N,N*-dimethyl methanesulfonamide (Fig. 3d) has a base peak *m/z* 44 corresponding to the cleavage α to the sulfur and loss of a neutral methyl sulfone moiety. The presence of a low abundance fragment ion *m/z* 79 (10%) corresponds to the loss of a neutral *N,N*-dimethylamine group from the molecular ion. It is noteworthy that the presence of fragment ion *m/z* 81 indicates that, as observed for aliphatic sulfones, that β-hydrogens can be transferred to the oxygens of the sulfones to form alcohol groups (Truce et al., 1968). The loss of hydrogens from the *N,N*-dimethylamine is also indicated by the presence of fragment ions *m/z* 42 and *m/z* 43.

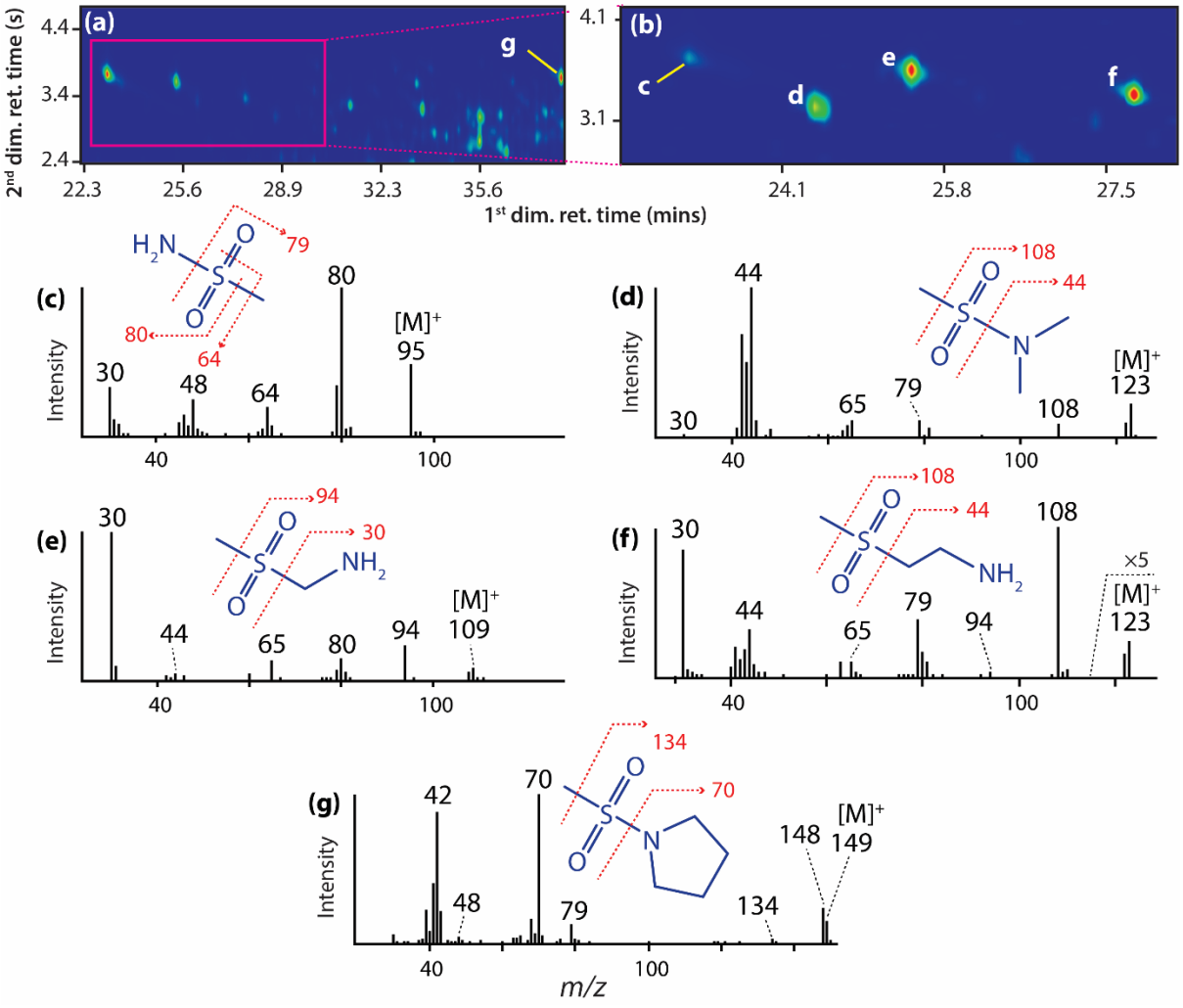

Figure 3: **(a)** GC×GC-TOF-MS partial summed mass ion chromatogram of *m/z* 70 + *m/z* 80 + *m/z* 95. (b) GC×GC-TOF-MS partial summed mass ion chromatograms *m/z* 44 (x10 intensity) + *m/z* 70 + *m/z* 80 + *m/z* 95 + *m/z* 108, which are major mass fragmentation ions for amino sulfones. **(b)** is expanded from the box in (a). **(c), (d), (e), (f), (g)** Mass spectra of peaks c, d, e, f, and g, respectively, and their tentative structures.

The mass spectra of peak e (Fig. 3b) is tentatively assigned as 2-(methylsulfonyl) methanamine on the basis of characteristic mass fragmentation. The presence of *m/z* 94, $[M\text{-}CH_3]^+$ (Fig. 3e) suggests the occurrence of a methyl substituent on the sulfur. The base peak *m/z* 30 corresponds to the loss of a methylsulfone neutral fragment. In the mass spectra of all tentatively identified methyl alkylaminosulfones, alkyl amine groups such as *m/z* 30 (methylamine) and *m/z* 44 (ethylamine) are abundant or are the base peaks in their mass spectra. In the mass spectra of peak f (Fig. 3b), the base fragment ion *m/z* 108 $[M^+\text{-}CH_3]$ and the fragment ion *m/z* 79, methylsulfonyl ion $[CH_3SO_2]^+$ suggest the presence of a methyl substituent on the sulfone. The presence of abundant fragment ions *m/z* 30 and *m/z* 44 indicate that the second substituent of the sulfone is an ethylamine. As a result, we tentatively assign this peak to 2-(methylsulfonyl)ethaneamine (Fig. 3f).

Because the fragment ion *m/z* 108 is larger than that of *m/z* 79, by analogy with aliphatic amines, we conclude that the substituent on the methyl sulfone is a straight-chain aliphatic amine rather than a branched alkyl amine. It is interesting to note that this compound is a structural analog of taurine (2-aminoethanesulfonic acid), a non-proteinogenic amino acid.

Another putative amino sulfone, peak 2g has a fragment ion *m/z* 70, characteristic of a pyrrolidinyl heterocycle (Fig. 3g). We tentatively assigned this compound as 1-(methylsulfonyl)-pyrrolidine. The location of the nitrogen is currently unknown and cannot be discerned with its mass spectra alone (Fig. 3g). Depending on the location of the nitrogen,

this compound bears structural similarity to proline, which is an amino acid that can also serve as an osmoprotectant (Margesin et al., 2008).

3.4.3. Cyclic and aromatic sulfones

The mass spectral fragmentation pattern of cyclic sulfones exhibit hydrogen rearrangement similar to those observed for acyclic aliphatic sulfones (Smakman and de Boer, 1970). Here, we observed the presence of three cyclic sulfones (Fig. 4a) that were identified by comparison of their mass spectra to the mass spectra of authentic standards reported in Smakman and de Boer (1970): tetrahydro-1,1-dioxide thiophene (Fig. 4b), tetrahydro-1,1-dioxide thiopyran (Fig. 4d), and 1,1-dioxide thiepane (Fig. 4e) (p = 1.4×10^{-6}, 6.5×10^{-10}, 2×10^{-4}, respectively; Fig. 2e, Fig. 2f, Fig. 2g). In addition, we tentatively identified one novel acyclic aliphatic sulfone, methyl tetrahydro-1,1,-dioxide thiophene (Fig 4e), based on relative retention time and mass fragmentation pattern.

In addition to the hydrogen transfer(s) as observed for the acyclic aliphatic sulfones, cyclic sulfones also undergo a single or double C-S cleavage upon electron impact, before and after the transfer of hydrogens, thus, providing aliphatic fragment ions with neutral losses of 64 for $[M\text{-}SO_2]^+$, 65 for $[M\text{-}SO_2H]^+$, or 66 $[M\text{-}SO_2H_2]^+$ (Smakman and de Boer, 1970). For the C_4 cyclic sulfone (Fig. 4b), losses of 64 and 65 are similar in abundance, giving rise to fragment ions *m/z* 55 and *m/z* 56, whereas C_5 cyclic sulfone (Fig. 4c and 4d), and C_6 cyclic sulfone (Fig.

4f) predominately loses [SO_2H], giving rise to *m/z* 69 and *m/z* 83, respectively. This suggests that, much like the methyl aliphatic sulfones, the H migration is nonspecific, but may increase with increasing size of the alkyl group (Smakman and de Boer, 1970).

The tentative identification of two aromatic sulfones is evidenced by the high abundance of mass fragments containing an aromatic ring, their relatively high first dimension retention time (over 40 mins; Fig. 4f), as well as comparisons of mass spectra to the literature and the NIST database. We structurally assign (methylsulfonyl)benzene (Fig. 4g) based on the observed mass fragmentation patterns and rearrangements as described in Bowie et al. (1966). This compound exhibits the characteristic *m/z* 77 base peak for the phenyl moiety fragment $[C_6H_5]^+$. Upon electron impact, it can also rearrange where a C-O bond is formed via the migration of the phenyl group from the S to the O, inducing a loss of 79 for the methyl sulfone [CH_3SO_2] and subsequent H rearrangement, thus forming a phenol ion *m/z* 94 $[C_6H_5OH]^+$ (Bowie et al., 1966; Kinstle and Oliver, 1972). The tentative identification of [(methylsulfonyl)methyl]benzene (Fig. 4h) is demonstrated by the presence of the base peak *m/z* 91 for $[CH_6H_5CH_2]^+$ and their molecular ions *m/z* 170. These two mass spectra do not seem to show a characteristic loss of methyl group $[M-15]^+$ like the other methyl sulfones discussed in this study. Furthermore, the presence of *m/z* 65 is attributed to the loss of one carbon from the phenyl ring, forming a cyclopentadiene carbocation much like in the mass spectrum of toluene (Rinehart et al., 1968).

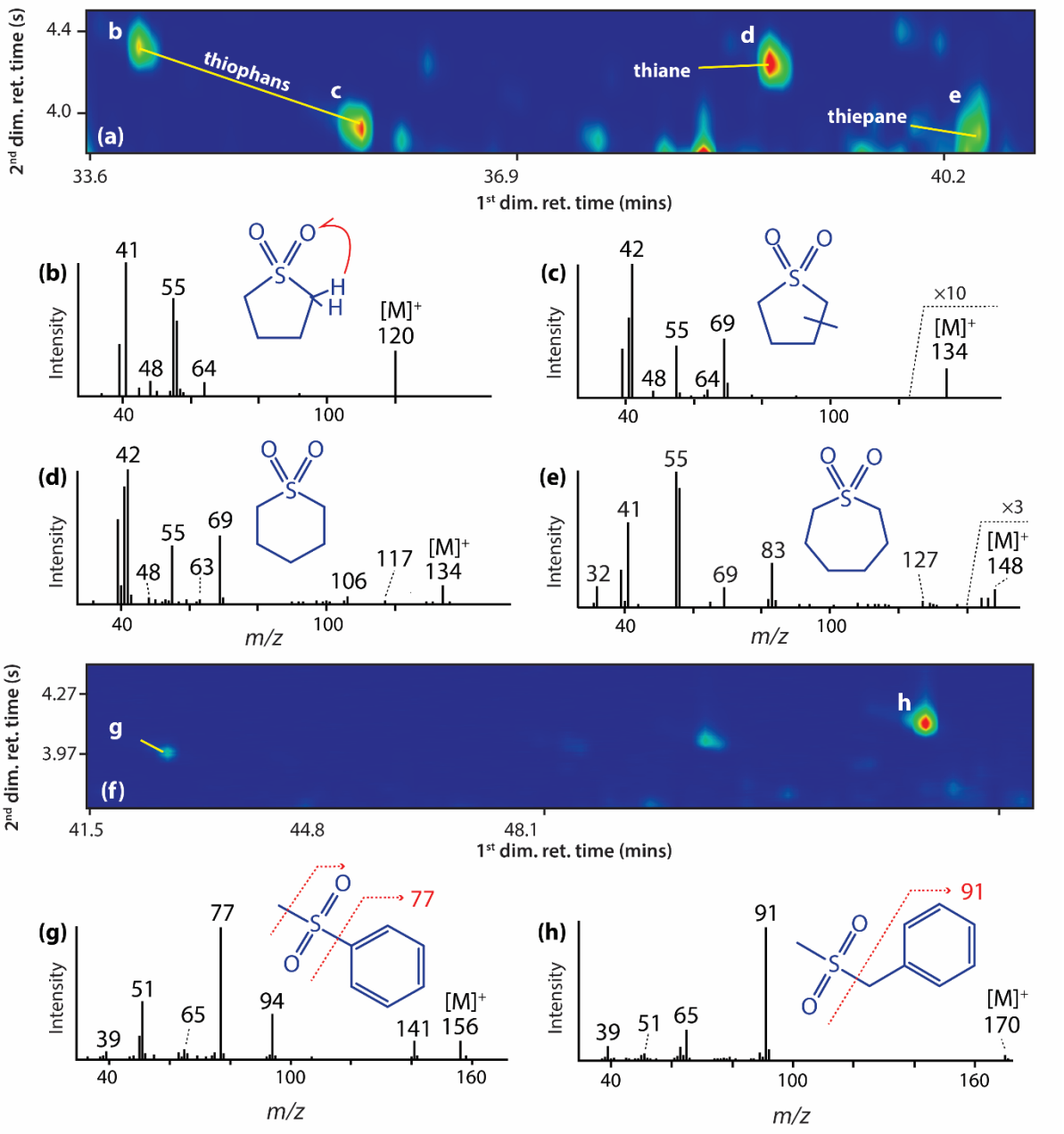

Figure 4: **(a)** GC×GC-TOF-MS partial summed mass ion chromatogram for *m/z* 39 + *m/z* 41 + *m/z* 42 + *m/z* 55 + *m/z* 56 + *m/z* 69, major fragments of alicyclic sulfones. **(b), (c), (d), (e)** Mass spectra of peaks b, c, d, and e, respectively, and their tentative structures. **(f)** GC×GC-TOF-MS partial summed mass ion chromatogram for *m/z* 77 + *m/z* 91 + *m/z* 156 (x20 intensity), major fragments of aromatic sulfones. **(g)**, (h) Mass spectra of peak g and h, respectively, and their tentative structures.

3.4.4. Other sulfur-bearing organic compounds

We also observed other OSCs while mass monitoring for sulfone moieties such as *m/z* 64 and 81 (Fig. 5a and 5b). We tentatively assign the structures of these compounds based on our understanding of the mass fragmentation patterns of sulfones previously described here, the abundance of isotopic peaks, and by comparisons to mass spectra published in the literature (Fig. 2 g and 2i). Two of the sulfones observed here contain chlorine. The mass fragment *m/z* 49 suggests the presence of a methyl chloride $[CH_2Cl]^+$ constituent, in which the monoisotopic peak was accompanied by *m/z* 51 due to the natural abundance of ^{37}Cl (~32% relative to ^{35}Cl). Methane chloro(methylsulfone) (Fig. 5d) and chloromethanesulfonamide (Fig. 5e) are structurally similar and contain methyl sulfone moiety like other OSCs compounds discussed in this study.

An OSC was tentatively identified here as methyl (methylthio)methyl disulfide (Fig. 5d), which is the only compound described in this study that does not contain oxidized sulfur. This compound fragments at the C-S bond adjacent to the S-S bond, giving rise to the stable $[CH_3S_2CH_2]^+$ fragment ion, *m/z* 61 (Dickschat et al., 2010). Within the entire GC×GC chromatogram, we observed only one other peak that contained a relatively high abundance of the $[CH_3S_2CH_2]^+$ *m/z* 61 fragment, which remains unidentified (data not shown), though this suggests that the DCM-extractable organic matter of LVBr is dominated by oxidized sulfur-bearing OSCs.

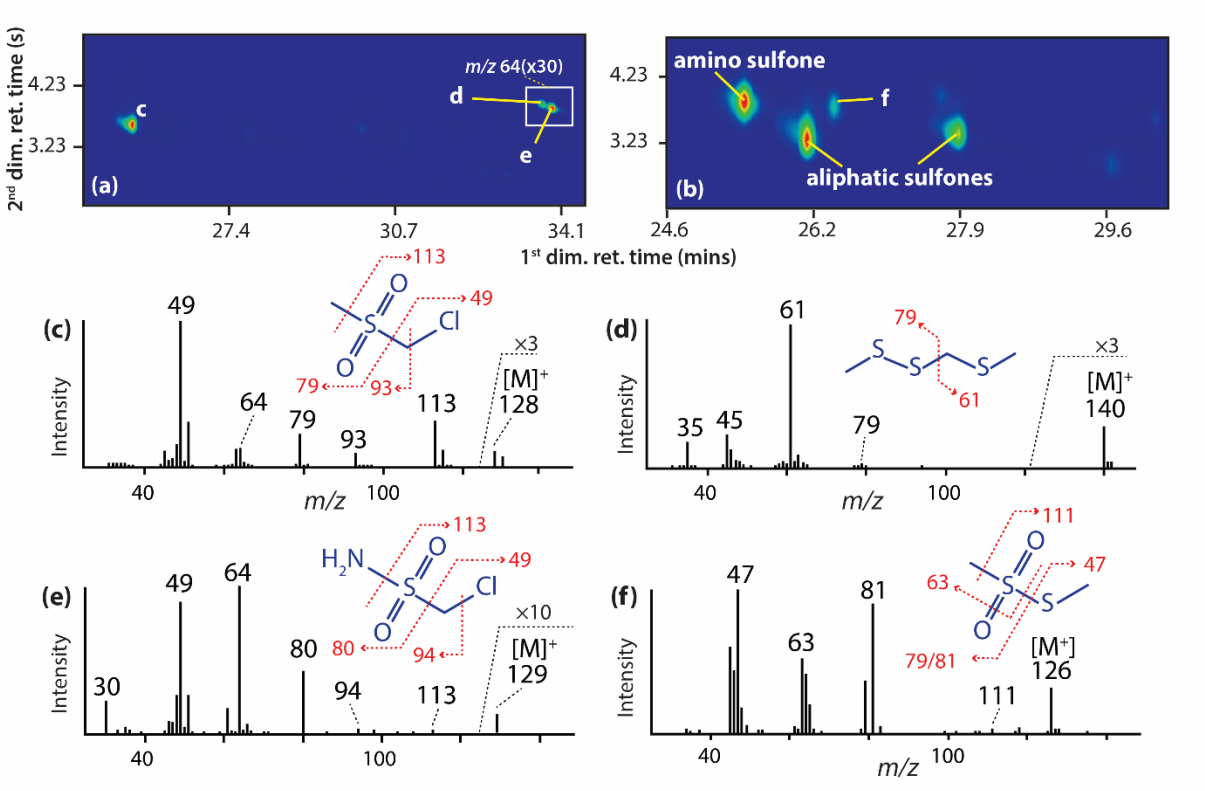

Figure 5: (a) GC×GC-TOF-MS partial summed mass ion chromatogram for *m/z* 49 + *m/z* 64, showing *m/z* 64 with 30x intensity in white box. **(b)** GC×GC-TOF-MS partial summed mass ion chromatogram for *m/z* 81. **(c), (d), (e), (f)** Mass spectra of peaks c, d, e, and f, respectively, and their tentative structures.

The tentative identification of S-methyl methanethiosulfonate (Fig. 5f) is supported by mass spectral fragmentation studies by Dickschat et al. (2010). Analysis of isotopically labelled S-methyl methanethiosulfonate shows that just this compound also undergo hydrogen rearrangement upon electron ionization, where up to two hydrogens at the β-position are transferred to the oxygen on the sulfone, producing the *m/z* 81 fragment (Dickschat et al., 2010). Furthermore, this compound is only one among other OSCs within the GC×GC chromatogram that contain a methyl sulfide moiety (*m/z* 47). Attempts to identify sulfonic acids, thiosulfonate, thioesters, or other organic sulfides via mass ion monitoring were unsuccessful in LVBr.

3.5. Discussion

Nineteen sulfones were observed in the DCM extract of the brine of Lake Vida. If the tentative structure of these compounds could be reported here, their origins are unclear. Organic compounds containing sulfur-bearing functional groups, such as sulfonic acids, are relatively common in marine sediments (Vairavamurthy et al., 1994) and can make up a large fraction of the dissolved organic sulfur in marine water columns (Pohlabeln and Dittmar, 2015), though the exact structures of these compounds have yet to be investigated.

In the following sections, several mechanisms for sulfur incorporation into organic matter (biotically or abiotically) in the LVBr system are discussed (Figure 6). We review the

potential pathways by which organic sulfur compounds form and be preserved in ancient Lake Vida (past) or its cryoencapsulated residual brine (present), as well as hypothesize on how those compounds could be oxidized to sulfones observed in this study.

3.5.1. Oxidation as part of a legacy process

In Antarctic aquatic environments, primary producers are a major source of sulfur bearing organic metabolites, such as DMSP. For example, the water column of Lake Bonney, a perennially ice-covered lake of the McMurdo Dry Valleys, has elevated concentrations of dissolved DMS, DMSP, and DMSO (up to 337 $nmol \cdot L^{-1}$, 20.4 $nmol \cdot L^{-1}$, and 270 $nmol \cdot L^{-1}$, respectively)(Lee et al., 2004). DMSP and DMSO were also observed in the meltwaters of the McMurdo Ice Shelf (up to 8.4 $nmol \cdot L^{-1}$ and 184.5 $nmol \cdot L^{-1}$, respectively)(De Mora et al., 1996), as well as DMSP (dissolved and particulates) in the pack ice from East Antarctica (268 $nmol \cdot L^{-1}$)(Trevena et al., 2000). High concentrations of DMS (up to 5,000 $nmol \cdot L^{-1}$) were observed in lakes of the Vestfold Hills (Gibson et al., 1991; Yau et al., 2013). In general, DMSO is derived from DMS itself, a metabolite of DMSP (Lomans et al., 2002).

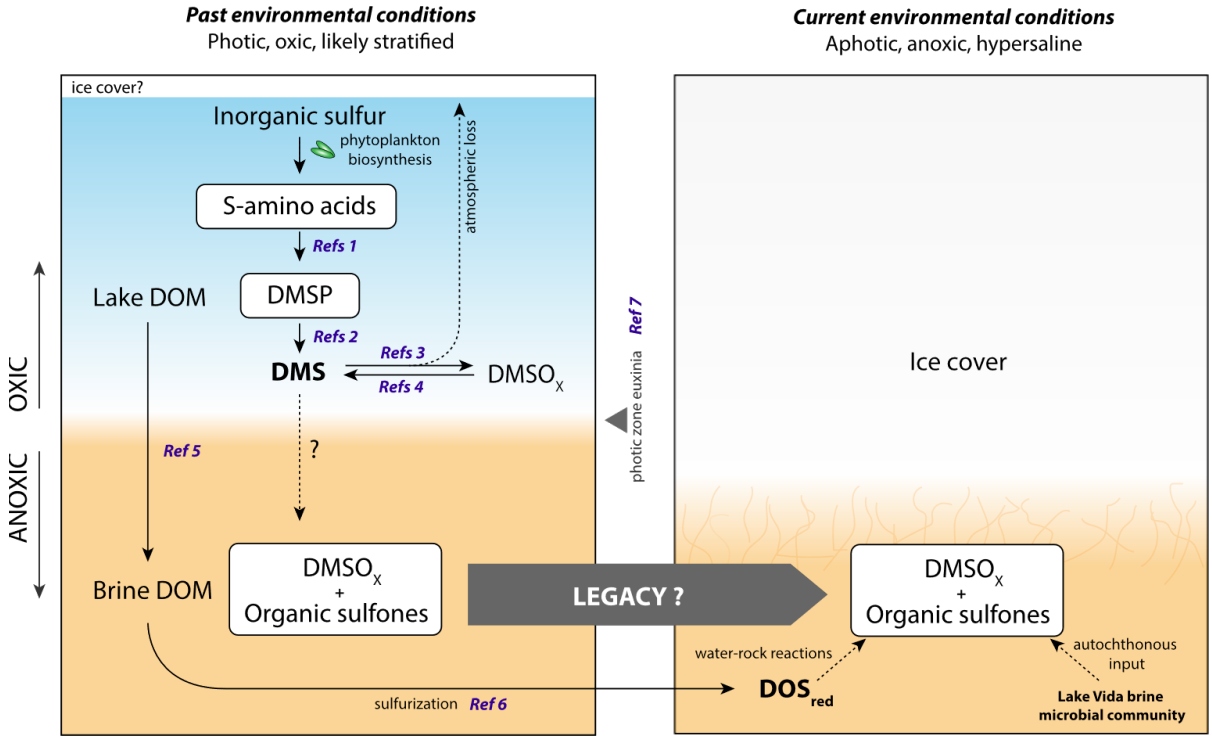

Figure 6: Schematic diagram detailing the potential sources and transformations of organic sulfone in Lake Vida brine. Solid arrows represent processes that are well described in the referenced literature. Dashed arrows indicate processes that are not-well understood but could potentially take place in LVBr, or throughout the history of the brine. **Refs 1:** Stefels (2000), Lee et al. (2004) **Refs 2:** Kiene and Taylor (1988), Taylor and Gilchrist (1991) **Ref 3:** Zinder and Brock (1978) **Refs 4:** Brimblecombe and Shooter (1986), Zeyer et al. (1987), Vila-Costa et al. (2006), Juliette et al. (1993), Koch and Dahl (2018) **Ref 5:** Cawley et al. (2016) **Ref 6:** Heitmann and Blodau (2006) **Ref 7**: Chou et al. (2018)

In oxygenated conditions, DMS is transformed to DMSO via abiotic photochemical oxidation (Brimblecombe and Shooter, 1986)(Fig. 6). Oxidation of DMS also occurs in purple phototrophic bacteria (Zeyer et al., 1987), some methylotrophic bacteria (Vila-Costa et al., 2006), ammonia oxidizing bacteria (Juliette et al., 1993) as well as potentially other organisms

that harbor the recently characterized heterodisulfide reductase genes of the DMS-degrader *Hyphomicrobium denitrificans* (Koch and Dahl, 2018) (Fig. 6). It was also recently reported that marine bacteria is capable of producing the metabolite dimethylsulfoxonium propionate, a precursor to DMSO, which had only been previously described in phytoplankton (Thume et al., 2018). In contrast, aerobic and anaerobic bacteria, as well as eukaryotes, can reduce DMSO back to DMS (Zinder and Brock, 1978) (Fig. 6).

Based on our understanding of the history of the biological community that inhabited Lake Vida basin prior to cryoencapsulation of the brine, the ancient Lake Vida environment was likely supported by primary productivity, much like other modern perennially ice-covered lakes of the Dry Valleys (Priscu et al., 1999). This assumption was validated by the detection of photosynthetically-derived paleometabolites such as maleimides and norisoprenoids in the same LVBr sample as analyzed here (Chou et al., 2018). The presence of these paleometabolites indicate that the ancient Lake Vida had fully oxygenated surface waters in contact with the atmosphere and experienced solar irradiation prior to its evaporation and cryoencapsulation. Some paleometabolite signatures, such as those deriving from bacteriochlorophyll *c* or *d,* indicate that previous Lake Vida had, at least temporarily, a stratified water column where light penetrated into an euxinic lower water column (Chou et al., 2018) as is observed in Lake Fryxell now, another lake of the MDVs (Jungblut et al., 2016).

In the ancient Lake Vida, like in modern MDV lakes, sulfur was assimilated to form sulfur bearing metabolites that are operationally defined here as primary biogenic sulfur (i.e.,

methionine, cysteine). These biogenic OSCs are degraded into VOSC's, some of which can reach the atmosphere, such as DMS and DMSO. The concentration of DMS in LVBr is 0.1 $\mu mol \cdot L^{-1}$ and DMSO is 25 $\mu mol \cdot L^{-1}$ (Murray et al., 2012). If the ancient Lake Vida is like the modern Lake Bonney, these VOSC's are likely remnants of DMSP degradation products that remained in the brine after the ancient Lake Vida's evaporation and permanent cryoencapsulation. In addition, a fraction of the biogenic sulfur produced in surface waters (under a perennial ice cover or not) in the past can also be biotically and abiotically transformed into non-labile dissolved organic matter (Dittmar, 2015) and further altered throughout the lake history leading to the sulfones detected in this study.

In contrast to primary sulfur incorporation via biosynthesis, sulfur incorporation into the DOM by diagenetic processes is also possible via the interaction with reduced sulfur species (i.e. H_2S) in sulfidic waters (Sleighter et al., 2014; Pohlabeln and Dittmar, 2015; Poulin et al., 2017). Reduced DOS could potentially be transformed into oxidized DOS by diffusion into the oxidizing water column (Sleighter et al., 2014). If sulfur incorporation into DOM through this mechanism took place in the ancient Lake Vida, it would have to occur much lower in the water column. The S-rich DOM could then be transported to more oxygenated conditions. The certainty of this postulation is hinged upon the diffusion or mixing mechanisms that drove the ancient lake dynamics. Owing to our understanding of LVBr and other McMurdo Dry Valley lakes, it is likely that ancient Lake Vida was stratified, though periodic turnover (if the lake was ice-free) may be a path for reduced DOS to come in contact with oxygenated waters, but this

is highly unlikely as other MDV lakes have been shown to have a stable water column for long periods of time (Lyons et al., 2000).

The brine of Lake Vida is currently aphotic and anoxic (Murray et al., 2012). Thus, any abiotic oxidation reactions (autooxidation or photooxidation) that could have transformed OSCs to the sulfones characterized in this study must have taken place before the brine became permanently encapsulated from external inputs. It has been shown that solar irradiation can induce direct or indirect photooxidation of DOM making it less (Stubbins and Dittmar, 2015) or more bioavailable depending on the reactivity of the DOM to solar irradiation and on bacterial organic carbon demand (Obernosterer et al., 1999). However, incomplete knowledge of former conditions in ancient Lake Vida (e.g. solar irradiation and oxygen) limit our abilities to predict how much the ancient water column experienced irradiating conditions. If ancient Lake Vida resembled other modern dry valley lakes, it is likely that it was partially or fully perennially ice-covered. It has been demonstrated that the optical properties of the water columns and ice cover of the McMurdo Dry Valley lakes can depreciate levels of incident irradiation up to 99% (Howard-William et al., 1998), which may play a role in regulating photochemical transformation of DMS to DMSO (Lee et al., 2004) and, by proxy, any potential photooxidation of more complex OSCs. Furthermore, a full or partial ice-cover can influence the levels of ventilation and thus affect the turn over time for volatile OSCs such as DMS.

If the source of sulfone-bearing organic compounds identified in this study is through the photooxidation or autooxidation of the pool of DOS that derived from autochthonous

biological activity, it is, thus, appropriate to assume that the OSCs in the total solvent-soluble extract of LVBr originated from past conditions in the lake prior to the cryoevaporation and complete encapsulation of the brine within the + 27 m of ice. As such, these organic molecules would be considered legacy biomarkers, biomarkers produced in a previous ecosystem that survived the ecosystem change(s). This deduction is supported by the study of radiocarbon ages of LVBr DOM, which show that a significant fraction of LVBr DOM are much older than the age of the ice cover (obtained from particulate organic matter trapped in the lake ice at 12 m, which provided a conservative estimate for the age of the ice-encapsulation; Doran et al., 2003) and that the LVBr is rich in legacy organic molecules (Cawley et al., 2016; Chou et al., 2018).

It is, however, especially interesting to note that characterization of the fluorescence properties of a sizable fraction of the LVBr DOM demonstrated that it has experienced much higher amounts of oxidative processes compared to other dry valley lakes (Cawley et al., 2016). These same DOM fractions are also among the oldest in radiocarbon age, which may in part explain the high degree of oxidation. Thus, the evidence for anomalously oxidized LVBr may suggest other abiotic or microbial oxidative factors are at play.

3.5.2. Long-term oxidation of organic sulfur compounds from byproducts of water-rock reactions

In isolated, aphotic, anoxic, subzero environments such as Lake Vida brine, the interplay between biotic and abiotic reactions can significantly influence the cycling of organic materials in the system. In LVBr, microbial activity is exceptionally limited by the environmental stress imposed by the very cold temperature (-13.4°C) and high salinity (188 psu). Based on respiration rate measured by leucine incorporation, it was estimated that the generation time of the LVBr microbial assemblage averages to be ~120 years (Murray et al., 2012). With this slow metabolic lifestyle, the present LVBr microbial community are existing mostly at maintenance level (Price and Sowers, 2004) and probably allocate their energy towards various measures to deal with the environmental stress (i.e. DNA repair) rather than on growth and division. Consequently, due to the slow turnover rate, abiotic reactions become a much more significant control on the alteration of dissolved organic constituents in the brine than biotic reactions. One such mechanism may be long-term abiotic brine-rock interactions that produce reactive oxygen and hydrogen species (Fig. 6).

Like serpentinization (Stevens and Mckinley, 1995) or radical reactions on mineral surfaces (Kita et al., 1982), water radiolysis can produce substantial energy substrates, such as H_2, that could drive microbial metabolism within subsurface environments (Lin et al., 2005). Along with these substrates, other byproducts of the dissociation of water molecules can play a role in the geochemistry of the system, especially over long timescales (millennial scale for

LVBr). These byproducts can include oxygen and hydrogen radicals (i.e. H•, OH•), H_2O_2, and H^+. Previous studies have demonstrated that water radiolysis from the decay of radioactive elements in subsurface environments can induce the oxidation of sulfide minerals producing oxidized sulfur species that can support microbial metabolism (Lin et al., 2005; Chivian et al., 2008). On the other hand, abiotic H_2 production via the comminution of silicate rocks reacting with water is also an important mechanism to consider in subsurface systems (Kita et al., 1982). This process is linked to the mechanical degradation of silicate crystals, where water can react with surface radicals such as Si• and Si-O• formed from the breaking of silica bonds thus producing H_2 (Kita et al., 1982). The conditions examined in these subsurface systems (i.e. deep in Precambrian rocks (Lin et al., 2005), or in active fault zones (Kita et al., 1982) are typically much warmer relative to polar temperatures like Antarctica. Recent laboratory experiments, however, have shown that brine-rock reactions from rock comminution could theoretically occur in subglacial environments (Telling et al., 2015) and may be significant in subglacial aquatic environments (Tranter, 2015).

Concentrations of H_2 in Lake Vida brine (10.47 $\mu mol \cdot L^{-1}$) and its isotopic value of δ^2H (-704‰) is consistent with radiolysis of water, with little to no contribution from biotic reactions such as microbial hydrogenase activities (Murray et al., 2012). This is because the isotopic disequilibrium of δ^2H in LVBr compared to seawater is in juxtaposition to the rate of metabolic reactions at LVBr temperatures (assuming there was H_2 consumption) and the time it would take for the brine to reach δ^2H isotopic equilibrium after its isolation from the

atmosphere. Consequently, the production of H_2 is likely abiotic. This abiotic reaction can generate reactive species such as radicals H•, OH•, H_2O_2, that could consequently react with LVBr organic matter.

Interestingly, a serpentinization-like activity in extremely cold hypersaline brines has also been proposed (Samarkin et al., 2010), providing an additional geochemical mechanism that could affect Antarctic systems and that may have been previously overlooked. Don Juan Pond, a shallow $CaCl_2$ hypersaline lake in nearby Wright Valley to the west of Lake Vida's Victoria Valley, produces a considerable amount of N_2O and molecular hydrogen via the oxidation of ferrous iron (Fe^{2+}) coupled to the reduction of nitrate or nitrite. This mechanism is particularly significant as Don Juan Pond is situated within a regolith composed of Fe^{2+}-rich Ferrar dolerite (Samarkin et al., 2010; Zavala et al., 2011). Indeed, Lake Vida brine is also in contact with iron-rich minerals deriving from weathering products of the same Ferrar dolerite sills in Victoria Valley (Turnbull et al., 1994; Dugan et al., 2015b). This chemodenitrification reaction, can proceed even at temperatures of Lake Vida (-13°C), with the lowest experimentally recorded at -20°C (Samarkin et al., 2010), and has been proposed be the source of supersaturated N_2O in LVBr (Ostrom et al., 2016).

Tranter (2015) speculated on another potentially important consequence of the weathering of igneous and high-grade metamorphic rocks in subglacial environments. Reactions between water and metal ions within the crystal lattice of silicate minerals upon fresh surface exposures have the ability to generate •OH radicals that can subsequently form H_2O_2,

a potent oxidizing agent. H_2O_2 can drive further oxidative Fenton or Fenton-like chemistry, especially with the co-occurrence of iron in the same system (Winterbourn, 1995), at concentrations as low as 2 μM (Sutton et al., 1987). The concentration of total iron in LVBr (308 μM) exceeds that of other dry valley lakes by several orders of magnitude (Murray et al., 2012), except for the Blood Fall subglacial brine discharge in Taylor Valley, where iron was measured to be 418 – 476 μM (Mikucki et al., 2009; Lyons et al., 2019).

Though this pathway of formation remains to be proven experimentally, it could provide an explanation for the highly oxidizing species of dissolved organic sulfur compounds within the LVBr system. The presence of oxidants like those deriving from these abiotic water-rock reactions as well as redox sensitive species such as iron or sulfur can drive complex geochemical reactions that make LVBr a unique ecosystem where abiotic chemistry dominates biological ones. The source of oxidants in LVBr is not known, though it is likely one or a combination of the processes described above.

3.5.3. Biological source of oxidized organic sulfur

We also consider potential biological sources of the sulfones described in this study as a biosynthetic intermediate or product of the past or extant microbial community that inhabited or are inhabiting LVBr. Biological systems that undergo oxidative stress are described as those that are exposed to an excessive amount of reactive oxygen species (ROS),

resulting of the imbalance between the generation of ROS and their destruction via cellular antioxidant regulations (Mukwevho et al., 2014). Sulfur-bearing biomolecules can act as a line of defense against reactive nitrogen and oxygen species, which is attributed to sulfur being multi-valent and highly reactive to oxidants. Amino acids cysteine and methionine, the primary S-bearing OSCs biosynthesized by organisms, can undergo reversible oxidation and reduction (Stadtman, 1993; Cabiscol et al., 2000). In proteins, these amino acid residues can act as an active site for oxidation via a variety of reactive species, including H_2O_2, O_2, peroxynitrite, superoxide, or reduced metals such as Fe^{2+} or Cu^{+} (see review by Levine et al., 2001). The resulting modification of methionine is methionine sulfoxide, which can be further oxidize to methionine sulfone (Vogt, 1995). These modified amino acid residues can be detrimental to enzymatic functions by changing the conformational structures of proteins, thus, cells regulate the reduction of methionine sulfoxide by methionine sulfoxide reductase (Vogt, 1995; Davies, 2005). However, oxidation of methionine to methionine sulfone under severe oxidative stress is irreversible (Ejiri et al., 1979; Vogt, 1995). Cysteine, on the other hand, is oxidized to cysteine sulfinic acid and cysteic acid, or can be implicated in disulfide bridges formation (Friguet, 2006). The structures of organic sulfones described in this study cannot be directly extrapolated to the amino acids methionine or cysteine due to their differences in the numbers of carbon or positions of the nitrogen. For example, we have observed the presence of 2-(methylsulfonyl)ethaneamine (Fig. 3f), a structural analog of taurine (2-aminoethanesulfonic acid), a non-proteinogenic amino acid. Taurine is an important

osmoregulator (Huxtable, 1992) and anti-oxidant compound found in animal cells (Hansen et al., 2006). It has been also been described, though less commonly, in bacteria as an osmoprotectant (Graham and Wilkinson, 1992) as well as a potential sole carbon energy source, and nitrogen or sulfur source (McLaggan and Epstein, 1991; Lie et al., 1999).

However, these S-bearing amino acids may be autochthonous sources of organic sulfur compounds found in the suite of LVBr metabolites that may have been further altered throughout LVBr history by biological or abiotic processes, contributing to the organic sulfone pool detected during our study.

S-methyl methanethiosulfonate (Fig. 4f) has been previously described as a secondary metabolite sulfur compound emitted by *Stigmatella aurantiaca* (Dickschat et al., 2005), *Loktanella*, and *Dinoroseobacter shibae* (Dickschat et al., 2005), marine bacteria that were isolated from the North Sea. Neither of these organisms are present in the LVBr active microbial assemblages (Murray et al., 2012), though the *Loktanella* genus has been previously cultured from various Antarctic lake systems (Van Trappen et al., 2004). We cannot completely rule out the possibility that the past or current LVBr microbial assemblage has the genetic capacity to produce *S*-methyl methanethiosulfonate. Furthermore, this compound has been shown to exhibit slight antioxidant activity in the presence of Fe^{2+} (Ramoutar and Brumaghim, 2008).

3.6. Conclusions

In this paper, we report on the detection of 19 organic sulfones in Lake Vida brine that have not been previously described in any other ecosystem. We suggest that these organic sulfones are legacy from a previous ecosystem that occupied Lake Vida basin in the past. These compounds were produced and altered in this ancient lake while in contact with atmospheric oxygen, either via photooxidation or autooxidation. We posit that a legacy of oxidized OSCs is more likely the explanation for the presence of these compounds detected in our sample, as the slow-growing ecosystem of the Lake Vida brine seems to be dominated by a legacy component (Cawley et al., 2016; Chou et al., 2018). However, we cannot completely rule out other abiotic mechanisms that may be at play. The organic sulfones described in this study could have also derived from long-term brine-rock reactions that generate reactive oxygen species as a byproduct of water radiolysis, reacting with S-bearing dissolved organic matter, or inducing biological oxidative stress and their subsequent S-metabolite alteration. Further studies on long-term brine-rock reactions can reveal the nature of organic matter alteration, especially in slow-growing, isolated, ecosystems with low metabolic activities. One avenue to explore the sources of sulfur in the organic matter derived from Lake Vida is via the investigation of compound-specific isotope analysis of the sulfur in these organic sulfones, as radiolytic oxidation has been shown to significantly affect the isotopic composition of sulfur oxidation products (Lefticariu et al., 2010). The presence of organic sulfones may not be exclusive to

LVBr. Subsurface glacial environments and other extremely cold and isolated ecosystems where abiotic geochemical reactions dominate the biological cycling of organic matter, may also host suites of organic sulfones or other oxidation products of surface legacy, or long-term water-rock reactions, thereby, pointing to a crucial pathway for organic matter alteration that may have been previously overlooked. These organic compounds could represent the potential constituents of non-volatile dissolved organic sulfur compounds suggested to persist in aquatic ecosystems via studies using FT-ICR-MS.

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Work Cited

Asher, E.C., Dacey, J.W.H., Mills, M.M., Arrigo, K.R., Tortell, P.D., 2011. High concentrations and turnover rates of DMS, DMSP and DMSO in Antarctic sea ice. Geophysical Research Letters 38, 1–5.

Blunden, G., Gordon, S.M., McLean, W.F.H., Guiry, M.D., 1982. The distribution and possible taxonomic significance of quaternary ammonium and other Dragendorff-positive compounds in some genera of marine algae. Botanica Marina XXV, 563–567.

Bowie, J.H., Williams, D.H., Lawesson, S.O., Madsen, J.O., Nolde, C., Schroll, G., 1966. Studies in mass spectrometry—XV : Mass spectra of sulphoxides and sulphones. The formation of C-C and C-O bonds upon electron impact. Tetrahedron Letters 22, 3515–3525.

Brimblecombe, P., Shooter, D., 1986. Photo-oxidation of dimethylsulfide in aqueous solution, Marine Chemistry 19(4), 343-353.

Burkins, M.B., Virginia, R.A., Chamberlain, C.P., Wall, D.H., Ross, A., 2000. Origin and Distribution of Soil Organic Matter in Taylor Valley, Antarctica. Ecology 81, 2377–2391.

Cabiscol, E., Tamarit, J., Ros, J., 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. International Microbiology 3, 3–8.

Cawley, K.M., Murray, A.E., Doran, P.T., Kenig, F., Stubbins, A., Chen, H., Hatcher, Patrick G, Mcknight, Diane M, Hatcher, P G, Mcknight, D M, 2016. Characterization of dissolved organic material in the interstitial brine of Lake Vida, Antarctica. Geochimica et Cosmochimica Acta 183, 63–78.

Chivian, D., Brodie, E.L., Alm, E.J., Culley, D.E., Dehal, P.S., Z., D.T., Gihring, T.M., Lapidus, A., Lin, L.-H., Lowry, S.R., Moser, D.P., Richardson, P., Southam, G., Wanger, G., Pratt, L.M., Andersen, G.L., Hazen, T.C., Brockman, F.J., Arkin, A.P., Onstott, T.C., 2008. Environmental genomics reveals a single species ecosystem deep within the Earth. Science 322, 275–278.

Chou, L., Kenig, F., Murray, A.E., Fritsen, C.H., Doran, P.T., 2018. Effects of legacy metabolites from previous ecosystems on the environmental metabolomics of the brine of Lake Vida, East Antarctica. Organic Geochemistry 122, 161-170.

Curson, A.R.J., Liu, J., Bermejo Martínez, A., Green, R.T., Chan, Y., Carrión, O., Williams, B.T., Zhang, S.H., Yang, G.P., Bulman Page, P.C., Zhang, X.H., Todd, J.D., 2017. Dimethylsulfoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process. Nature Microbiology 2: 17009.

Davies, M.J., 2005. The oxidative environment and protein damage. Biochimica et Biophysica Acta 1703, 93–109.

De Mora, S.J., Lee, P.A., Grout, A., Schall, C., Heumann, K.G., 1996. Aspects of the biogeochemistry of sulphur in glacial melt water ponds on the McMurdo Ice Shelf, Antarctica. Antarctic Science 8, 15–22.

Dickschat, Jeroen S., Bode, H.B., Wenzel, S.C., Müller, R., Schulz, S., 2005. Biosynthesis and identification of volatiles released by the myxobacterium Stigmatella aurantiaca. ChemBioChem 6, 2023–2033.

Dickschat, J.S., Rabe, P., Citron, C.A., 2015. The chemical biology of dimethylsulfoniopropionate. Organic & Biomolecular Chemistry 13, 1954–1968.

Dickschat, Jeroen S, Wagner-D Bler, I., Schulz, S., 2005. The Chafer Pheromone Buibuilactone and Ant Pyrazines are also produced by marine bacteria. Journal of Chemical Ecology 31. doi:10.1007/s10886-005-3553-9

Dickschat, J.S., Zell, C., Brock, N.L., 2010. Pathways and substrate specificity of DMSP catabolism in marine bacteria of the Roseobacter clade. ChemBioChem 11, 417–425.

Dittmar, T., 2015. Reasons Behind the Long-Term Stability of Dissolved Organic Matter, in: Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 369–388.

Doran, P., Kenig, F., Knoepfle, L.J., Mikucki, A.J., Lyons, W.B., 2014. Radiocarbon distribution and the effect of legacy in lakes of the McMurdo Dry. Limnology and Oceanography 59, 811–826.

Doran, P.T., Fritsen, C.H., McKay, C.P., Priscu, J.C., Adams, E.E., 2003. Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "'ice-sealed'" east Antarctic lake. Proceedings of the National Academy of Sciences 100, 26–31.

Doran, T.P., Fritsen, C.H., Murray, A.E., Kenig, F., Mckay, C.P., Kyne, J.D., 2008. Entry approach into pristine ice-sealed lakes - Lake Vida , East Antarctica , a model ecosystem. Limnology and Oceanography: Methods 6, 542–547.

Drotar, A., Burton, G.A., Tavernier, J.E., Fall, R., 1987. Widespread Occurrence of Bacterial Thiol Methyltransferases and the Biogenic Emission of Methylated Sulfur Gases. Applied and Environmental Microbiology 53, 1626–1631.

Dugan, H.A., Doran, P.T., Tulaczyk, S., Mikucki, J.A., Arcone, S.A., Auken, E., Schamper, C., Virginia, R.A., 2015a. Subsurface imaging reveals a confined aquifer beneath an ice-sealed Antarctic lake. Geophysical Research Letters 42, 96–103.

Dugan, H.A., Doran, P.T., Wagner, B., Kenig, F., Fritsen, C.H., Arcone, S.A., Kuhn, E., Ostrom, N.E., Warnock, J.P., Murray, A.E., 2015b. Stratigraphy of Lake Vida, Antarctica: hydrologic implications of 27 m of ice. The Cryosphere 9, 439–450.

Ejiri, S.-I., Weissbach, H., Brot, N., 1979. Reduction of Methionine Sulfoxide to Methionine by Escherichia coli. Journal of Bacteriology 139, 161–164.

Friguet, B., 2006. Oxidized protein degradation and repair in ageing and oxidative stress. FEBS Letters 580, 2910–2916.

Gibson, J.A.E., Garrick, R.C., Franzmann, P.D., Deprez, P.P., Burton, H.R., 1991. Reduced sulfur gases in saline lakes of the Vestfold Hills, Antarctica. Palaeogeography, Palaeoclimatology, Palaeoecology Elsevier Science Publishers B.V 84, 131–140.

Gomez-Saez, G. V, Niggemann, J., Dittmar, T., Pohlabeln, A.M., Lang, S.Q., Noowong, A., Pichler, T., Wö Rmer, L., Bü Hring, S.I., 2016. Molecular evidence for abiotic sulfurization of dissolved organic matter in marine shallow hydrothermal systems. Geochimica et Cosmochimica Acta 190, 35–52.

Graham, J.E., Wilkinson, B.J., 1992. Staphylococcus aureus Osmoregulation: Roles for Choline, Glycine Betaine, Proline, and Taurine. Journal of Bacteriology 174, 2711–2716.

Green, W.J., Gardner, T.J., Nixon, P., Ferdehnan, G., Angle, M.P., Fryxell, L., 1989. Geochemical processes in the Lake Fryxell Basin (Victoria Land ,. Hydrobiologia 172, 129–148.

Hansen, S.H., Andersen, M.L., Birkedal, H., Cornett, C., Wibrand, F., 2006. The Important Role of Taurine in Oxidative Metabolism, in: Taurine 6. Springer US, pp. 129–135.

Heitmann, T., Blodau, C., 2006. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. Chemical Geology 235, 12–20.

Hollibaugh, J.T., Wong, P.S., Bano, N., Pak, S.K., Prager, E.M., Orrego, C., 2001. Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event, in: Developments in Hydrobiology: Saline Lakes. Springer Netherlands, pp. 45–60.

Howard-William, C., Schwarz, A.-M., Hawes, I., Priscu, J.C., 1998. Optical Properties of the McMurdo Dry Valley Lakes, Antarctica, in: Priscu, J. (Ed.), Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys, Antarctica. American Geophysical Union, pp. 189–203.

Huxtable, R.J., 1992. Physiological Actions of Taurine, Physiological Reviews 72(1), 101-163.

Jokic, A., Cutler, J.N., Ponomarenko, E., van der Kamp, G., Anderson, D.W., 2003. Organic carbon and sulphur compounds in wetland soils: Insights on structure and transformation processes using K-edge XANES and NMR spectroscopy. Geochimica et Cosmochimica Acta 67, 2585–2597.

Juliette, L.Y., Hyman, M.R., Arp, Daniel, J., 1993. Inhibition of Ammonia Oxidation in Nitrosomonas europaea by Sulfur Compounds: Thioethers Are Oxidized to Sulfoxides by

Ammonia Monooxygenase Downloaded from, Applied and Environmental Microbiology 59(11), 3718-3727.

Jungblut, A.D., Hawes, I., Mackey, T.J., Krusor, M., Doran, P.T., Sumner, D.Y., Eisen, J.A., Hillman, C., Goroncy, A.K., 2016. Microbial mat communities along an oxygen gradient in a perennially ice-covered Antarctic lake. Applied and Environmental Microbiology 82, 620–630.

Karr, E. a, Sattley, W.M., Rice, M.R., Jung, D.O., Madigan, M.T., Laurie, a, Achenbach, L. a, 2005. Diversity and Distribution of Sulfate-Reducing Bacteria in Permanently Frozen Lake Fryxell , McMurdo Dry Valleys , Antarctica Diversity and Distribution of Sulfate-Reducing Bacteria in Permanently Frozen Lake Fryxell , McMurdo Dry Valleys , Antarctica. Applied and environmental microbiology 71, 6353–6359.

Kasamatsu, N., Hirano, T., Kudoh, S., Odate, T., Fukuchi, M., 2004. Dimethylsulfoniopropionate production by psychrophilic diatom isolates. Journal of Phycology 40, 874–878.

Kenig, F., Huc, A.H., 1990. Incorporation of sulfur into recent organic matter in a carbonate environment (Abu Dhabi, United Arab Emirates), in: ACS Symposium Series, Vol. 429. American Chemical Society, pp. 170–185.

Kertesz, M.A., 1999. Riding the sulfur cycle -- metabolism of sulfonates and sulfate esters in Gram-negative bacteria. FEMS Microbiology Reviews 24, 135–175.

Kingston, D.G.I., Bursey, J.T., Bursey, M.M., 1974. Intramolecular Hydrogen Transfer in Mass Spectra. II. The McLafferty Rearrangement and Related Reactions. Chemical Reviews 74, 215–242.

Kinstle, T.H., Oliver, W.R., 1972. The mass spectra of styryl sulfoxides and sulfones. Organic Mass Spectrometry 6, 699–714.

Kirst, G.O., Thiel, C., Wolff, H., Nothnagel, J., Wanzek, M., Ulmke, R., 1991. Dimethylsulfoniopropionate (DMSP) in icealgae and its possible biological role. Marine Chemistry 35, 381–388.

Kita, I., Matsuo, S., Wakita, H., 1982. H2 generation by reaction between H2O and crushed rock: An experimental study on H2 degassing from the active fault zone. Journal of Geophysical Research 87, 10789–10795.

Knoepfle, L.J., Doran, T.P., Kenig, F., Lyons, W.B., Galchenko, V.F., 2009. Particulate organic and dissolved inorganic carbon stable isotopic compositions in Taylor Valley lakes , Antarctica : the effect of legacy. Hydrobiologia 632, 139–156.

Koch, T., Dahl, C., 2018. A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. The ISME Journal 12, 2479–2491.

Kohnen, M.E.L., Sinninghe Damsté, J.S., ten Haven, H.L., de Leeuw, J.W., 1989. Early incorporation of polysulphides in sedimentary organic matter. Nature 341, 640–641.

Ksionzek, K.B., Lechtenfeld, O.J., Mccallister, S.L., Schmitt-Kopplin, P., Geuer, J.K., Geibert, W., Koch, B.P., 2016. Dissolved organic sulfur in the ocean: Biogeochemistry of a petagram inventory. Science 354, 456–459.

Kuhn, E., Ichimura, A.S., Peng, V., Fritsen, C.H., Trubl, G., Doran, P.T., Murray, A.E., 2014. Brine Assemblages of Ultrasmall Microbial Cells within the Ice Cover of Lake Vida, Antarctica. Applied and Environmental Microbiology 80, 3687–3698.

Lee, P.A., Priscu, J.C., DiTullio, G.R., Riseman, S.F., Tursich, N., de Mora, S.J., 2004. Elevated Levels of Dimethylated-Sulfur Compounds in Lake Bonney, a Poorly Ventilated Elevated levels of dimethylated-sulfur compounds in Lake Bonney, a poorly ventilated Antarctic lake. Limnology and Oceanography 49, 1044–1055.

Lefticariu, L., Pratt, L.A., Laverne, J.A., Schimmelmann, A., 2010. Anoxic pyrite oxidation by water radiolysis products — A potential source of biosustaining energy. Earth and Planetary Science Letters 292, 57–67.

Levine, R., Moskovitz, J., Stadtman, E., 2001. Oxidation of Methionine in Proteins: Roles in Antioxidant Defense and Cellular Regulation. IUBMB Life 50, 301–307.

Lie, T.J., Clawson, M.L., Godchaux, W., Leadbetter, E.R., 1999. Sulfidogenesis from 2-Aminoethanesulfonate (Taurine) Fermentation by a Morphologically Unusual Sulfate-Reducing Bacterium, Desulforhopalus singaporensis sp. nov. Applied and Environmental Microbiology 65, 3328–3334.

Lin, L.-H., Slater, G.F., Lollar, B.S., Lacrampe-Couloume, G., Onstott, T.C., 2005. The yield and isotopic composition of radiolytic H 2 , a potential energy source for the deep subsurface biosphere. Geochimica et Cosmochimica Acta 69, 893–903.

Lomans, B.P., Van Der Drift, C., Pol, A., Op Den Camp, H.J.M., 2002. Microbial cycling of volatile organic sulfur compounds. Cellular and Molecular Life Sciences 59, 575–588.

Lyons, B.W., Mikucki, J.A., German, L.A., Welch, K.A., Welch, S.A., Gardner, C.B., Tulaczyk, S.M., Pettit, E.C., Kowalski, J., Dachwald, B., Team, T.E., 2019. The Geochemistry of Englacial Brine from Taylor Glacier, Antarctica. Journal of Geophysical Research: Biogeosciences 124(3), 633-648.

Lyons, W.B., Fountain, A., Doran, T.P., Priscu, J.C., Neumann, K., 2000. Importance of landscape position and legacy : the evolution of the lakes in Taylor Valley , Antarctica. Freshwater Biology 43, 355–367.

Malone, J.L., Castro, M.C., Hall, C.M., Doran, P.T., Kenig, F., Mckay, C.P., 2010. New insights into the origin and evolution of Lake Vida, McMurdo Dry Valleys, Antarctica — A noble gas study in ice and brines. Earth and Planetary Science Letters 289, 112–122.

Manceau, A., Nagy, K.L., 2012. Quantitative analysis of sulfur functional groups in natural organic matter by XANES spectroscopy. Geochimica et Cosmochimica Acta 99, 206–223.

Margesin, R., Schinner, F., Marx, J.C., Gerday, C., 2008. Psychrophiles: From biodiversity to biotechnology, Psychrophiles: From Biodiversity to Biotechnology. doi:10.1007/978-3-540-74335-4

McLafferty, F.W., 1959. Mass Spectrometric Analysis. Molecular Rearrangements. Analytical Chemistry 31, 82–87.

McLaggan, D., Epstein, W., 1991. Escherichia coli accumulates the eukaryotic osmolyte taurine at high osmolarity, FEMS Microbiology Letters 81(2), 209-213.

Mikucki, J.A., Foreman, C.M., Sattler, B., Berry Lyons, W., Priscu, J.C., 2004. Geomicrobiology of Blood Falls: An Iron-Rich Saline Discharge at the Terminus of the Taylor Glacier, Antarctica. Aquatic Geochemistry 10, 199–220.

Mikucki, J.A., Pearson, A., Johnston, D.T., Turchyn, A. V., Farquhar, J., Shrag, P.D., Anbar, A.D., Priscu, J.C., Lee, P.A., 2009. A Contemporary Microbially Maintained Subglacial Ferrous "Ocean." Science 324, 397–400.

Mukwevho, E., Ferreira, Z., Ayeleso, A., 2014. Potential role of sulfur-containing antioxidant systems in highly oxidative environments. Molecules 19, 19376–19389.

Murray, A.E., Kenig, F., Fritsen, C.H., Mckay, C.P., Cawley, K.M., Edwards, R., Kuhn, E., Mcknight, D.M., Ostrom, N.E., Peng, V., Ponce, A., Priscu, J.C., Samarkin, V., Townsend, A.T., Wagh, P., Young, S.A., To, P., Doran, T.P., 2012. Microbial life at – 13 ° C in the brine of an ice-sealed Antarctic lake. Proceedings of the National Academy of Sciences 109, 20626–20631.

Obernosterer, I., Reitner, B., Herndl, G.J., 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnology and Oceanography 44, 1645–1654.

Ostrom, N.E., Gandhi, H., Trubl, G., Murray, A.E., 2016. Chemodenitrification in the cryoecosystem of Lake Vida, Victoria Valley, Antarctica. Geobiology 14, 575–587.

Pohlabeln, A.M., Dittmar, T., 2015. Novel insights into the molecular structure of non-volatile marine dissolved organic sulfur. Marine Chemistry 168, 86–94.

Pohlabeln, A.M., Gomez-Saez, G. V., Noriega-Ortega, B.E., Dittmar, T., 2017. Experimental Evidence for Abiotic Sulfurization of Marine Dissolved Organic Matter. Frontiers in Marine Science 4, 1–11.

Poulin, B.A., Ryan, J.N., Nagy, K.L., Stubbins, A., Dittmar, T., Orem, W., Krabbenhoft, D.P., Aiken, G.R., 2017. Spatial Dependence of Reduced Sulfur in Everglades Dissolved Organic Matter Controlled by Sulfate Enrichment. Environmental Science and Technology 51, 3630–3639.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proceedings of the National Academy of Sciences 101, 4631–4636.

Priscu, J.C., Wolf, C.F., Takacs, C.D., Fritsen, C.H., Laybourn-Parry, J., Roberts, E.C., Sattler, B., Lyons, W.B., 1999. Carbon Transformations in a Perennially Ice-Covered Antarctic Lake. BioScience 49, 997–1008.

Proemse, B.C., Murray, A.E., Schallenberg, C., McKiernan, B., Glazer, B.T., Young, S.A., Ostrom, N.E., Bowie, A.R., Wieser, M.E., Kenig, F., Doran, P.T., Edwards, R., 2017. Iron cycling in the anoxic cryo-ecosystem of Antarctic Lake Vida. Biogeochemistry 1–11.

Ramoutar, R.R., Brumaghim, J.L., 2008. Investigating the antioxidant properties of oxo-sulfur compounds on metal-mediated DNA damage. Main Group Chemistry 6, 143–153.

Rinehart, K.L., Buchholz, A.C., Van Lear, G.E., Cantrill, H.L., 1968. A Randomly Labeled Tropylium Ion in the Mass Spectrum of Toluene-a,1-13C2. Journal of the American Chemical Society 90, 2983–2985.

Samarkin, V.A., Madigan, M.T., Bowles, M.W., Casciotti, K.L., Priscu, J.C., Mckay, C.P., Joye, S.B., 2010. Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica. Nature Geoscience 3, 341–344.

Schulz, S., Dickschat, J.S., 2007. Bacterial volatiles: the smell of small organisms. Natural Product Reports 24, 814–842.

Sela-Adler, M., Said-Ahmad, W., Sivan, O., Eckert, W., Kiene, R.P., Amrani, A., 2015. Isotopic evidence for the origin of dimethylsulfide and dimethylsulfoniopropionate-like compounds in a warm, monomictic freshwater lake. Environmental Chemistry 13. doi:10.1071/EN15042

Sinninghe Damsté, J.S., Rijpstra, I.W.C., Dalen, K.A.C., De Leeuw, J.W., Schenck, P.A., 1989. Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the

formation of sedimentary organic sulphur compounds at the early stages of diagenesis. Geochimica et Cosmochimica Acta 53, 1343–1355.

Sleighter, R.L., Chin, Y.P., Arnold, W.A., Hatcher, P.G., McCabe, A.J., McAdams, B.C., Wallace, G.C., 2014. Evidence of Incorporation of Abiotic S and N into Prairie Wetland Dissolved Organic Matter. Environmental Science and Technology Letters 1, 345–350.

Smakman, R., de Boer, T.J., 1970. The mass spectra of some aliphatic and alicyclic sulphoxides and sulphones. Organic Mass Spectrometry 3, 1561–1588.

Stadtman, E.R., 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annual Review of Biochemistry 62, 797–821.

Stefels, J., 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. Journal of Sea Research 43.

Stevens, T. O., & McKinley, J. P. (1995). Lithoautotrophic microbial ecosystems in deep basalt aquifers. Science, 270(5235), 450-455.

Stubbins, A., Dittmar, T., 2015. Illuminating the deep: Molecular signatures of photochemical alteration of dissolved organic matter from North Atlantic Deep Water. Marine Chemistry 177, 318–324.

Sunda, W.G., Kieber, D.J., Kiene, R.P., Huntsman, S., 2002. An antioxidant function of DMSP and DMS in marine algae. Nature 418, 317–320.

Sutton, H.C., Vile, G.F., Winterbourn, C.C., 1987. Radical driven fenton reactions—Evidence from paraquat radical studies for production of tetravalent iron in the presence and absence of ethylenediaminetetraacetic acid. Archives of Biochemistry and Biophysics 256, 462–471.

Takacs, C.D., Priscu, J.C., 1998. Bacterioplankton Dynamics in the McMurdo Dry Valley Lakes, Antarctica: Production and Biomass Loss over Four Seasons. Microbial Ecology 36, 239–250.

Taylor, B.F., Gilchrist, D.C., 1991. New routes for aerobic biodegradation of dimethylsulfoniopropionate. Applied and environmental microbiology 57, 3581–4.

Telling, J., Boyd, E.S., Bone, N., Jones, E.L., Tranter, M., MacFarlane, J.W., Martin, P.G., Wadham, J.L., Lamarche-Gagnon, G., Skidmore, M.L., Hamilton, T.L., Hill, E., Jackson, M., Hodgson, D.A., 2015. Rock comminution as a source of hydrogen for subglacial ecosystems. Nature Geoscience 8, 851–855.

Thume, K., Gebser, B., Chen, L., Meyer, N., Kieber, D.J., Pohnert, G., 2018. The metabolite dimethylsulfoxonium propionate extends the marine organosulfur cycle. Nature 563, 412–415.

Tranter, M., 2015. Grand challenge for low temperature and pressure geochemistry—sparks in the dark, on Earth, Mars, and throughout the Galaxy. Frontiers in Earth Science 3, 1–4.

Trevena, A.J., Jones, G.B., Wright, S.W., Van Den Enden, R.L., 2000. Profiles of DMSP, algal pigments, nutrients and salinity in pack ice from eastern Antarctica. Journal of Sea Research 43, 265–273.

Tripp, H.J., Kitner, J.B., Schwalbach, M.S., Dacey, J.W.H., Wilhelm, L.J., Giovannoni, S.J., 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. Nature 452, 741–744.

Truce, W.E., Campbell, R.W., Madding, G.D., 1968. Mass Spectral Study of Alkyl Alkanesulfonates. The Journal of Organic Chemistry 32, 308–317.

Turnbull, I.M., Allibone, A.H., Forsyth, P.J., Heron, D.W., 1994. Geology of the Bull Pass-St Johns Range area, southern Victoria Land, Antarctica, scale 1:50,000, Institute. ed. Institute of Geological & Nuclear Sciences.

Vairavamurthy, A., Zhou, W., Eglinton, T., Manowitz, B., 1994. Sulfonates: A novel class of organic sulfur compounds in marine sediments. Geochimica et Cosmochimica Acta 58, 4681–4687.

Van Diggelen, J., Rozema, J., Dickson, D., R, B., 1986. Beta-3-dimethylsulfoniopropionate, proline, and quaternary ammonium compounds in Spartina Anglica in relation to sodium chloride, nitrogen, and sulphur. New Phytologist 103, 573–589.

Van Trappen, S., Mergaert, J., Swings, J., VanTrappen, S., Mergaert, J., Swings, J., 2004. Loktanella salsilacus gen. nov., sp. nov., Loktanella fryxellensis sp. nov. and Loktanella vestfoldensis sp. nov., new members of the Rhodobacter group isolated from microbial mats in Antarctic lakes. International Journal of Systematic and Evolutionary Microbiology 54, 1263–1269.

Vila-Costa, M., Del Valle, D.A., González, J.M., Slezak, D., Kiene, R.P., Sánchez, O., Simó, R., 2006. Phylogenetic identification and metabolism of marine dimethylsulfide-consuming bacteria. Environmental Microbiology 8, 2189–2200.

Vogt, W., 1995. Oxidation of methionyl residues in proteins: Tools, targets, and reversal. Free Radical Biology and Medicine 18, 93–105.

Webster, J.G., 1994. Trace-metal behaviour in oxic and anoxic Ca-Cl brines of the Wright Valley drainage, Antarctica. Chemical Geology 112, 255–274.

Werne, J.P., Lyons, T.W., Hollander, D.J., Formolo, M.J., Sinninghe Damsté, J.S., 2003. Reduced sulfur in euxinic sediments of the Cariaco Basin: sulfur isotope constraints on organic sulfur formation. Chemical Geology 195, 159–179.

Winterbourn, C.C., 1995. Toxicity of iron and hydrogen peroxide: the Fenton reaction. Toxicology Letters 8283, 969–974.

Yau, S., Lauro, F.M., Williams, T.J., Demaere, M.Z., Brown, M. V., Rich, J., Gibson, J.A.E., Cavicchioli, R., 2013. Metagenomic insights into strategies of carbon conservation and unusual sulfur biogeochemistry in a hypersaline Antarctic lake. ISME Journal 7, 1944–1961.

Yoch, D.C., 2002. Dimethylsulfoniopropionate : Its Sources , Role in the Marine Food Web , and Biological Degradation to Dimethylsulfide Dimethylsulfoniopropionate : Its Sources , Role in the Marine Food Web , and Biological Degradation to Dimethylsulfide. Society 68, 5804–5815.

Zavala, K., Leitch, A.M., Fisher, G.W., 2011. Silicic Segregations of the Ferrar Dolerite Sills, Antarctica. Journal of Petrology 52, 1927–1964.

Zeyer, J., Eicher, P., Wakeham, S.G., Schwarzenbach, R.P., 1987. Oxidation of Dimethyl Sulfide to Dimethyl Sulfoxide by Phototrophic Purple Bacteria. Applied and Environmental Microbiology 53, 2026–2032.

Zhu, M.-X., Chen, L.-J., Yang, G.-P., Huang, X.-L., Ma, C.-Y., 2014. Humic sulfur in eutrophic bay sediments: Characterization by sulfur stable isotopes and K-edge XANES spectroscopy. Estuarine, Coastal and Shelf Science 138, 121–129.

Zinder, S.H., Brock, T.D., 1978. Dimethyl Sulphoxide Reduction by Micro-organisms. Journal of General Microbiology 105, 335–342.

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CHAPTER IV

METAGENOMIC EVIDENCE FOR LIPID LEGACY: THE BRINE OF LAKE VIDA, ANTARCTICA

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The contents of Chapter IV will be reformatted for submission to *Geobiology*

Abstract

Lake Vida brine is a cold, anoxic, aphotic, hypersaline, interstitial brine trapped beneath the thick (27+ m) ice of Lake Vida, McMurdo Dry Valleys, East Antarctica. This brine is an ideal astrobiological analog for studying the biogeochemistry of isolated, cryogenic ecosystems. The brine currently hosts a slow-growing, cold-limited bacterial ecosystem and has been isolated from the atmosphere for at least ~2800 years. The dissolved organic matter (DOM) of Lake Vida brine contains legacy metabolites deriving from an ancient ecosystem that inhabited the lake in the past. The particulate organic matter (POM) of Lake Vida brine was investigated in this study to determine the lipid biosignature composition of the Lake Vida brine modern microbial community. Lipidomics analysis demonstrated that legacy organic biosignatures persist in the POM fraction. Large, detrital, eukaryotic cells were detected by scanning electron microscopy, and lipids belonging to eukaryotes were observed in the Lake Vida brine lipidome, despite the lack of detection of eukaryotes via 18S rRNA gene survey. Targeted metagenomics analysis confirmed the presence of all the genes necessary to biosynthesize the majority of the lipids in the lipidome (i.e., fatty acids), but not others (i.e., sterols). Thus, we suggest that sterols in the lipidome of Lake Vida brine POM are legacy lipids. This study highlights the usefulness of combined lipidomics and metagenomics analysis and can provide important insight into lipid biosignatures detection and interpretation in other subzero environments on earth and on extraterrestrial icy bodies.

Keywords: lipidomics, metagenomics, legacy, biosignatures, Antarctica

4.1. Introduction

The McMurdo Dry Valleys of East Antarctica are considered among the best Earth analogs for astrobiological investigations of icy planetary worlds. Lake Vida, located in Victoria Valley, the northernmost of the dry valleys, encapsulates a pristine, cryogenic (-13.4°C), anoxic, hypersaline (188 psu) interstitial brine that has been isolated for millennia, and is known to support an active bacterial ecosystem (Murray et al., 2012). Geochemical and biological investigations of this environment can provide a crucial understanding of the potential habitability of extraterrestrial cryogenic environments. Lake Vida brine is also an ideal terrestrial analog for examining the fate of organic biosignatures, and cryogenic preservation of putative subsurface biosignatures that could be present in other icy extraterrestrial environments, such as in the polar regions of Mars, or in the oceans of Europa and Enceladus.

Previous environmental metabolomic analysis of the dissolved organic matter of Lake Vida brine (LVBr) revealed the presence of a complex mixture of metabolites that contains, in part, a legacy component (i.e, chlorophyll- and carotenoid-derivatives), which derived from a previous ecosystem that occupied the Lake Vida basin (Chou et al., 2018, Chapter II of this dissertation). This presence of legacy is further supported by the detection of organic sulfones that are thought to be the products of photo- or auto- transformations of sulfur-bearing metabolites produced in a previous, photosynthetically dominated ecosystem (Chapter III of

this dissertation). The presence of legacy organic molecules presents a tremendous challenge to our ability to observe modern metabolites or organic biosignatures that solely belong to the modern microbial community. To potentially overcome this, we exclusively investigate the particulate organic matter (POM) of Lake Vida brine that was collected on microbial filters. This POM includes intact cells that are living in the brine as well as any other particulate matter that are ~0.22+ μm in size.

Microbial life in environments such as Lake Vida brine are challenged not only by the extremely cold temperature, but also by the osmotic stress imposed by high salinity. Analysis of the small subunit (SSU) rRNA genes and complementary genes of the rRNA obtained from a Lake Vida brine sample collected in 2005 revealed the presence of eight bacterial phyla (32 sequences), of which eight 16S rRNA sequences were inferred to be biologically active (Murray et al., 2012). *Gammaproteobacteria*-associated *Psychrobacter sp.* and *Marinobacter sp*. dominate the SSU rRNA gene sequences obtained from LVBr (39%). Several lineages from the *Epsilonproteobacteria* were also relatively abundant (16%), in addition to the *Lentisphaera, Firmicutes, Actinobacteria, Spirochaeta, Verrucomicrobia*, and *Candidate Division TM*7. Representatives of the LVBr microbial assemblage include bacteria that are capable of growing heterotrophically, chemolitoheterotrophically, or chemolitho-autotrophically, as well as fermentatively. Surveys of the 16S and 18S SSU rRNA genes did not detect archaeal or eukaryotic signals (Murray et al., 2012). Although biological activity was inferred via ^{3}H-leucine incorporation, protein synthesis rates were measured to be very low (Murray et al.,

2012). Based on this ^{3}H-leucine incorporation rate, it was estimated that the average generation time of the LVBr microbial assemblage is ~120 years (Murray et al., 2012), which suggest that the community currently exists slightly above maintenance metabolism (Price and Sowers, 2004). Despite the extremely cold and isolated conditions of the current brine and given the presence of high levels of dissolved resources (i.e., inorganic and organic compounds), we expect the LVBr community to be self-sustaining via heterotrophic metabolism, along with at least some chemoautotrophic component driven by the *Epsilonproteobacteria*.

Microorganisms that live in conditions similar to Lake Vida brine possess a variety of mechanisms to deal with the adverse effects of cold temperatures on the physical properties of their cell membranes. One strategy is the increased production of polyunsaturated, monounsaturated, and methyl-branched fatty acids, as well as fatty acids with shorter acyl-chain length, which induce steric interference that alters the packing order of the membrane, thereby increasing membrane fluidity (Russell, 1997; Chintalapati et al., 2004). Branched fatty acids found in membrane alterations due to cold temperatures are usually *iso*- or *anteiso*-substituted fatty acids. *Anteiso*-substituted fatty acids typically induce more steric hindrance than *iso*-substituted acids, although an increase of either types of fatty acids lead to more flexible and fluid membranes (Russell, 1997). Microorganisms can also alter the structures of other membrane lipids to adapt to decreasing temperatures. One example is the production of sterols in eukaryotes, which can influence membrane fluidity, solute permeability, and other transport functions (Bloch, 1992). Cholesterol, in particular, is known to cause the membrane

structures to behave differently at different temperatures (Cooper and Hausman, 2007). At high temperatures, cholesterol can hinder the membrane, making it more rigid, and reducing permeability to small molecules, whereas at low temperatures, cholesterol can prevent membranes from freezing by maintaining membrane fluidity. Other sterols (i.e. ergosterol, stigmasterol), especially those found in plants and algae, also perform a similar function. Although bacteria lack sterols, some bacteria are known to produce a different family of lipids, hopanoids, which are considered functional analogs of sterols (Rohmer et al., 1979; Ourisson et al., 1987). Hopanoids are pentacyclic tritrerpenoids that are involved in regulating membrane fluidity and stability and are known to contribute to a significant portion of the hydrocarbons found in ancient sediments (Ourisson et al., 1987). Membrane lipid components such as steroids, and hopanoids can provide important taxonomical and environmental information in ancient samples as the hydrocarbon backbone of these compounds are known to be extremely resistant to degradation and may survive for billions of years (Brocks and Summons, 2004).

Lipidomics is an "-omics" platform characterized by the system-level analysis of cellular lipid profiles, their functional roles, including interactions with other biomolecules, and the genetic capacity for the synthesis and catabolism of those lipids (Wenk, 2005; Pearson, 2013). An example of using lipidomics as a framework to elucidate important biochemical reactions is the seminal work on determining the O_2-dependent reaction pathways for the formation of steroids (Bloch, 1964), where the biosynthesis of this class of lipids was investigated in

enzymatic studies *in vitro*. Metagenomics, on the other hand, is the study of the genes obtained from the sequencing all of the environmental DNA collected from a particular environment. This method allows for the comprehensive analysis of the abundance and diversity of the inhabiting microbial communities and the genetic potential for that community to carry out functions that are important to the ecosystem (Turnbaugh and Gordon, 2008). In the context of cold, hypersaline extreme environments such as Lake Vida brine, lipids can serve as a functional indicator of physiological adaptations to such conditions and, whereas their genetic abilities hold clues for the organisms' adaptation potential. In the context of extraterrestrial environments, lipids present valuable information on the biological and biochemical state of the environment and can be used as biosignatures of present or ancient life. Thus, studying the lipidomics in combination with the metagenomics of an icy planetary analog, Lake Vida brine, can enhance our understanding of the habitability of extremely cold, isolated, cryo-habitats of the solar system.

In this study, we analyzed the lipidome of Lake Vida brine microbial assemblage POM, the morphology of POM collected on glass fiber and polytetrafluoroethylene (PTFE) filters, and the targeted metabolic pathways for lipid biosynthesis as revealed by metagenomics. To understand the origins of the lipids detected in the lipidome, we identified the genes necessary to encode for the enzymes required to produce each lipid class identified in the POM of LVBr. If the coding genes necessary to synthesize a given lipid class were present in the metagenome, we considered their possible biosynthesis by members of the modern microbial cohort. In

contrast, if the coding genes necessary for the synthesis of a given lipid group were not present in the metagenome, we hypothesized that these lipids may be legacy from a previous ecosystem. In this report, fatty acids, isoprenoids, steroids and hopanoids, all observed in the lipid extract of the brine particulates were investigated.

4.2. Materials and Methods

4.2.1. Sample collection

Samples were collected during the 2010 expedition to Lake Vida. The brine was sampled at 18.5 m depth from a 20.4 m hole drilled for geochemical and microbial analysis (Murray et al., 2012). Clean sampling strategies to minimize forward contamination is described in Doran et al. (2008). POM was collected on ~0.7 μm (142 mm diameter) baked (450°C for 6 hours) glass fiber filter (GFF, Whatman®) using a stainless steel (142 mm diameter; solvent-triple washed, dichloromethane (DCM):methanol (MEOH)1:1) filter holder (Millipore®) attached to a peristaltic pump. Filtrate was then passed through a ~0.22 μm (142 mm diameter) PTFE filter (solvent triple-washed) until samples were no longer able to pass through the filter. All filters were then folded and stored in baked (500°C for 12 hours) Pyrex® glass petri dishes (10 cm diameter), wrapped in baked aluminum foil and stored at -20°C until

analysis. Lipid extraction and scanning electron microscopy were performed on these filters, whereas the metagenome was obtained from a separate DNA extraction analysis (Figure 1).

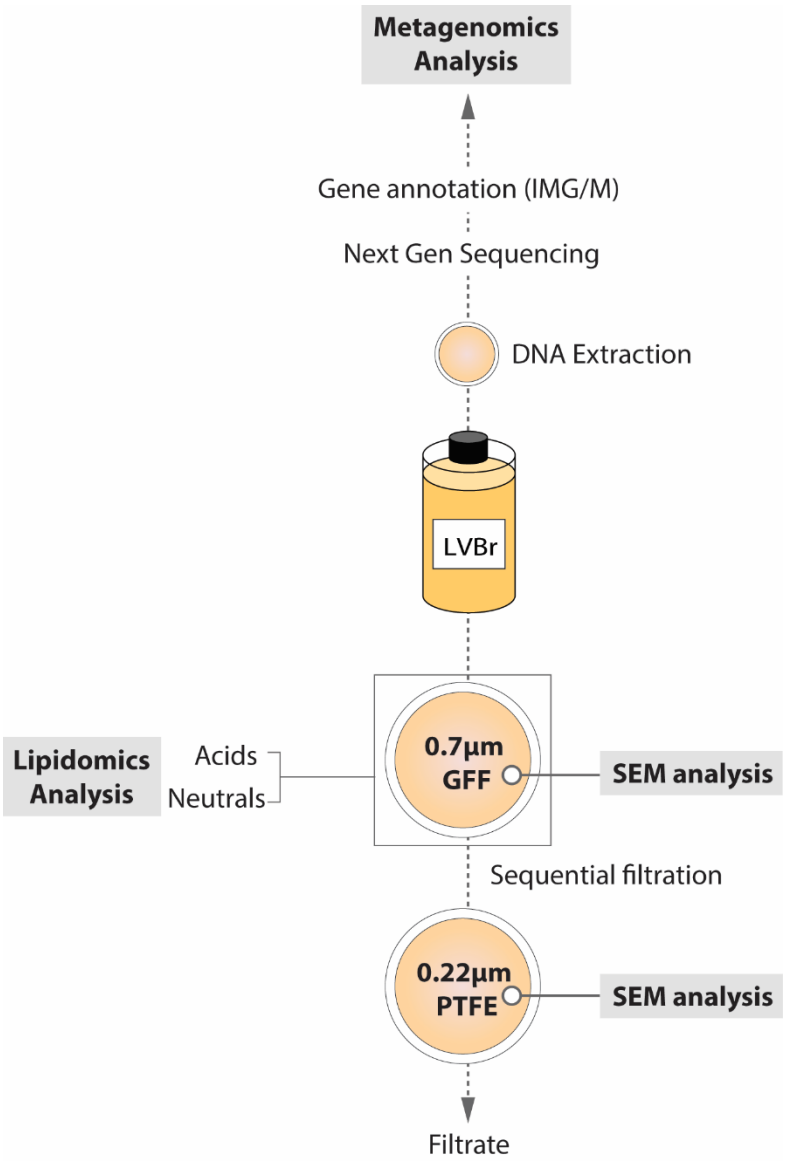

Figure 1: Analytical flow diagram of particulate organic matter analysis of Lake Vida brine.

4.2.2. Lipid extraction

Total lipids were extracted from the Lake Vida brine particulate and cellular material collected on the 0.7 μm GFF and 0.22 μm PTFE filter using a modified Bligh-Dyer procedure (Bligh and Dyer, 1959). Briefly, about ¾ of the glass fiber filter (9.7 L of brine) and 1 and ¼ of two different PTFE filters (combined 1.57 L of brine), respectively, were cut into small ~0.5 cm^2 pieces for lipid extraction using tweezers and scissors that have been baked at 500°C overnight, and solvent triple-washed (DCM:MeOH, 1:1, vol/vol). A mixture of MeOH:DCM: phosphate buffer solution (PBS) pH 7.4 (2:1:0.8) was added (28.5 mL) with filter sample into a 50 mL extraction tube (baked at 500°C overnight). The sample were ultrasonicated (Sonic Dismembrator, Fischer Scientific®) for 15 minutes on ice at 50% amplitude. After, the samples were centrifuged at 7,000 rpm for 7 minutes (Centra CL3, Thermo Scientific®), and the supernatant was collected in a separatory funnel. The extraction and centrifugation were repeated once more. A final extraction step was performed using a mixture of MeOH:DCM:5% trichloroacetic acid H_2O to enhance the extraction of any archaeal glyceroldialkyl-glyceroltetraether (GDGT) lipids that could be present in the sample (Nishihara and Koga, 1987). These steps were repeated 3-4 times or until the supernatant was colorless.

A separatory funnel was used to recover the total lipid extract (TLE) from the solvent phases mixed with aqueous phase (7.5 mL of DCM and 6 mL of PBS or 5% trichloroacetic acid was added per sample). The solvent phase was collected in a round bottom flask. This step was

repeated three times, each step using 7.5 mL of DCM. The TLE was evaporated using a Buchi R-114 rotary evaporator, and transferred to a 4 mL vial, dried under a gentle flow of N_2, and weighed.

4.2.3. Base Hydrolysis and Neutral and Acid extraction

The TLE was added to a 15 mL glass centrifuge tube and reduced to dryness under a gentle flow of N_2. 6 mL of 0.5 M KOH in MeOH and 2 mL of dH_2O were added to each sample vial along with boiling chips (extracted with DCM:MeOH 7.5:1 for 48 hours). The vials were then blown under N_2 to remove air in the headspace, then capped. Vials were then placed on heating blocks for 2 hours at 70°C after which the saponified solution was allowed to cool for 24 hours.

Once the samples were saponified, 3mL of hexanes and 2.5 mL of saturated NaCl solution were added to the vial, and samples shaken vigorously several times for 30 – 40 seconds. The solution was allowed to separate, and the top hexane layer was recovered to obtain the neutral fraction. The process was repeated three times to ensure maximum recovery of neutral lipids. An internal standard, 2.68 µg of deuterated cholesterol-d7 (Avanti Polar Lipids) was added to the sample prior to gas chromatography-mass spectrometry (GC-MS) for semi-quantitation of neutral lipids.

The saponified samples were then acidified by adding 6M of HCl. Once the solution reached pH ~2, 3 mL of hexanes were added, vials were capped, and shaken vigorously several times for 30 – 40 seconds and the acid fraction were recovered from the top hexane layer. This hexanes extraction was repeated three times.

A 50% aliquot of the acid fraction was derivatized with BF_3 methanol to generate fatty acid methyl esters (FAMEs). An internal standard, 1.02 mg of deuterated undecanoic-d21 acid (C/D/N Isotopes®) was added to the sample prior to the derivatization for semi-quantitation. Samples were transferred to a 15 mL glass tube, and excess solvent was dried off under a low flow of N_2. 2 mL of 10% BF_3-MeOH was added, and the tubes were placed on a heating block for 10 minutes at 60°C. The samples were allowed to cool and then 1 mL of dH_2O and 1 mL of hexane was added. The vials were shaken vigorously for 30-40 seconds. The upper layer was removed, and trace dH_2O was removed over anhydrous sodium sulfate (solvent extracted and baked at 100°C) packed in a Pasteur pipette. Samples were recovered and excess hexanes were removed under a low flow of N_2. Samples were weighted and redissolved in cyclohexane at 10 μg/μL and then injected into the GC-MS.

4.2.4. Gas chromatography-mass spectrometry

GC-MS was performed on a Hewlett Packard (HP) 6890 GC coupled to a HP-5973 mass selective detector. The electron ionization energy was 70 eV and the carrier gas was

helium (>99.999% ultra-high purity, Praxair®). The capillary column used was a 30 m Agilent HP-5 MS column (0.25 mm internal diameter and 0.25 μm film thickness). The flow rate was held constant at 1mL/min. The samples were injected in pulsed splitless mode at 320 °C (40 psi). The oven temperature was kept isothermal at 60 °C for 2 min, then ramped at 10 °C/min to 150 °C and further ramped at 3 °C/min to 320 °C and held constant at 320 °C for 20 min. Mass scans were performed between *m/z* 40 and *m/z* 650 at a rate of 3 scans per second.

4.2.5 Field Emission Scanning Electron Microscopy (FESEM) and X-ray Energy Dispersive Spectroscopy (XEDS)

The filter samples (0.7 μm on GFF, and 0.22 μm on PTFE filter) containing the LVBr microbial assemblage and the same blank filters were subsampled using a stainless-steel coring device (2.4 cm diameter) with a sharpened circumference to obtain smaller filters (solvent-tripled washed, DCM:MeOH 1:1). Samples were fixed in 2% glutaraldehyde. To enhance surface charging of biological material, the filter samples were secondary fixed with osmium tetroxide. Samples were then dehydrated with a series of increasing ethanol concentrations, and hexamethyldisilazane was used as a transition fluid during drying of the sample. Fixed filters were then placed on aluminum mounts using a double-sided carbon tape adhesive. Samples were then sputter-coated with platinum/palladium vapor at low vacuum to a thickness of 10.0 nm. Samples were viewed with a secondary electron detector for surface morphology. Operating conditions of the electron beam on the FESEM were 10 kV at a working distance

between 7 to 10 mm. Operating conditions of the electron beam for XEDS analysis were 15 kV at a working distance of 15 mm.

Electron imagining was performed on a JOEL JSM-6320F field emission scanning electron microscope and a Hitachi S-3000N variable pressure scanning electron microscope located at the Electron Microscopy Core facility at the University of Illinois at Chicago's Research Resources Center. The XEDS analysis was performed on the Hitachi S-3000N using an Oxford Inca EDX system with a light element X-ray detector to analyze bulk elemental composition.

4.2.6. Targeted Metagenomics analysis

Metagenome annotation was conducted by the Joint Genome Institute (JGI) using the Integrated Microbial Genomes and Microbiomes (IMG/M) pipeline (Huntemann et al., 2015, Markowitz et al., 2014). Briefly, the total environmental DNA was extracted in the field and submitted to IMG/M. Raw sequences were obtained on the Illumina and Illumina HiSeq (2500 and 2000) platforms (Illumina, Inc., San Diego, CA, USA). Metagenomes are pre-processed before annotation by removal of low-quality read regions, any duplicate sequence, and by masking particularly low-complexity regions that could artificially inflate biologically insignificant comparisons to high-scoring matches using the DUST algorithm (Morgulis et al., 2006). Protein coding genes (coding sequences; CDS) are identified using GeneMark,

Metagene (Noguchi et al., 2006), Prodigal (Hyatt et al., 2010) and FragGeneScan (Rho et al., 2010), and compared to existing databases: the Clusters of Orthologous Groups (COG), Protein Families (Pfam), and Kyoto Encyclopedia of Genes and Genomes (KEGG). These genes are then associated with KEGG Orthology term, assigned an Enzyme Commissioner (EC) number and phylogeny. Gene identity is assigned by computing protein sequence similarities between genes in the samples and all the genes in reference genomes in the IMG database. The Lake Vida brine combined metagenome (IMG Genome ID: 3300001097) was analyzed using the browser and comparative search tools native to IMG. To verify the presence of lipid biosynthesis genes that do not have an associated EC or KEGG orthologs, we used the amino acid sequences of coding regions obtained from previously published work (Welander et al., 2010; Welander and Summons, 2012; Banta et al., 2015) and performed a Basic Local Alignment Search Tool (BLAST) protein (BLASTp) analysis against the LVBr metagenome in IMG. BLASTp was performed by using NCBI Blast+ command-line application (Camacho et al., 2009), and a cutoff e-value of 1e-5 with a 30% or better alignment percent identity, covering at least 70% over the query gene was set at the same parameters as KEGG Orthology assignment in IMG (Huntemann et al., 2015).

4.3. Results

4.3.1. The lipidome of Lake Vida brine microbial assemblage

The lipidome of the Lake Vida brine POM contains lipids belonging to three major lipid classes: (1) fatty acids, (2) pentacyclic-, and (3) tetracyclic-triterpenoids, as well as *n*-alkanes, *n*-alkenes ($C_{16:1}$ and $C_{18:1}$), and isoprenoid. Fatty acids methyl esters (FAMEs) dominate the GC amenable LVBr lipidome as they represent ~99% of the observed lipid fraction. In particular, straight chain hexanoic acid (C_{16}) and octanoic acid (C_{18}) account for 34.4% and 58.7% of the total lipids, respectively (Table 1). Other fatty acids such as branched fatty acids, including, *iso-*, and *anteiso-* substituted compounds, monounsaturated fatty acids, and polyunsaturated fatty acids ($C_{18:2}$ and $C_{19:2}$) constitute 6.9% of the lipidome (Table 1).

The remaining ~1% if the observed lipids are found in the neutral fraction. Steroids identified in the LVBr lipidome include cholest-5-en-3β-ol (cholesterol; C_{27}, **I**, Appendix IV), 24-methyl-cholest-5,22-dien-3β-ol (brassicasterol; $C_{28}\Delta^{5,22}$, **IIa**), 24-methyl-cholest-5,22-en-3β-ol (campesterol; $C_{28}\Delta^{5}$, **IIb**), 24-ethyl-cholest-5,22-dien-3β-ol (stigmasterol; $C_{29}\Delta^{5,22}$, **IIIa**), and 24-ethyl, cholest-5-en-3β-ol (sitosterol; $C_{29}\Delta^{5}$, **IIIb**) (Table 2). Hopanoids were also observed in the LVBr lipidome including diplopterol (**IVa**), 2-methyl diplopterol (**IVb**, Appendix V), and gammaceran-3β-ol (tetrahymanol; **V**) (Table 2). The distribution of hopanoids and steroids observed in the extract of LVBr particulate fraction bears remarkable

resemblance to steroids and hopanoids observed in the Lake Vida sediments embedded within the lake ice and sediments found in those ice (data not shown).

Here, we only consider the relative abundance of lipids on the 0.7 μm GFF filter as it contains both large cellular-detritus as well as ultrasmall microbial cells which can be analyzed in concert with the combined LVBr metagenome, whereas the smaller particulate fraction from the 0.22 μm does not have complementary metagenome data.

Table 1: Lipids detected in the acid fraction of Lake Vida lipidome.

	Compound Name	Numbering	Total FA content (ng/mL)	% of FA Total
Straight-chain FA	dodecanoic acid	*n*-12:0	1.79	**0.03**
	tridecanoic acid	*n*-13:0	0.55	**0.01**
	tetradecanoic acid	*n*-14:0	34.18	**0.65**
	pentadecanoic acid	*n*-15:0	21.44	**0.41**
	hexadecanoic acid	*n*-16:0	1802.73	**34.43**
	heptadecanoic acid	*n*-17:0	12.90	**0.25**
	octadecanoic acid	*n*-18:0	3077.85	**58.77**
	nonadecanoic acid	*n*-19:0	1.67	**0.03**
	eicosanoic acid	*n*-20:0	9.15	**0.17**
	heneicosanoic acid	*n*-21:0	0.53	**0.01**
	docosanoic acid	*n*-22:0	1.28	**0.02**
	tetracosanoic acid	*n*-24:0	0.63	**0.01**
Branched-chain FA	methyl-dodecanoic acid	13	0.49	**0.01**
	methyl-tridecanoic acid	14	2.18	**0.04**
	13-methyl-tetradecanoid acid	iso-15:0	3.89	**0.07**
	12-methyl- tetradecanoid acid	anteiso-15:0	16.3	**0.31**
	14-methyl-heptadecanoic acid	16	3.57	**0.07**
	methyl-hexadecanoic acid	17	7.75	**0.15**
	14-methyl- hexadecanoic acid	iso-17:0	1.79	**0.03**
	13-methyl- hexadecanoic acid	anteiso-17:0	4.34	**0.08**
Monounsaturated FA	tetradecenoic acid	*n*-14:1	3.07	**0.06**
	pentadecenoic acid	*n*-15:1	5.20	**0.10**
	hexadecenoic acid	*n*-16:1	98.15	**1.87**
	heptadecenoic acid	*n*-17:1	4.13	**0.08**
	octadecenoic acid	*n*-18:1	66.38	**1.27**
	octadecenoic acid	*n*-18:1	20.26	**0.39**
	eicosenoic acid	*n*-20:1	3.65	**0.07**
	docosenoic acid	*n*-22:1	26.34	**0.50**
Polyunsaturated FA	octahexenoic acid	*n*-18:2	2.98	**0.06**
	nonahexenoic acid	*n*-19:2	1.50	**0.03**

Table 2: Lipids detected in the neutral fraction of Lake Vida lipidome.

	Compound Name	Common Name	Total Lipid Content (pg/mL)	% of Ster.+Hop. Total
Steroids	cholest-5-en-3*β*-ol	cholesterol	2.7	**31.93**
	24-methyl-cholest-5,22-dien-3*β*-ol	brassicasterol	1.7	**19.82**
	24-methyl-cholest-5-en-3*β*-ol	campesterol	0.6	**7.07**
	24-ethyl-cholest-5,22-dien-3*β*-ol	stigmasterol	0.8	**9.72**
	24-ethyl-cholest-5-en-3*β*-ol	sitosterol	1.5	**18.38**
Hopanoids	2-methyl-hopan-22-ol		0.9	**11.04**
	hopan-22-ol	diplopterol	0.1	**1.01**
	gammaceran-3β-ol	tetrahymanol	0.1	**1.03**

4.3.2. Scanning electron microscopy and elemental analysis of Lake Vida brine POM

Electron microscopy analysis of the Lake Vida brine glass fiber filter (0.7μm) revealed the presence of amorphous granular particles (Figure 2a), a population of small coccoid cells and cell-like particles (<1 μm; Figure 2b and 2c), and numerous large (>100 μm), degraded, cell-like detritus (Figure 3) within a thick network of glass fibers. The amorphous particles do not have a distinct crystalline structure, nor do they resemble any known cell morphology. Some particles (Figure 2b) are within range of the known ultrasmall microbial cell of Lake Vida brine (Kuhn et al., 2014), whereas others (Figure 2c) are extremely small, nano-sized cell-like particles ranging down to ~30 nm. Large cell-like structures (>100 μm) were observed, though appear to be severely degraded. These structures are oval or rhombus in shape, exhibiting ridges on the outer edges and sharp apexes at corners (Figure 3).

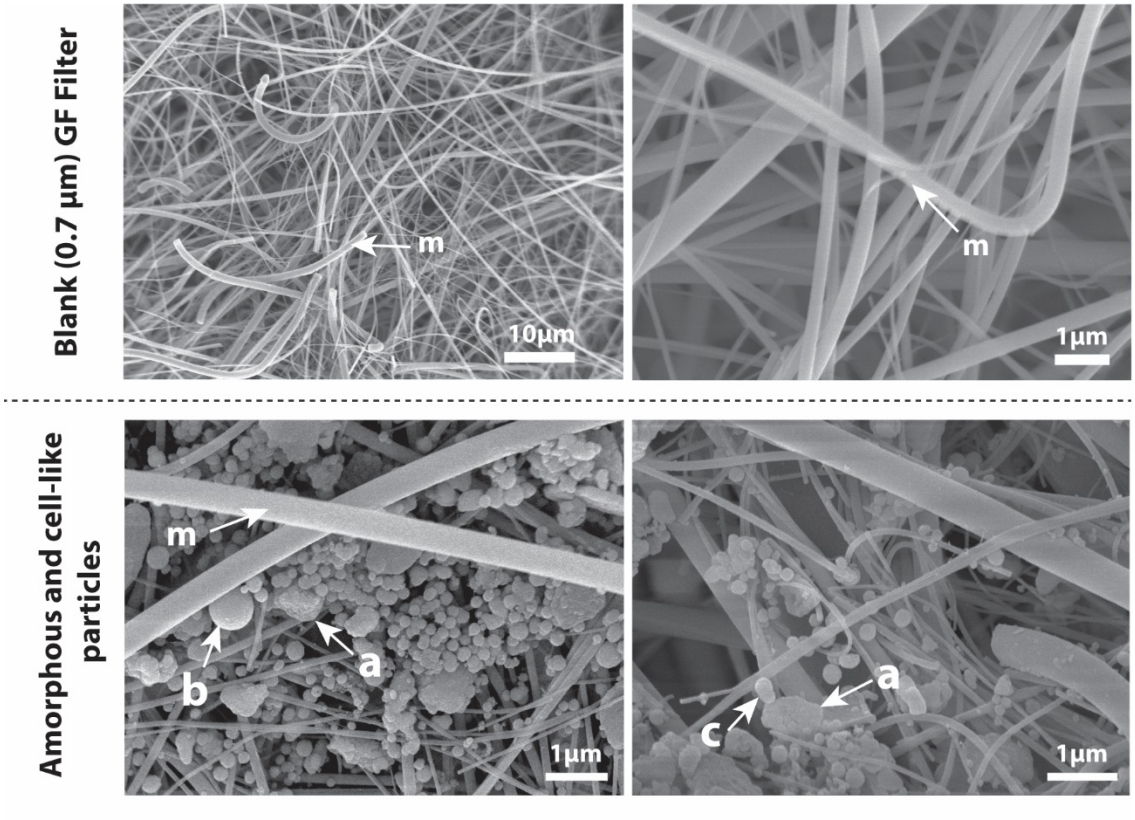

Figure 2: SEM images of the particulate organic matter collected on the 0.7 µm glass fiber filter. **Top**: blank glass fiber filter (baked at 450°C for 6 hours). **Bottom: "a"** amorphous granular particles, **"b"** coccoid cells ~1 µm in size, and **"c"** ultrasmall microbial cell or nano-sized cell-like structure that appear to be dividing. The matrix **"m",** glass fiber, is indicated. Scale bars are shown in each micrograph.

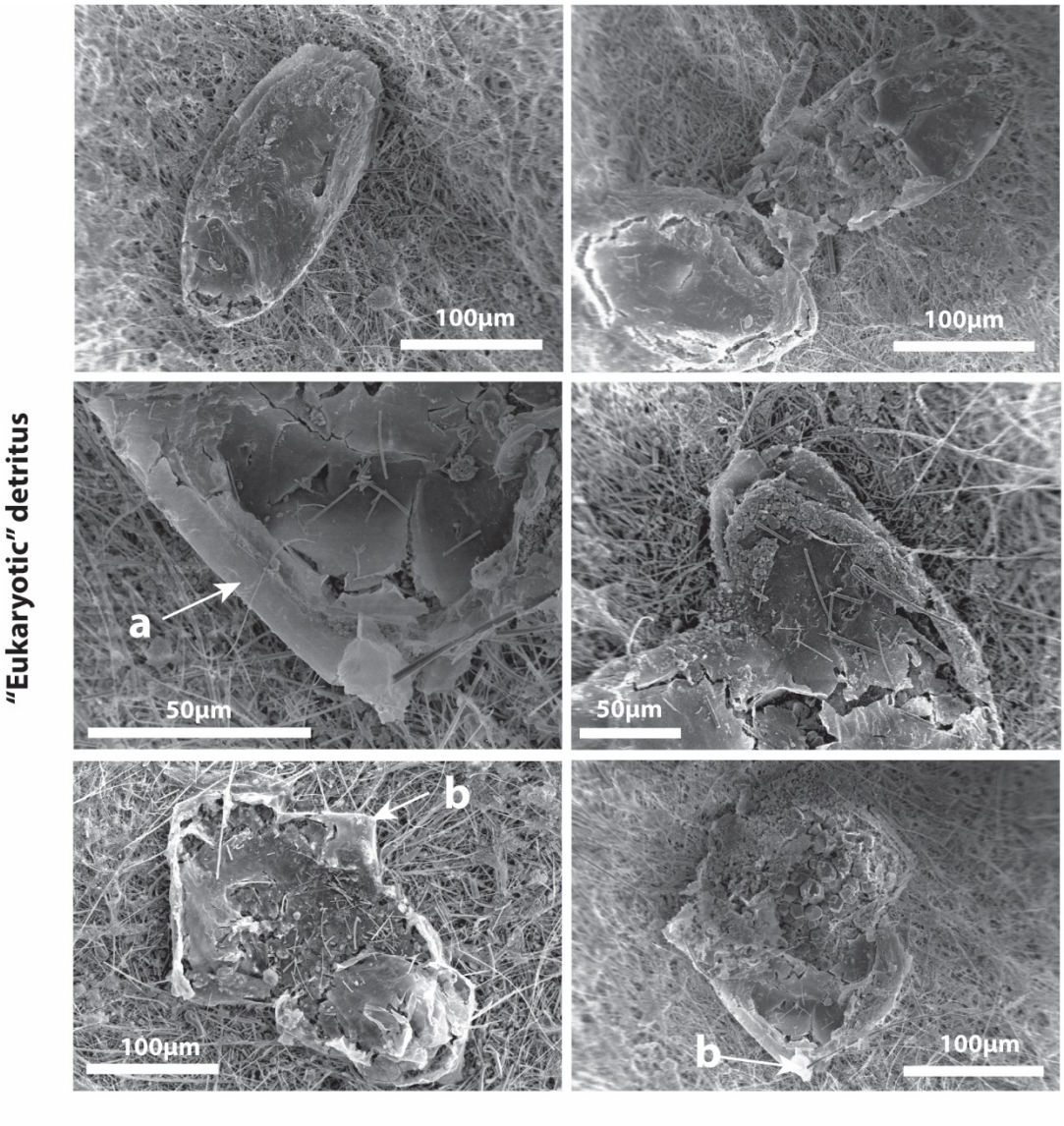

Figure 3. SEM images of the particulate organic matter collected on the 0.7 µm glass fiber filter. Large, degraded oval and rhombus "eukaryotic" detritus structures showing **"a"** raised ridges on collapsed edge and **"b"** sharp apexes of the rhombus shape. Scale bars are shown in each micrograph.

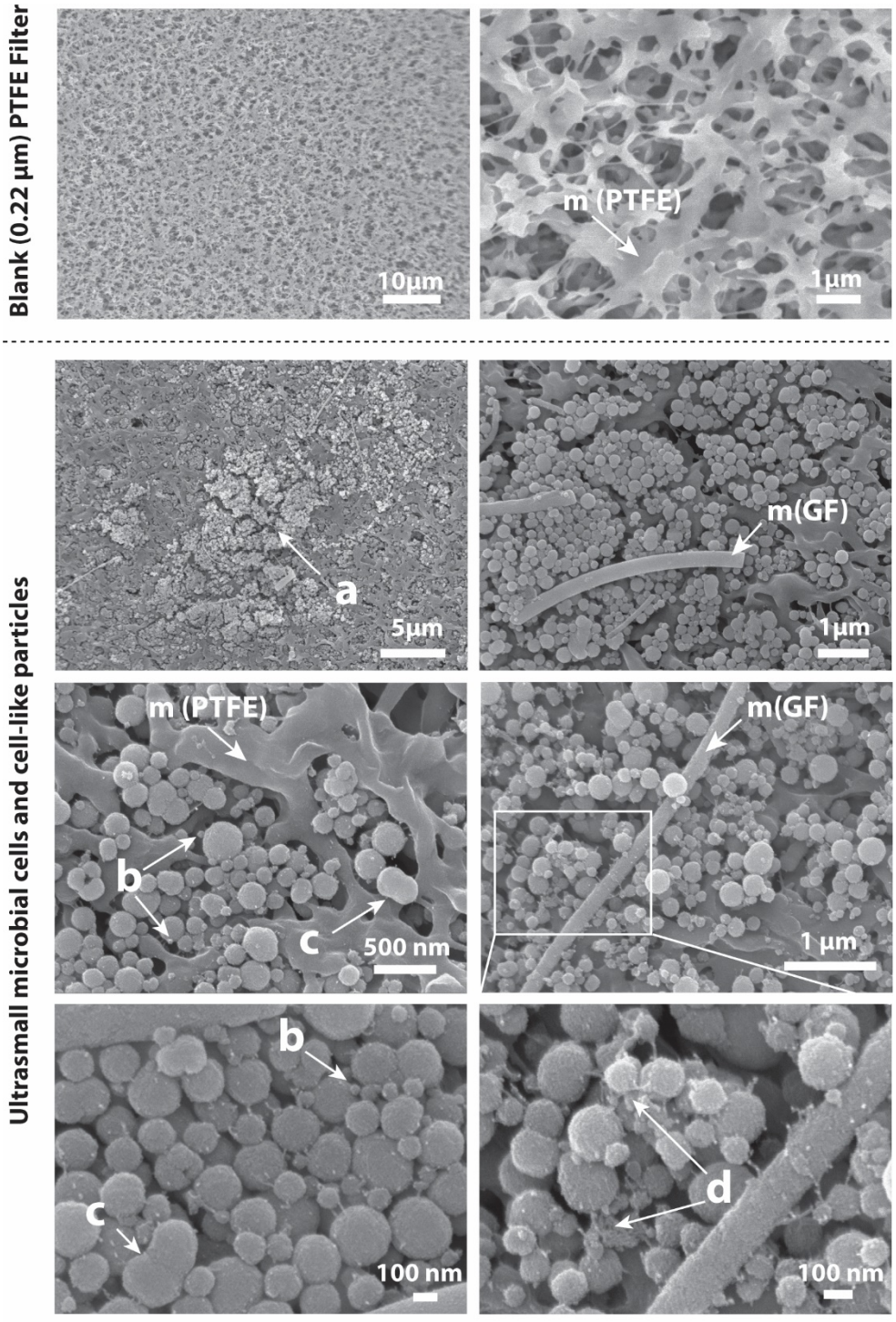

Figure 4: SEM images of the particulate organic matter collected on the 0.22 µm PTFE filter. **Top:** Blank PTFE (0.22 µm) filter. **Bottom: "a"** Ultrasmall microbial cells and small coccoid-shaped cell-like particles dominate this filter fraction. **"b"** Some particles appear to be budding off a larger cell or cell-like particle. **"c"** Some cells appear to be dividing. **"d"** Filamentous material appear to connect some cells. The matrix **"m(GF)"** and **"m(PTFE)"**, glass fiber and PTFE, respectively, are indicated. Scale bars are shown in each micrograph.

The SEM images of the PTFE (0.22 μm) filter show the presence of a large number of ultrasmall microbial cells and an abundance of nano-sized cell-like particles (Figure 4a) that were also retained on the glass fiber filter (Figure 2). Most of the cell and cell-like particles have uniform coccoid morphology. Very small nanoparticle (Figure 4b) are typically found associated with a larger cell and may constitute membrane vesicle expressions previously observed in LVBr (Kuhn et al., 2014). Some cells appear to be pinching around the circumference, which is interpreted, in this study, as cells dividing (Figure 4c). A network of unidentified filamentous material appears to connect some cells and cell-like particles (Figure 4d) and have also been previously observed by Murray et al. (2012).

An XEDS point analysis of a spot on the large (> 100 μm) cell-like structures shows high peak intensities in P signal at 2.01 keV (K_α) region, Ca signal at 3.7 keV (K_α) region, and Si signal at 1.7 keV (K_α) (Figure 5a). When this structure was analyzed by XEDS mapping, a region of P and Ca show the same shape as the structure observed in SEM, confirming that the cell-like structure contain high levels of P and Ca relative to the background. In contrast, the map for Si revealed the inverse relationship, where the Si signal is more intense outside of the structure than inside (Figure 5a). Bulk elemental analysis of the XEDS map shows relatively high C (10.89 wt%), P (16.38 wt%) and Ca (25.75 wt%), and lower Si (5.67 wt%) compared to the blank filter (P is 3%, Ca is 2.6%, and C is not detected, whereas Si is 50.3%) (Table 3). Analysis of another large cell-like structure revealed a similar trend (Figure 5b). As a result, we hypothesize here that that these cell-like structures are remnants of calcareous eukaryotic cells.

The elemental compositions of the ultrasmall microbial cells and nano-sized cell-like particles on the PTFE filter were distinctly different from the larger cell structures observed on the glass fiber filter. We detected large, smooth, rod-like structures with sharp edges that we postulated to be glass fibers that were transferred to the PTFE filter during sequential glass fiber-PTFE filtration. An XEDS point analysis of one of these rod-like structures confirmed our prediction with the energy spectrum showing peaks in Si signal region (bulk abundance of 13.23 wt%) (Figure 6a) compared to other spectra obtained on field of ultrasmall microbial cells only, which contain <5wt% of Si (Table 3), and lower bulk P abundances (2.86 wt%) compared to the large cell-like structures described previously (P was between ~14 – 22%). Furthermore, the XEDS mapping analysis of the structure revealed high Si signals in the same region as the rod structure, and this spatial correlation was not observed in any other elements (Figure 6b). The spectra of the point XEDS analysis of the rod structure also show a peak in the Fe region of 6.4 keV (K_{α}). Both point and mapping analyses of the particulates on the PTFE filter show a relatively high abundance of Fe (13.96 wt% and 24.61 wt%, respectively) compared to abundances of Fe on the glass fiber filter, with bulk values of less than 4 wt% (Table 3).

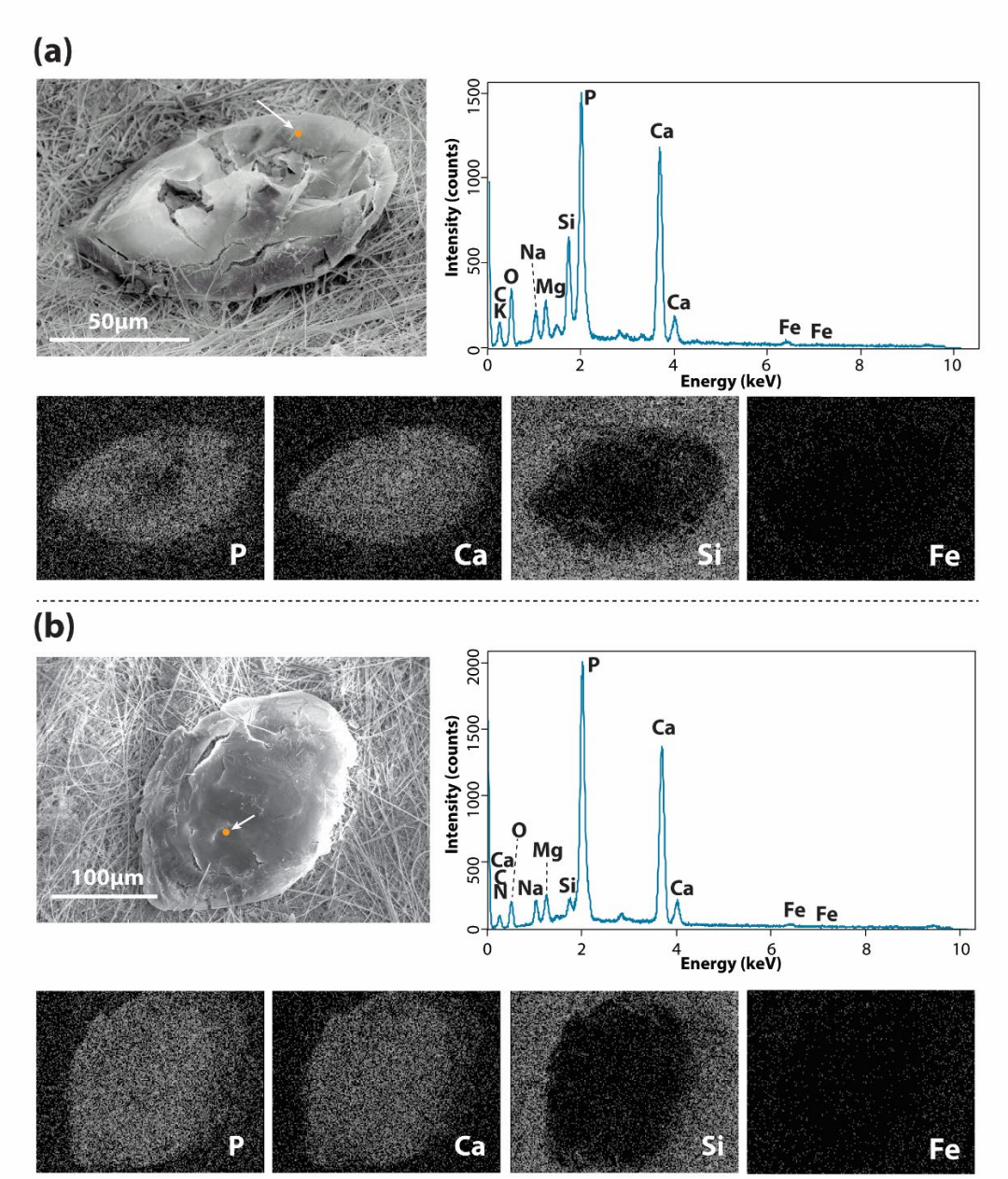

Figure 5: **(a)** SEM and XEDS analysis of large "eukaryotic" detritus on glass fiber filter with point spectra showing relatively high peak intensities in the P, Si, and Ca regions. Complementary spectral mapping of the large structure reveal concentrations of P and Ca that align with the structure in SEM image and an inverse relationship with Si. Mapping of Fe does not reveal any significant contribution to the structure. **(b)** SEM and XEDS analysis of another "eukaryotic" detritus with point spectra showing relatively high peak intensities in the P and Ca regions. Complementary spectral mapping of the large structure reveal concentrations of P and Ca that align with the structure in SEM image and an inverse relationship with Si. Mapping of Fe does not reveal any significant contribution to the structure. White points relative to the black surface in mapping spectra represents higher electron intensities (KeV) relative to the background.

Because individual ultrasmall microbial cell, and nano-sized cell-like particle were too small to be confidently analyzed individually with the XEDS electron beam, we chose to perform a point analysis on the center of a large field of thick material consisting mainly of the ultrasmall microbial cell and nano-sized cell-like particles. The XEDS spectra show high intensity in the region for P, as well as Fe peak in the 6.4 keV (K_α) region (Figure 6b). Bulk elemental analysis show relatively high amounts of P (13.7 wt%), O (12.95 wt%), and Fe (34.25 wt%) relative to the bulk analysis of blank PTFE filter, although we cannot definitively determine whether a majority of the C (19.54 wt%) was derived from the conglomeration of cells or from the PTFE filter beneath (Table 3). Mapping XEDS analysis of a field region containing large conglomeration of ultrasmall microbial cells further show a uniform distribution and co-location of high abundance of C, Fe and P intensities (Figure 6c).

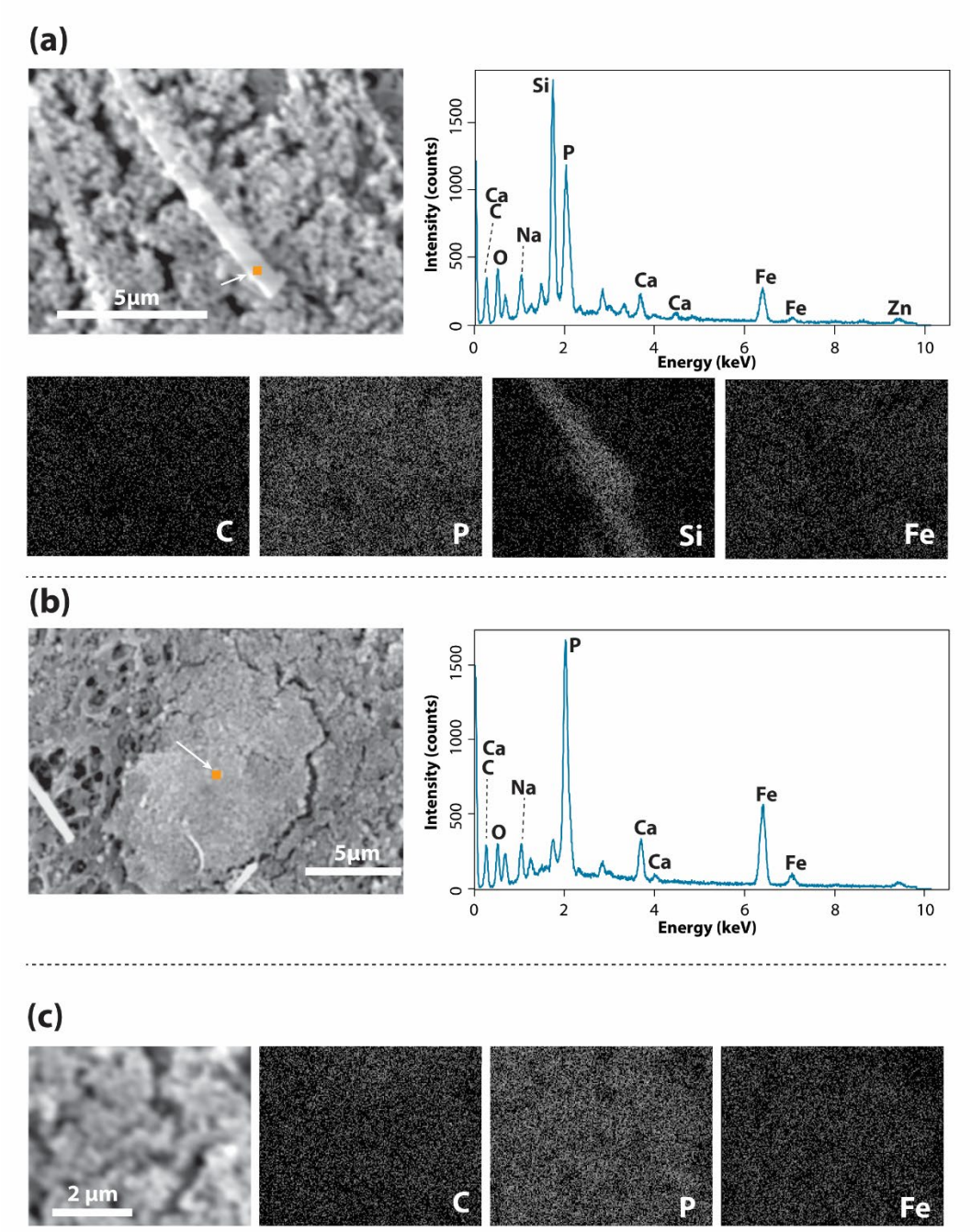

Figure 6: **(a)** SEM and XEDS of a glass fiber caught on the PTFE filter with point spectra (orange square in SEM) showing relatively high peak intensities in the Si and P regions. Complementary spectral mapping of the structure confirm that it is made mostly of Si. Maps of P, C, and Fe show a relatively uniform distribution. **(b)** Point XEDS of a large conglomeration of ultrasmall microbial cells and cell-like particles with point spectra (orange square in SEM) showing relatively high peak intensities in P, Ca, and Fe. **(c)** Mapping XEDS of a large conglomeration of ultrasmall microbial cells and cell-like particles with mapping spectra showing uniform abundance of C, P and Fe. White points relative to the black surface in mapping spectra represents higher electron intensities (KeV) relative to the background.

Table 3: Elemental weight (%) of particulate organic matter collected on glass fiber filter and PTFE filter. Each data column refers to specific elemental abundances obtained by point XEDS spectral or mapping XEDS spectral analysis of SEM images shown in Figure 5 and Figure 6. Map of blank glass fiber and blank PTFE filter SEM images are not shown.

			Glass Fiber Filter XEDS (Figure 5)				PTFE Filter XEDS (Figure 6)			
			a		b		a		b	c
Element (Weight %)	**Blank GFF**	**Blank PTFE**	**Point**	**Mapping**	**Point**	**Mapping**	**Point**	**Mapping**	**Point**	**Mapping**
C	nd	56.2	10.9	8.6	9.9	9.9	23.0	35.3	19.5	43.6
F	nd	41.6	nd	nd	nd	nd	nd	3.7	2.5	5.5
N	nd	nd	7.5	nd	8.7	8.6	nd	nd	5.9	nd
O	28.6	nd	27.2	26.1	20.3	20.3	16.6	12.2	13.0	10.3
Na	4.6	nd	2.3	2.5	2.4	2.4	2.9	2.6	3.4	1.9
Mg	nd	nd	2.4	1.7	2.3	2.3	0.4	0.8	1.3	0.7
Al	nd	nd	nd	nd	0.3	nd	1.3	nd	nd	nd
Si	50.3	nd	5.7	18.9	0.8	0.9	13.2	4.1	1.5	1.2
P	3.0	2.2	16.4	14.5	22.2	22.2	2.9	12.3	13.7	11.1
S	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cl	0.4	nd	nd	nd	nd	nd	nd	nd	0.2	nd
K	5.4	nd	0.5	2.4	nd	nd	1.3	0.6	nd	nd
Ca	2.6	nd	25.8	19.0	31.9	32.0	2.6	3.7	4.7	3.2
Fe	nd	nd	1.4	4.0	1.4	1.4	14.0	24.6	34.3	22.6
Zn	5.0	nd	nd	2.4	nd	nd	nd	nd	nd	nd

* nd = not detected

4.3.3. The metagenome of the Lake Vida brine microbial assemblage

The gene abundance of each coding sequences (CDS) was determined based on the KEGG orthology assignment in IMG. The total gene abundance of coding sequences assigned to Type II fatty acid biosynthesis range from 7 to 149, except for one gene, the acyl-CoA Δ^6-desaturase (FADS2), which was not detected using this method (Table 4). Coding sequences associated with mevalonate pathway ranged from 4 to 119. Two genes coding for enzymes HMG-CoA synthase and HMG-CoA reductase were not detected using this method (Table 5). On the other hand, all of the genes necessary to code for enzymes involved in the mevalonate-independent pathway were detected, at abundances between 1 to 47 (Table 5). Of the 14 enzymes associated the sterol biosynthesis pathway, only 2 were detected in the LVBr metagenome, sterol-4α-carboxylate 3-dehydrogenase (ERG26), and 4-methylsterol oxidase 2 (SMO2) (Table 6). The coding genes associated with hopanoid and tetrahymanol biosynthesis vary in abundance. Though the genes for squalene-hopene cyclase and hopanoid 3-methyltransferase were detected, the gene associated with bacterial tetrahymanol synthesis (squalene-tetrahymanol cyclase) was not detected. Tetrahymanol synthase, the enzyme implicated in the synthesis of tetrahymanol in eukaryotes (i.e., freshwater ciliates) has not been previously characterized, thus, we were unable to determine of this gene was present in the LVBr metagenome.

Table 4: Enzymes involved in type II fatty acid synthesis and their name, EC number, KEGG Orthology, and abundance in the Lake Vida brine metagenome

Type II Fatty Acid Synthesis	Enzyme	Name	EC Number	KEGG Orthology	Gene Abundance
	ACP synthase	**ACPS**	EC 2.7.8.7	K00997	7
	Acetyl-coenzyme A carboxylase (ACC)	**accC**	EC 6.3.4.14	K01961	36
		accA	EC 2.1.3.15	K01962	32
		accD	EC 2.1.3.15	K01963	32
		accB	-	K02160	33
	Malonyl-CoA–ACP transacylase	**FabD**	EC 2.3.1.39	K00645	33
	β-Oxoacyl synthase III	**FabH**	EC 2.3.1.180	K00648	92
	β-Oxoacyl synthase I	**FabB**	EC 2.3.1.41	K00647	40
	β-Oxoacyl synthase II	**FabF**	EC 2.3.1.179	K09458	57
	β-Oxoacyl reductase	**FabG**	EC 1.1.1.100	K00059	149
	β-Hydroxydecanoyl dehydratase	**FabA**	EC 4.2.1.59	K01716	9
	β-Hydroxyacyl dehydratase	**FabZ**	EC 4.2.1.59	K02372	33
	Enoyl reductase	**FabI**	EC 1.3.1.9	K00208	47
	fatty acyl-ACP thioesterase B	**FATB**	EC 3.1.2.14	K10781	0
	long-chain acyl-CoA synthetase	**ACSL**	EC 6.2.1.3	K01897	131
	acyl-CoA Δ^9-desaturase	**SCD**	EC 1.14.19.1	K00507	17
	acyl-CoA Δ^6-desaturase	**FADS2**	EC 1.14.19.3	K10226	0
	acyl-lipid ω-6 12-desaturase	**FADS6**	EC 1.14.19.23	K10255	27

Table 5: Enzymes involved in triterpenoid backbone synthesis and their name, EC number, KEGG Orthology, and abundance in the Lake Vida brine metagenome

Triterpenoid Backbone Biosynthesis	Enzyme	Name	EC Number	KEGG Orthology	Gene Abundance
	Mevalonate pathway				
	Acetyl-CoA-thiolase	**AtoB**	EC 2.3.1.9	K00626	119
	HMG-CoA synthase	**HMGCS**	EC 2.3.3.10	K01641	0
	HMG-CoA reductase	**HMGCR**	EC 1.1.1.34	K00021	0
	mevalonate kinase	**MVK**	EC 2.7.1.36	K00869	17
	phosphomevalonate kinase	**PMK**	EC 2.7.4.2	K00938	4
	mevalonate diphosphase decarboxylase	**MVD**	EC 4.1.1.33	K01597	17
	Mevalonate-independent pathway				
	1-deoxy-*D*-xylulose 5-phosphate synthase	**Dxs**	EC 2.2.1.7	K01662	42
	2*C*-methyl-*D*-erythritol 4-phosphate synthase	**IspC**	EC1.1.1.267	K00099	25
	4-diphosphocytidyl-2*C*-methyl-*D*-erythritol synthase	**IspD**	EC 2.7.7.60	K00991	14
	4-diphosphocytidyl-2*C*-methyl-*D*-erythritol	**IspE**	EC 2.7.1.148	K00919	24
	2*C*-methyl-*D*-erythritol 2,4-diphosphate synthase	**IspF**	EC 4.6.1.12	K01770	18
	2*C*-methyl-*D*-erythritol 2,4-cyclodiphosphate reductase	**IspG**	EC 1.17.7.1	K03526	30
	1-hydroxy-2methyl-butenyl 4-diphosphate reductase	**IspH**	EC 1.17.7.4	K03527	31
	isopentenyl-diphosphate isomerase	**IDI**	EC 5.3.3.2	K01823	14
	farnesyl diphosphate synthase	**IspA**	EC:2.5.1.1	K00795	47
	farnesyl-diphosphate farnesyltransferase	**FDFT1**	EC:2.5.1.21	K00801	1

Table 6: Enzymes involved in sterol biosynthesis and their name, EC number, KEGG Orthology, and abundance in the Lake Vida brine metagenome

Sterol Biosynthesis	Enzyme	Name	EC Number	KEGG Orthology	Gene Abundance
	squalene monooxygenase	**SQLE, ERG1**	EC 1.14.14.17	K00511	0
	lanosterol synthase	**LSS**	EC 5.4.99.7	K01852	0
	sterol 14α- desmethylase	**CYP51**	EC 1.14.14.154	K05917	0
	Δ^{14} sterol reductase	**TM7SF2**	EC 1.3.1.70	K00222	0
	sterol-4α-carboxylate 3-dehydrogenase	**ERG26**	EC 1.1.1.170	K07748	1
	cholestenol Δ-isomerase	**EBP**	EC 5.3.3.5	K01824	0
	Δ^{7}-sterol 5-desaturase	**ERG3**	EC 1.14.19.20	K00227	0
	Δ^{24}- sterol reductase	**DHCR24**	EC 1.3.1.72	K09828	0
	7-dehydrocholesterol reductase	**DHCR7**	EC:1.3.1.21	K00213	0
	cycloartenol synthase	**CAS1**	EC 5.4.99.8	K01853	0
	sterol 24-C-methyltransferase	**SMT1**	EC:2.1.1.41	K00559	0
	4-methylsterol oxidase 1	**SMO1**	-	K14423	0
	cycloeucalenol cycloisomerase	**CPI1**	EC:5.5.1.9	K08246	0
	4-methylsterol oxidase 2	**SMO2**	-	K14424	1

Table 7: Enzymes involved in hopanoid biosynthesis and their name, EC number, KEGG Orthology, and abundance in the Lake Vida brine metagenome

Hopanoid Biosynthesis	Enzyme	Name	EC Number	KEGG Orthology	Gene Abundance
	squalene-hopene cyclase	**Shc**	EC 5.4.99.17	K06045	3
	tetrahymanol synthase	**Ths**	-	-	0
	squalene-tetrahymanol cyclase	**Stc**	EC 4.2.1.123	-	N/A
	hopanoid 2-methyltransferase	**HpnP**	EC:2.1.1.-	K22705	0
	hopanoid 3-methyltransferase	**HpnR**	EC:2.1.1.-	K22704	1

4.4. Discussion

4.4.1. The lipid composition and cell morphology of Lake Vida brine POM

Straight-chain FA (C_{16} and C_{18}) dominate the lipidome of LVBr POM, which is not surprising as these compounds are the most common lipids found in bacteria, and evidence obtained from 16S rRNA gene survey of the LVBr microbial assemblage suggest that the LVBr community is exclusively bacterial. Monounsaturated and polyunsaturated fatty acids are found in Antarctic marine bacteria at a wide range of concentrations (from ~3% to as high as 96%) (Nichols et al., 1993). Branched-chain fatty acids biosynthesis have also been observed as an adaptive response in culture-dependent analysis of a psychrophillic Antarctic bacterium (Nichols and Russell, 1996) as well as psychrotolerant bacteria from an Antarctic lake sediment (Fukunaga and Russell, 1990). However, at extremely low temperatures (-15°C), the permafrost bacterium *Planococcus halocryophilus* Or1 was observed to favor an increase in straight-chain FA compared to branched FA (Denich et al., 2003), despite the trends of increasing branched FA in other bacteria at cold temperatures (Mykytczuk et al., 2013). While imparting the unambiguous origin of these fatty acids detected in the LVBr sample is impossible due to the complex nature of the environmental sample, comparing the overall abundance and distribution of FA lipids in LVBr POM lipidome to the metagenome can shed light on the ability for the microbial community to biosynthesize these fatty acids *in situ*.

Low levels of sterols were also observed in the LVBr POM lipidome. The detection of sterol was unexpected, especially in an anoxic environment in which 18S rRNA genes associated with eukaryotes have not been detected (Murray et al., 2012). However, the observation of large detritus cell-like structures, along with evidence obtained from their elemental analysis (i.e., abundant phosphorous and calcium) points to the persistence of eukaryotic (potentially algae) cellular debris in LVBr likely deriving from previous environmental conditions. These detrital cells, though structurally degraded (Figure 5), can contribute to the lipidome of LVBr POM. Furthermore, the overall basic structures and the size of these eukaryotic debris is evocative of some uniquely shaped diatoms or dinoflagellates (i.e. *Cocconeis sp.*, *Rhabdonema sp., Ostreopsis sp.*) though they are relatively smaller in size than the degraded cells detected in LVBr (Appendix IV).

The observation of detritus eukaryotic cell-like material and sterol lipids points to the recurring problem in the analysis of modern biological materials that are being produced and processed by the modern Lake Vida brine ecosystem: legacy. The presence of legacy was clearly established in the analysis of the dissolved fraction of the organic matter present in LVBr, including small molecules (<500 Da; Chou et al., 2018) and larger, more complex organic molecules (between 500 and 1,000 Da; Cawley et al., 2016). This legacy signal complicates our ability to observe the metabolites that belong to the current microbial community. Based on analysis by electron microscopy of the particulate organic matter obtained from LVBr, showing the presence of putative degraded eukaryotic detritus, as well as the steroid lipids that were

detected in the lipidome of LVBr POM, we also suggest that the POM collected from LVBr, which should contain a majority of intact cells likely representative of the current ecosystem, may also, in part, be comprised of legacy material.

To determine if LVBr POM also contains legacy, we examine the genomic potential of the LVBr microbial assemblage to determine if the legacy lipids could hypothetically be biosynthesized by the extant microbial community. This necessitates a thorough examination of the biosynthetic pathways that would produce the lipids in question, and knowledge of the known proteins and enzymes that are responsible for catalyzing those biosynthetic reactions. As such, we generated a list of protein and gene sequences that can be queried against the LVBr combined metagenome to determine (1) if the coding genes necessary to catalyze a specific reaction in a lipid biosynthesis pathway are present in the metagenome, (2) if the genomic potential for the pathway is complete and thus the LVBr organisms theoretically could produce the lipids detected in the lipidome, and (3) if the relative abundances of those genes play a role in the completed biosynthetic pathway required to make the lipid biomolecules.

4.4.2 The genetic potential of the Lake Vida brine microbial assemblage

4.4.2.1. Genetic potential for fatty acid biosynthesis

De novo synthesis of saturated fatty acids (FA) in bacteria involves the Type II FA synthase system that consists of more than ten individual enzymes centralized on the acyl carrier protein (ACP) that bind to all fatty acyl intermediates (Prescott and Vagelos, 1972; Chan and Vogel, 2010) (Figure 7). The FA biosynthetic pathway is divided into two different steps, the initiation step, and the elongation, which is the cycle that continuously catalyze the condensation of 2-carbon subunits. Briefly, ACP synthase (ACPS) first activates ACP by attaching a Coenzyme A (CoA) group to the ACP to form a reactive center that is used to catalyze all other fatty acid reactions in a covalent, high-energy thioester bond (Jackowski and Rock, 1987; Zhang et al., 2001). Then, FA biosynthesis begins when an acetyl-CoA is converted to a malonyl-CoA with the acetyl-CoA carboxylase enzyme (ACC; EC 6.4.1.2), which consists of four proteins encoded by the *accABCD* gene cassette (EC 6.4.1.2) (Cronan and Waldrop, 2002). The malonyl-CoA is transferred to the ACP via a protein called malonyl-CoA-ACP transacylase (FabD, EC 2.3.1.39) (Serre et al., 1995). The first condensation reaction with an acetyl-CoA building block then takes place, which is catalyzed by the β-ketoxoacyl synthase (FabH, EC 2.3.1.180), forming β-ketoxobutyryl-ACP (Tsay et al., 1992). This compound, which is a four carbon (C_4) unit is the first to be generated in the fatty acid synthesis cycle,

where the same reactions are catalyzed in a cyclic manner, extending the saturated fatty acid acyl chain by two units of carbon in each cycle. At the start of the first cycle, the β-ketoxobutyryl-ACP is reduced to β-hydroxylbutyryl-ACP by the β-oxoacyl reductase (FabG). The hydroxyl group is removed to form *trans*-2-enoyl groups via either enzyme β-hydroxylacyl dehydratases, FabA or FabZ (both EC 4.2.1.59) (Rock and Jackowski, 2002) and the double bond is removed by an enoyl-reductase (FabI). During each of the cycle, the condensation reaction, however, is catalyzed by either β-oxyacyl synthase I or II (FabB or FabF), in which the acyl chain is detached from ACP and then condensed again by these two proteins, providing the addition of the two carbon group from "inside-out", rather than carbon groups being added to the tail end of the side chain (Jenni et al., 2006).

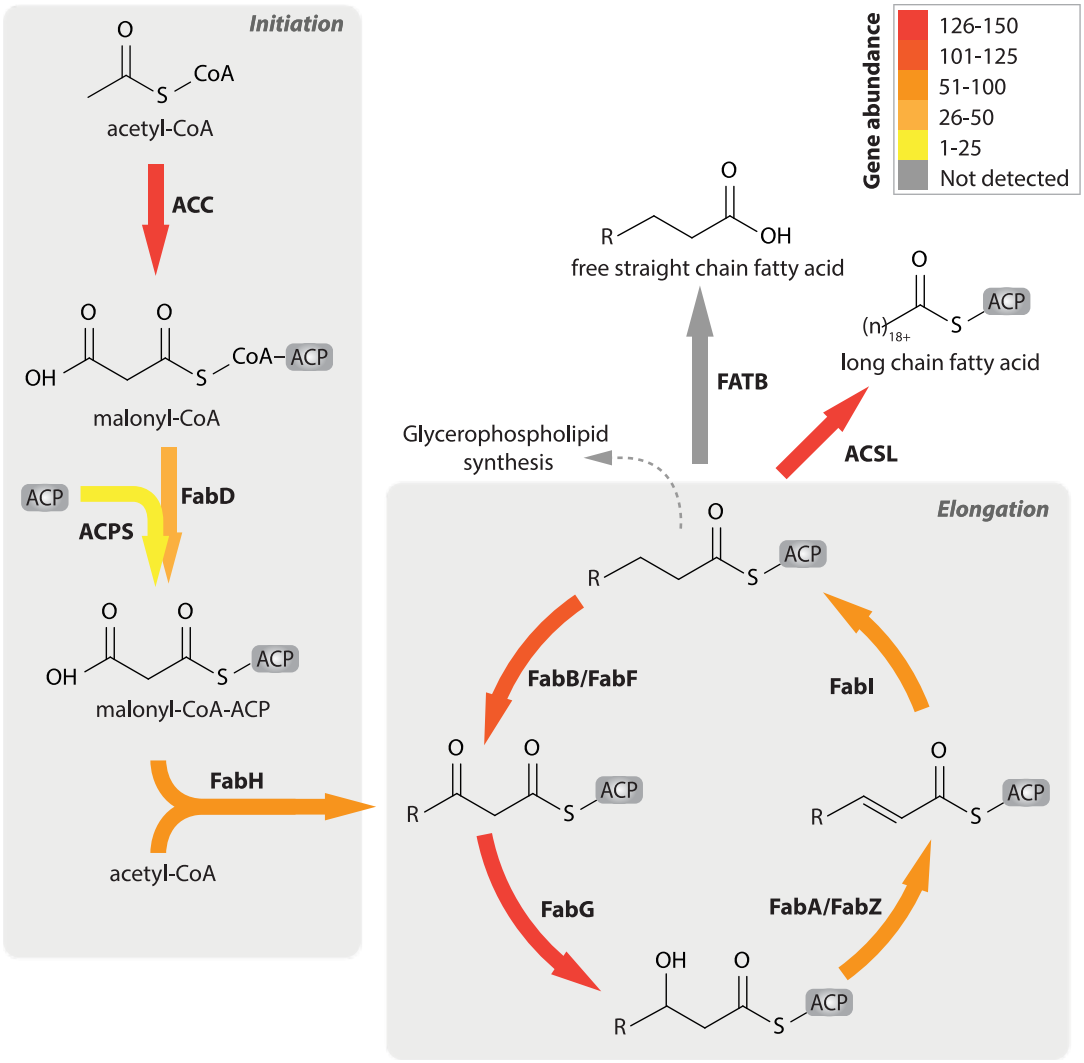

Figure 7: Type II Fatty Acid synthesis system showing the starting primers, final products, and intermediate molecules. Arrows represent the enzymes that catalyze each reaction step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

Finally, when the acyl chain reaches C_{16} or C_{18}, the long chain acyl-CoA-ACP can be used to (1) initiate glycerophospholipid metabolism, (2) form a free fatty acid by the hydrolysis of thioester catalyzed by the fatty acyl-ACP thioesterase B (FATB, EC 3.1.2.14) (Salas and Ohlrogge, 2002) or (3) further elongate the side chain through subsequence reactions in the Type II fatty acid system cycling via the long-chain acyl-CoA synthetase (ACSL, EC 6.2.1.3) (Black et al., 1992). All of the genes that encode for the enzymes involved in the biosynthesis of straight chain FA were detected in the LVBr metagenome (Figure 7). Though FATB was not detected, this enzyme is not crucial for the initiation and elongation cycle and leads to the synthesis of free acids only.

The production of monounsaturated fatty acids is similar to that described for saturated fatty acid, with a slightly different step using the β-hydroxylacyl dehydratase (FabA) (Cronan et al., 1975) (Figure 8a). FabA specifically catalyzes the dehydration reaction of the C_{10} substrate, the trans-2-decenoyl-ACP while simultaneously isomerizing it to a *cis*-2-decenoyl-ACP (Heath and Rock, 1996). The β-oxyacyl synthase I (FabB) then catalyze the condensation reaction to cis-2-dodecenoyl-ACP. This enzyme, FabB, essentially competes with the enoyl-reductase (FabI), ultimately determining the saturated to unsaturated fatty acids ratio in the cell membrane (Heath and Rock, 1996), whereas FabA is required for the introduction of the double bond during the 10-carbon stage of fatty acid elongation. Both FabA and FabB were detected in the LVBr metagenome.

Monounsaturated and polyunsaturated FAs can also be directly formed from saturated straight-chain FAs bound to either ACP, CoA, or to phospholipid headgroups via the enzymatic reaction of FA desaturases (Los and Murata, 1998) (Figure 8b). An example of a FA desaturase that catalyze CoA-bound FA is acyl-CoA Δ^9-desaturase (SCD, EC 1.14.19.1). Other FA desaturases can catalyze the introduction of subsequent unsaturation in addition to the first unsaturation to form polyunsaturated FA (PUFAs). An example is the ability for a Δ^6-desaturase (FADS2, EC 1.14.19.3) to transform a C18:2 fatty acid to C18:3 fatty acid (Reddy et al., 1993). Lastly, a phospholipid-bound monounsaturated FA can be desaturated by acyl-lipid ω-6 12-desaturase (FAD6, EC 1.14.19.23) though this ability is more often found in cyanobacteria and higher plants (Falcone et al., 2002). Though SCD and FAD6 were observed in the LVBr metagenome, FADS2 was not.

The branched-FA synthase system is also very similar to that of straight-chain FA (Kaneda, 1991). In this enzyme system, a branched acyl-CoA ester acts as a primer instead with malonyl-CoA as the extender, which can result in long chain branched-FA with an *iso*- or *anteiso*-substituted hydrocarbon chain (Figure 8). A homolog of β-ketoxoacyl synthase, FabH, is implicated in the utilization of *iso*- and *anteiso*-substituted acyl-CoA primers as the starting substrates for branched-FA biosynthesis (Choi et al., 2000). This protein can initiate FA synthesis from isovaleryl-CoA, isobutyryl-CoA, or 2-methylbutyryl-CoA to produce branched-FA (Choi et al., 2000). These α-keto acids are generally derived from the amino acids valine, leucine, and isoleucine (Wang et al., 1993; Klein et al., 1999). In support of the

branched-substituted FA in the LVBr POM lipidome, FabH was detected, suggesting that the LVBr microbial community has the ability to synthesize these FA acids.

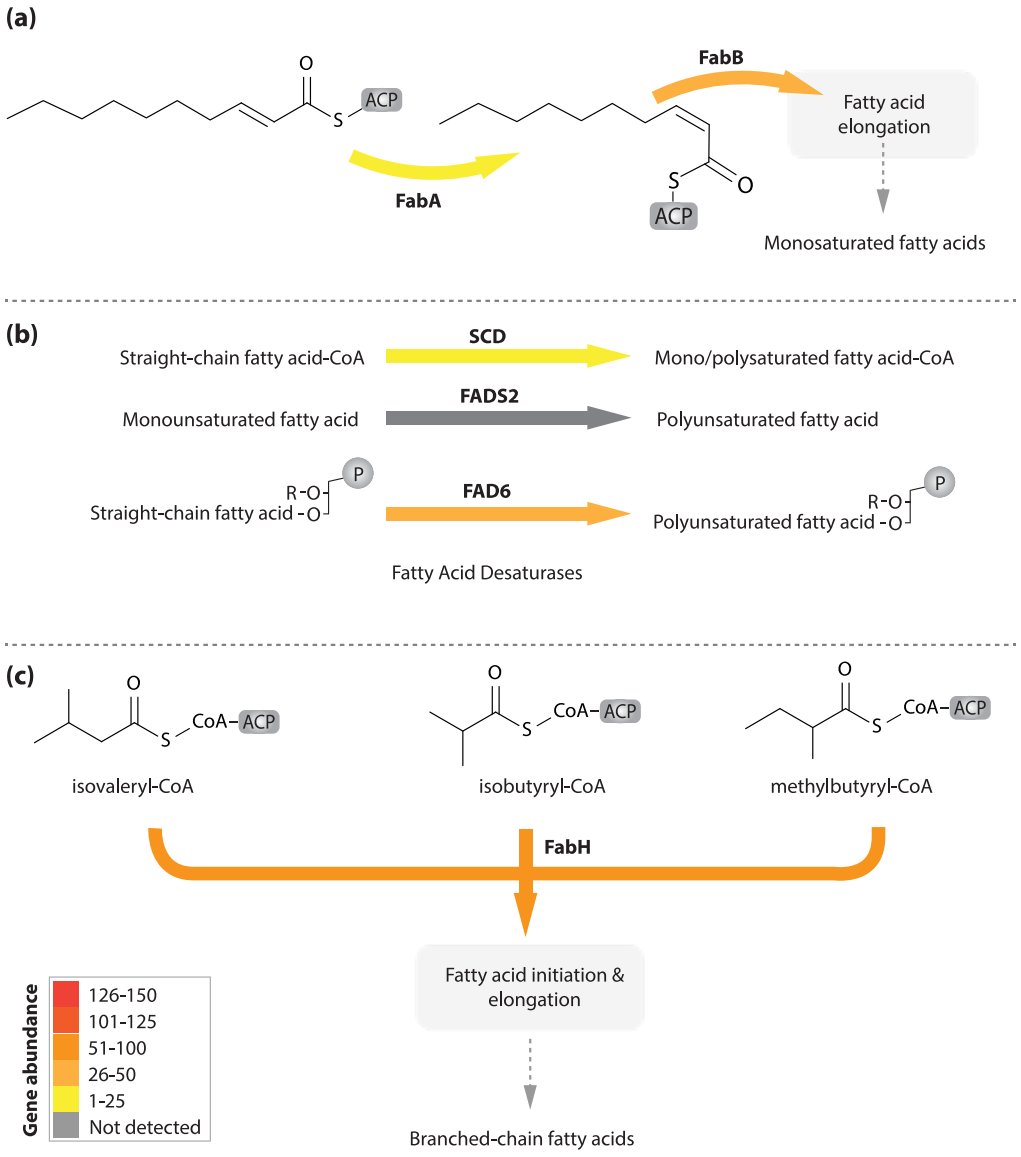

Figure 8: (a) Pathway for the *de novo* synthesis of monounsaturated fatty acids prior to continuing fatty acid elongation. **(b)** Enzymes involved in the desaturation of fatty acids-CoA, fatty acid-bound phospholipids, and free fatty acids. **(c)** Pathway for different primer utilization in the *de novo* biosynthesis of branched-chain fatty acids. Arrows represent the enzymes that catalyze each step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

4.4.2.2. Genetic potential for triterpenoid backbone biosynthesis

Tetracyclic and pentacyclic triterpenoids, such as steroids and hopanoids, respectively, are derived from the same universal building block, the C_5 isoprenoid subunit, isopentenyl diphosphate (IPP) (Ourisson et al., 1987). The synthesis of IPP can take place either via the mevalonate (MVA) pathway, or the 2-*C*-methyl-*D*-erythritol 4-phosphate/1-deoxy-*D*-xylulose 5-phosphate (MEP/DOXP) pathway (Figure 9). The MVA pathway is typically found in eukaryotes, archaea, and a few bacteria, whereas the MEP pathway is generally found in most bacteria (Lange et al., 2000). Eukaryotes that are capable of synthesizing IPP via the MEP pathway likely inherited their genes from bacteria, especially those that bear plastids, which were thought to have been acquired via endosymbiosis with cyanobacteria (Lange et al., 2000). In this section, we explore the biosynthetic potential for the formation of isoprenoid backbone of steroid and hopanoid biosynthesis.

The pathway for forming IPP units via the intermediate, mevalonate can be followed in Figure 9. The pathway begins with two C_2 units of acetyl-CoA that are combined to form acetoacetyl-CoA via the enzyme acetoacetyl-CoA thiolase (AtoB; EC 2.3.1.19) (Gehring and Lynen, 1972). The formation of *β*-Hydroxy *β*-methylglutaryl-CoA (HMG)-CoA takes place via the condensation of acetyl-CoA and acetoacetyl-CoA, which is catalyzed by an HMG-CoA synthase (HMGCS, EC 2.3.3.10) (Ferguson and Rudney, 1959). Then, a two steps reduction performed by HMG-CoA reductase (HMGCR; EC 1.1.1.34) generates mevalonate (Wilding et

al., 2000), which further is transformed to mevalonate-5-phosphate via the enzymatic activity of meavalonate kinase (MVK; EC 2.7.1.36) (Tchen, 1958). Another phosphate group is added to generate mevalonate-5-diphosphate via a phosphomevalonate kinase (Doun et al., 2005) (PMK; EC 2.7.4.2). Finally, a mevalonate diphosphate decarboxylase (MVD; EC 4.1.1.33) catalyzes the decarboxylation of the mevalonate-5-diphosphate to form isopentenyl 5-diphosphate (IPP). Further, IPP is can be interconverted to DMPP, another important precursor to triterpenoid biosynthesis, with the isopentenyl diphosphate isomerase enzyme (IDI, EC:5.3.3.2) (Muehlbacher and Poulter, 1985; Kuzuyama and Seto, 2003).

Isoprenoids can also be biosynthesized by an entirely different pathway, the 2-*C*-methyl-*D*-erythritol 4-phosphate/1-deoxy-*D*-xylulose 5-phosphate pathway (MEP/DOXP), or simply, mevalonate-independent pathway (Lütke-Brinkhaus and Kleinig, 1987; Rohmer, 2002; Eisenreich et al., 2004) (Figure 9). This pathway begins with the condensation of pyruvate and *D*-glyceraldehyde 3-phosphate to form DOXP via the DOXP synthase (DXS; EC 2.2.1.7) (Lois et al., 1998). This compound is then converted to MEP by the MEP synthase (ispC; EC 1.1.1.267), which then is transformed to a 4-diphosphocytidyl-2C-methyl-*D*-erythritol via a 4-diphosphocytidyl-2*C*-methyl-*D*-erythritol synthase (ispD, EC 2.7.7.60) (Rohdich et al., 1999). The phosphorylation of this compound then yield 4-diphosphocytidyl-2*C*-methyl-*D*-erythritol 2-phosphate via a kinase of the same name (IspE; EC 2.7.1.1.48) (Lüttgen et al., 2000). Then, the product of this reaction is converted to 2*C*-methyl-*D*-erythritol 2,4-cyclodiphosphate by the 2*C*-Methyl-*D*-erythritol 2,4-diphosphate synthase (IspF, E.C.

4.6.1.12) (Takagi et al., 2000). Furthermore, this intermediate is reduced to 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate by a 2*C*-Methyl-*D*-erythritol 2,4-cyclodiphosphate reductase (IspG, EC 1.17.7.1) (Seemann et al., 2002). Finally, a 1-hydroxy-2methyl-butenyl 4-diphosphate reductase (IspH; EC 1.17.12) catalyzes the conversion of 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate to IPP, the isoprenoid building block subunit (Rohdich et al., 2002).

All of the genes required for the biosynthesis of IPP were present in the LVBr metagenome (Figure 9). However, the lack of important enzymes in the catalysis of important intermediates, HMG-CoA and mevalonate, suggest that it is likely that IPP synthesis by the LVBr microbial assemblage takes place via the mevalonate-independent path. This agrees with the observation that the MEP pathway is more common in bacteria. However, IPP synthesis via the MEP pathway can also occur in plastid-bearing eukaryotes (i.e., algae, and higher plants).

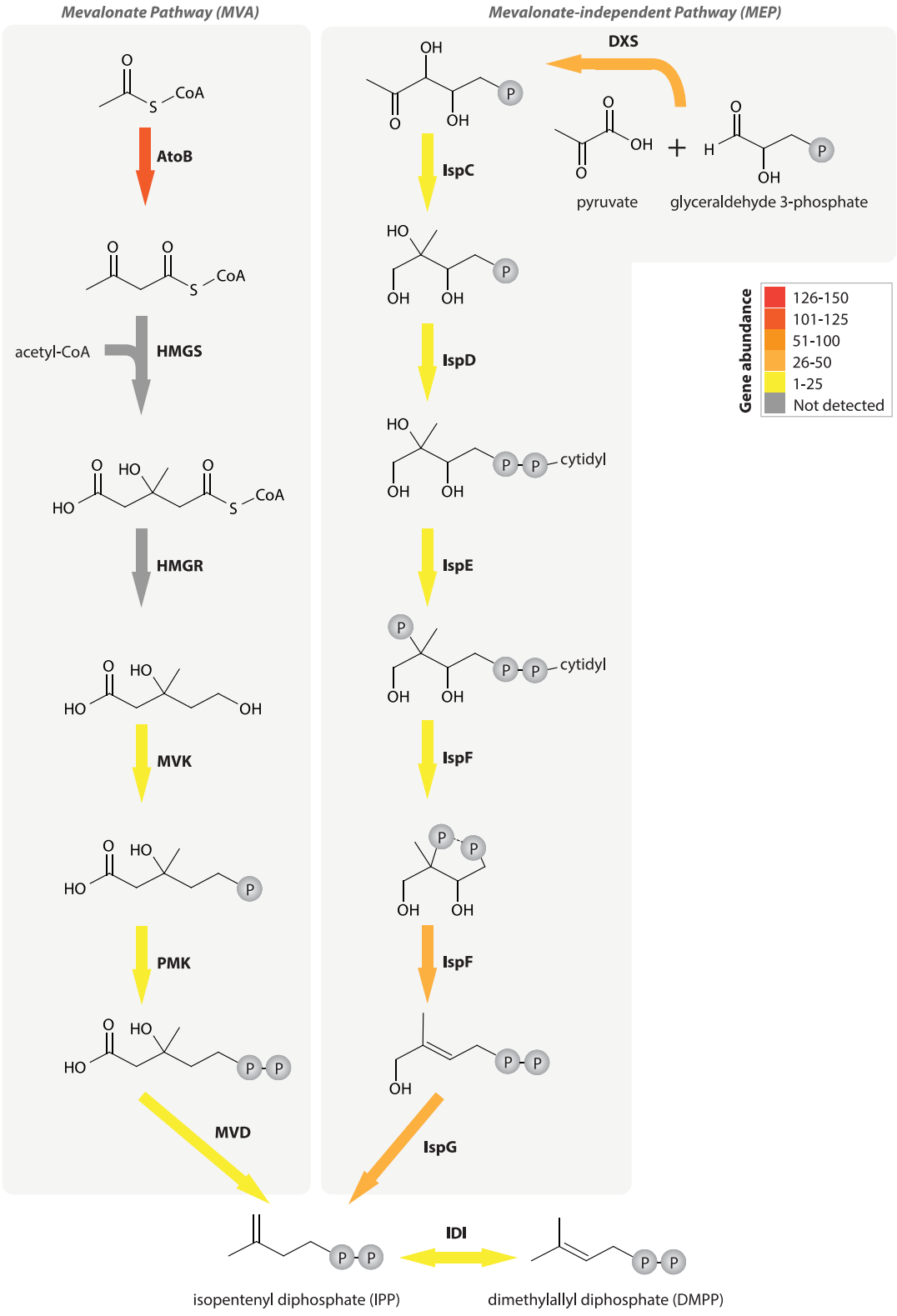

Figure 9: Mevalonate and mevalonate-intendent pathway for synthesis of isopentenyl diphosphate, the isoprenoid subunits for C_{30} tri terpenoid biosynthesis. Arrows represent the enzymes that catalyze each step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

Both the MVA and the MEP biosynthetic pathways lead to IPP, which is the substrate necessary to form the triterpenoid, squalene (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosahexane), the precursor for both steroids and hopanoids (Figure 10). The enzyme involved in the biosynthesis of squalene include farnesyl diphosphate synthase (IspA, EC:2.5.1.1), which catalyze the condensation of dimethylallylpyrophosphate (DMPP) with 2 units of IPP to form farnesyl pyrophosphate, a C_{15} intermediate (Figure 10). Farnesyl pyrophosphate then condenses with another farnesyl pyrophosphate to form pre-squalene diphosphate and then squalene via the activity of a farnesyl-diphosphate farnesyltransferase (FDFT1; EC:2.5.1.21) (Jennings et al., 2006). Squalene represents the compound that leads to diverging path between steroid and hopanoid synthesis.

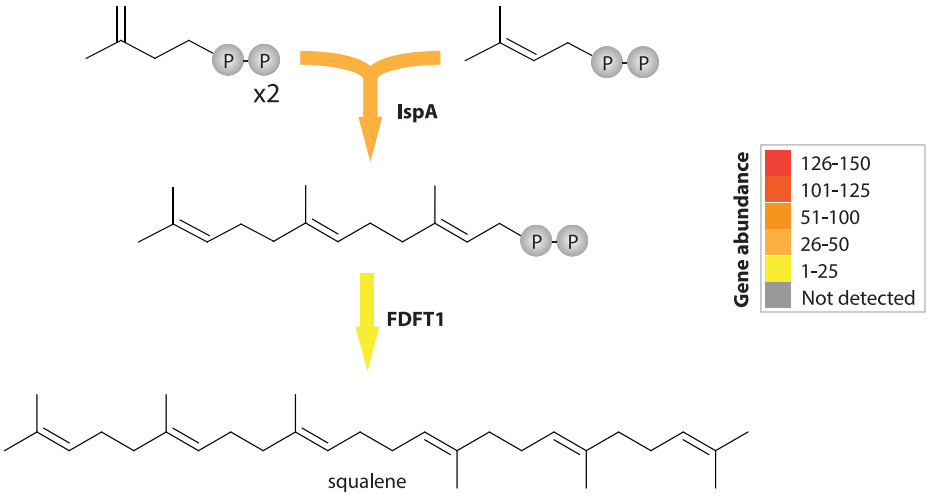

Figure 10: Squalene biosynthesis pathway. Arrows represent the enzymes that catalyze each step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

4.4.2.3. Genetic potential for steroid synthesis

Sterols are tetracyclic terpenoids that function as membrane stabilizers and are present in most eukaryotes (Demel and Kruyff, 1976; Bloch, 1992). Sterols are biosynthesized via three pathways that involve (1) formation and C_5 carbon skeleton of isoprene units from acetate, (2) the condensation and multistep additions of isoprene units to form C_{30} squalene, as described above, and (3) the epoxidation to and cyclization as well as rearrangements to form cholesterol (Bloch, 1992; Wright, 2003).

The biosynthesis of steroids require molecular oxygen (Bloch, 1983). There are generally two different pathways for the biosynthesis of sterols. The first pathway is more common to animals and fungi, whereas the second pathway is found photosynthetic eukaryotes such as algae and higher plants (Figure 11). The conversion of squalene to squalene-2,3-epoxide is catalyzed by the enzyme squalene monooxygenase (SQLE, or ERG1, EC:1.14.14.17), which is the first step in sterol synthesis that requires molecular oxygen (Jahnke and Klein, 1983). Squalene-2,3-epoxide is then converted to lanosterol with the enzyme lanosterol synthase (LSS, EC 5.4.99.7) (Corey et al., 1994). In animals and fungi, lanosterol is transformed to cholesterol via a complex pathway involving several enzymes: sterol 14α-demethylase (CYP51, EC:1.14.14.154), Δ^{14}-sterol reductase (TM7SF2, EC:1.3.1.70), sterol-4α-carboxylate 3-dehydrogenase (ERG26, E1.1.1.170), cholestenol Δ-isomerase (EBP, EC:5.3.3.5), Δ^{7}-sterol 5-desaturase (ERG3, EC:1.14.19.20), Δ^{24}-reductase (DHCR24, EC:1.3.1.72) and 7-

dehydrocholesterol reductase (DHCR7, EC:1.3.1.21) (Bloch, 1983; see review by Cerqueira et al., 2016) (Figure 11). There are three total demethylation steps: one methyl group is removed by CYP51 at the C14 position, and two methyl groups are removed by ERG26 at the C4 position, leaving a C_{27} carbon skeleton, cholest-5-en-3β-ol (cholesterol; **I**, Appendix IV). The genes for ERG26 was detected in low abundance in the LVBr metagenome (Table 4).

In photosynthetic organisms, biosynthesis of sterols with methyl- or ethyl-substituents begins with the conversion of squalene-2,3-epoxide to cycloartenol via cycloartenol synthase (CAS1; EC 5.4.99.8) (Goodwin, 1979) (Figure 11). A methylation step then takes place on the C24 position, generating 24-methylene cycloartenol via cycloartenol 24-C-methyltransferase (SMT1; EC 2.1.1.41). A C4 demethylation step is catalyzed by a 4-methylsterol oxidase 1 (SMO1), followed by the isomerization of the 4-desmethyl-24-methylene cycloartenol via cycloeucalenol cycloisomerase (CPI1, EC:5.5.1.9) to form a Δ^8-unsaturated intermediate, obtusifoliol (Heintz and Benveniste, 1974). From here, another demethylation step at C14 via the sterol 14α-demethylase (CYP51) can take place to form a $\Delta^{8,14,24}$-sterol. The unsaturation at C14 can be removed via the enzymatic reaction of Δ^{14} sterol reductase (TM7SF2), and a cholestenol Δ-isomerase (EBP) catalyzes the isomerization of Δ^8- to a Δ^7-sterol intermediate, forming a 24-methylene lophenol. These three enzymes, CYP51, TM7SF2, and EBP are shared with the pathway involving conversion from lanosterol to sterol (Nes, 2011) as described above. A second demethylation at the C4 position takes place via 4-methylsterol oxidase 2 (SMO2) to form episterol (Pascal et al., 1993). The conversionof Δ^7- to Δ^5-sterol follows the steps of the

conversion of lanosterol to cholesterol pathway via the enzymes ERG3, DHCR24, and DHCR7, ultimately forming the sterol 24-methyl-cholest-5-en-3β-ol (campesterol; **IIb**) (Piironen et al., 2000). Only one enzyme, SMO2, was observed to be present in the LVBr metagenome.

For 24-ethyl sterols, a second methylation step can branch off after the formation of 24-methylene lophenol. This reaction is catalyzed by the enzyme 24-methylene lophenol-C-24-methyl transferase (SMT2) (Piironen et al., 2000). These two different C24 methylation steps via SMT1 and SMT2 involve one or two molecules of SAM, respectively, and can then lead to subsequent sterol compounds with respective substituents at the C24 position. The same enzymes previously described, SMO2, ERG3, DHCR24, DHCR7, and DHCR7, then act to demethylate at C4 position, desaturate at Δ^5, reduce the double bond at C24, and C7, respectively to form the final sterol, 24-ethyl-cholest-5-en-3β-ol (sitosterol, **IIIb**, Appendix IV). In plants and algae, the desaturation of C22 position is catalyzed by the sterol 22-desaturase (CYP710A; EC:1.14.19.41) to form $\Delta^{5,22}$ sterols, 24-methyl-cholest-5,22-dien-3β-ol (brassicasterol**, IIa**) and 24-ethyl-cholest-5,22-dien-3β-ol (stigmasterol, **IIIa**) (Morikawa et al., 2006; Mthakathi et al., 2015).

It is important to note that only 2 of the 14 coding sequences involved in the entire steroid biosynthesis pathway are present in the LVBr metagenome (Table 6). The lack of genes necessary to generate the enzymes that can catalyze the biosynthesis of steroids suggest that the LVBr microbial assemblage does not have the capacity to produce steroids. This is further supported by the absence of eukaryotes in the 18S rRNA gene survey of the LVBr microbial

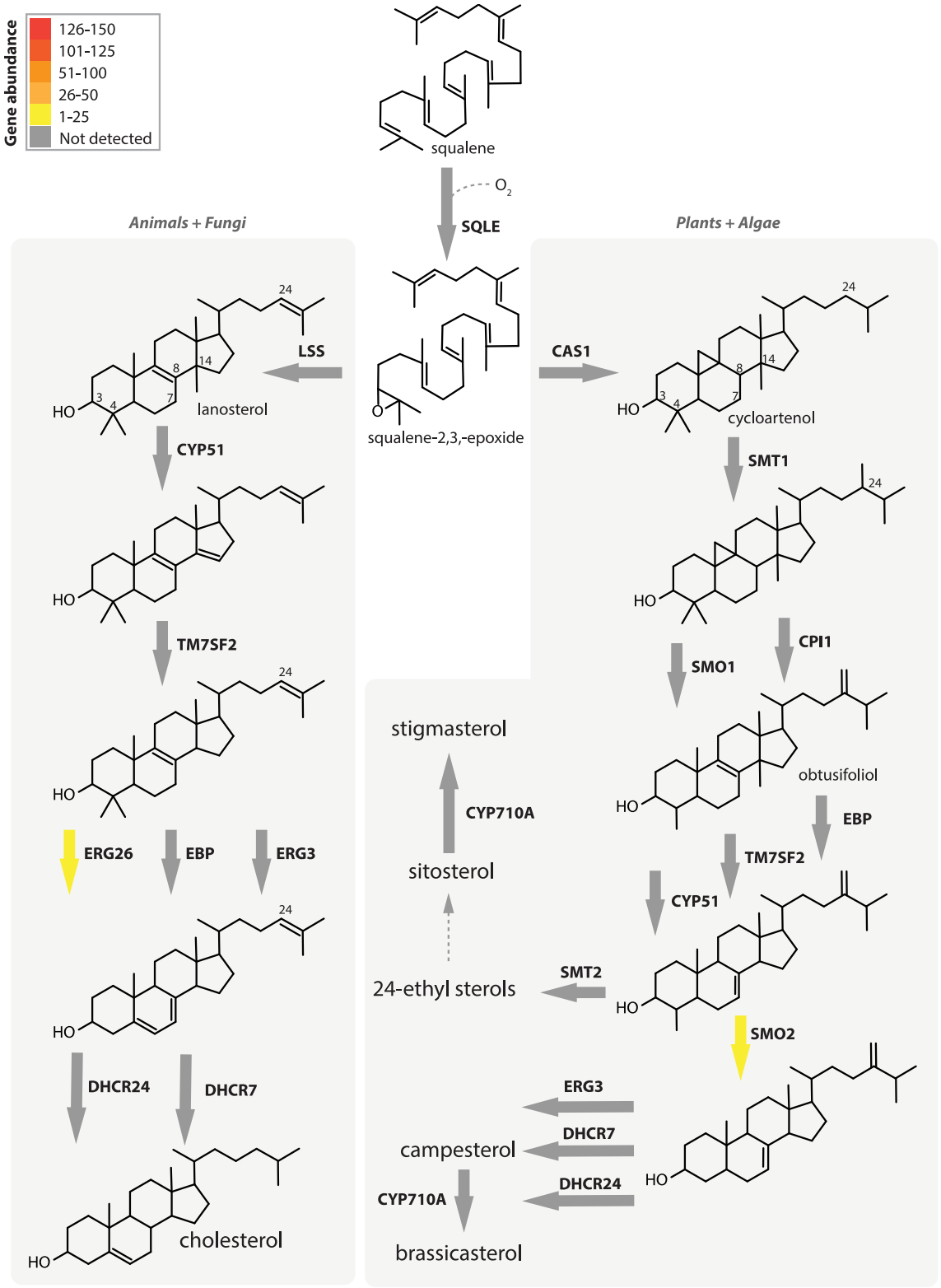

Figure 11: Steroid biosynthesis pathway for non-photosynthetic eukaryotes (animals and fungi) and for photosynthetic eukaryotes (plants and algae). Arrows represent the enzymes that catalyze each step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

community (Murray et al., 2012). Based on our results, we conclude that the steroids detected in the LVBr POM lipidome are not derived from the modern LVBr microbial community, but rather a legacy of eukaryotic community that existed in the Lake Vida basin in the past.

4.4.2.4. Genetic potential for hopanoid synthesis

Hopanoids are pentacyclic triterpenoids found mostly in bacteria, and serve a similar function as a membrane stabilizer as sterols in eukaryotes (Ourisson et al., 1987; Sahm et al., 2008). In contrast to the biosynthesis of steroids, hopanoids can be biosynthesized in the absence of molecular oxygen. The cyclization of squalene in hopanoid-synthesizing organisms can proceed via the enzyme squalene-hopene cyclase (Shc, EC 5.4.99.17), which has been found to be related to the oxidosqualene cyclases, such as squalene monooxygenase (SQLE) implicated in steroid biosynthesis (Rohmer et al., 1980; Ourisson and Nakatani, 1994; Poralla, 1994) (Figure 12). This enzyme is used to synthesize diplopterol (**IVa**, Appendix IV) or diploptene (Sato et al., 2004) (Figure 12). In bacteria, Shc is also capable of producing tetrahymanol (**V**) with the additional step catalyzed by a tetrahymanol synthase enzyme (Ths), although this function is restricted in the purple nonsulfur bacterium, *Rhodopseudomonas palustris, Methylomicrobium sp.*, nitrogen-fixing symbiont *Bradyrhizobium sp*. (Bravo et al., 2001; Welander et al., 2009; Banta et al., 2015). However, the cyclization of squalene directly to tetrahymanol in eukaryotes, especially the well-studied freshwater ciliate *Tetrahymena sp.*,

requires a different enzyme, the squalene-tetrahymanol cyclase (Stc; EC 4.2.1.123) (Saar et al., 1991).

The *shc* gene, which encodes the enzyme Shc, is part of a larger conserved *hpn* operon that contains genetic instruction for the triterpenoid synthesis and hopanoid structural modification (Perzl et al., 1998). One such modification is the addition of a C_5 ribose moiety, which would form C_{35} hopanoids (Rohmer et al., 1989). Another modification involves the methylation of either the C2 position via the hopanoid 2-methyltransferase (HpnP) (Welander et al., 2010), and the C3 position via the hopanoid 3-methyltransferase (HpnR).

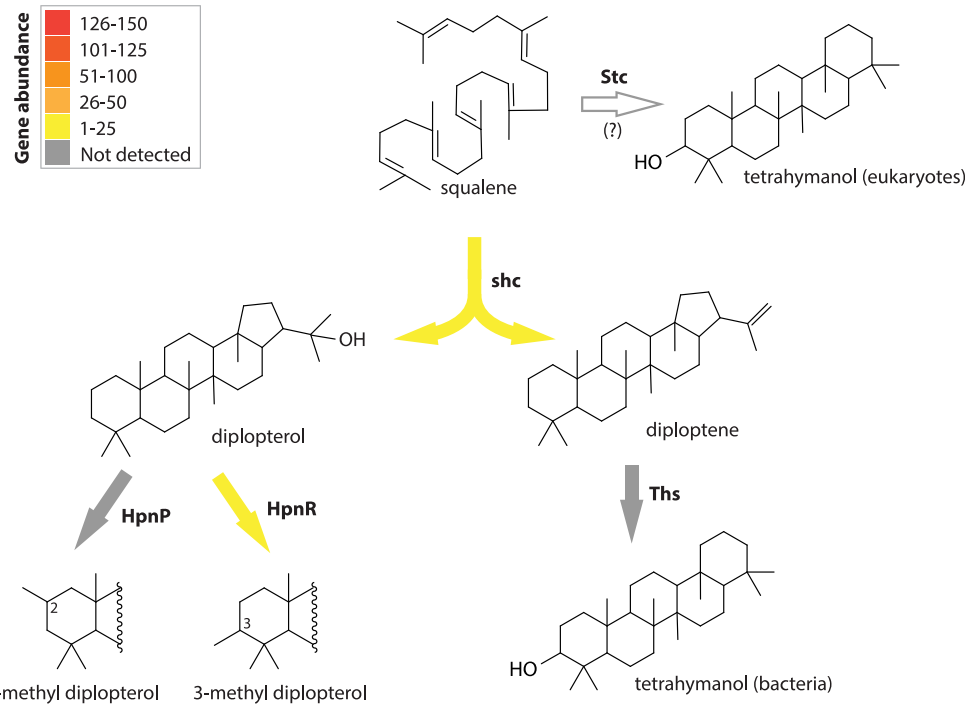

Figure 12: Hopanoid biosynthesis pathway. Arrows represent the enzymes that catalyze each step in the pathway and are colored based on gene abundance observed in the LVBr metagenome.

The gene encoding the enzyme for the cyclization of squalene in bacteria (shc) was present in LVBr metagenome. However, the pathway for tetrahymanol synthesis (in bacteria) was not detected. We were unable to test for the presence of Stc (squalene-tetrahymanol cyclase in eukaryotes) as the gene associated with this function has not yet been sequenced. We also detected the gene that catalyzes the addition of a methyl group in the C3 position. However, the LVBr POM lipidome contains 2-methyl diplopterol, for which the gene HpnP, implicated in its hopanoid modification, was not detected (Figure 12). This possibly suggests that 2-methyl diplopterol may potentially be legacy. 2-methyl hopanoids are biomarkers of cyanobacteria (Summons et al., 1999). Due to the presence of large structure of eukaryotic detritus that potentially represent remnant of dead algae, it is also possible that the LVBr POM retained legacy lipids from cyanobacteria that were present in the past Lake Vida conditions.

4.4.2.5. Implications for metagenomic evidence for legacy lipids

The Lake Vida brine metagenome contained all the genes that are necessary to carry out *de novo* fatty acid biosynthesis, but do not contain all the genes necessary to biosynthesize sterols. This unambiguously supports our hypothesis that the sterols detected in the Lake Vida brine lipidome are legacy. The detection of large (>100 μm) detrital eukaryotic cells, most likely algae or diatoms, suggest that their degraded cell components can contribute legacy lipids to the total lipidome pool of the POM. Consequently, the presence of legacy in both the DOM

and POM in Lake Vida brine necessitates a more cautious interpretation of modern organic biosignatures in environments where organic matter is degraded extremely slowly.

A particularly important lesson can be drawn from this study: though legacy is likely prevalent in slow-growing, cold-limited ecosystems, assignments of certain organic biosignatures to be extant or ancient can be supported by metagenomic evidence. An important context, provided by the genetic potential derived from the metagenome, is crucial in determining if a modern community is capable of producing such organic biosignatures.

The results of this work also prompt further investigations of the preservation potential of organic biosignatures in other cryogenic or metabolically-limited ecosystems. In the conditions of LVBr, legacy lipids persisted from past environmental conditions that may have potentially supported photosynthetic eukaryotes. Despite the preservation of legacy lipids, the DNA from these past environmental conditions may have been partially or fully degraded. The assertion that some biomolecules such as lipids are more recalcitrant than DNA or RNA is well recognized in studies of sedimentary hydrocarbons and ancient biomarkers (Brocks and Summons, 2004). However, the preservation of fragile biomolecules in cold environments likely increases with decreasing temperature, with frozen conditions to be the endmember of such preservation, where most or all biomolecules are preserved. This is due to the physiological constraints that cold temperatures impose on growth rates and metabolic activities, consequently limiting the processing and turnover of metabolites in a given biological system. Though the conditions of LVBr were conducive to the degradation of genetic

materials, other colder or more metabolically-limited systems, especially those that could exist on other extraterrestrial icy bodies, may be limited such that legacy DNA is also preserved. Studying the genetic potential of such environments, thus, warrants a contextual understanding of the *in situ* conditions as well as metabolic state of the extant community.

4.5. Conclusions

Lake Vida brine is a unique system that harbors an active microbial community capable of metabolizing under extremely cold and hypersaline conditions. Yet, the biological activity of this community proceeds very slowly, allowing for the accumulation of legacy biosignatures, both in the dissolved fraction and in the particulate fraction of the organic matter. The detection of legacy lipids in the Lake Vida brine POM suggest that large intact structures of organisms of the previously occupying, possibly photosynthetic, community may persist over long timescales. Evidence from the targeted metagenomic analysis further confirms that the modern Lake Vida microbial assemblage is unable to biosynthesize the legacy lipids. Based on the results of this study, we show that combined lipidomic and metagenomic analysis can be a powerful tool in elucidating the biogeochemistry of a cold-limited ecosystem. This approach allowed us to determine the provenance of lipids detected in the lipidome of the POM of Lake Vida brine, which, without the data obtained from the metagenome, may have been misinterpreted as belonging to the modern microbial community.

Acknowledgements

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Work Cited

Banta, A.B., Wei, J.H., Welander, P. V., 2015. A distinct pathway for tetrahymanol synthesis in bacteria. Proceedings of the National Academy of Sciences 112, 13478–13483.

Black, P.N., Dirusso, C.C., Metzger, A.K., Heimert, T.L., 1992. Cloning, Sequencing, and Expression of the fadD gene of Escherichia coli Encoding Coenzyme A Synthetase. The Journal of Biological Chemistry 267, 25513–25520.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Bloch, K., 1992. Sterol molecule : structure , biosynthesis , and function, in: Steroids. pp. 378–383.

Bloch, K.E., 1964. Nobel Lecture: The Biological Synthesis of Cholesterol".

Bloch, K.E., 1983. Sterol Structure and Membrane Function. Critical Reviews in Biochemistry and Molecular Biology 14, 47–92.

Bravo, J.M., Perzl, M., Härtner, T., Kannenberg, E.L., Rohmer, M., 2001. Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium Bradyrhizobium japonicum USDA 110. European Journal of Biochemistry 268, 1323–1331.

Brocks, J.J., Summons, R.E., 2004. Sedimentary Hydrocarbons, Biomarkers for Early Life, in: Treatise on Geochemistry. Elsevier Ltd, pp. 1–13.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC bioinformatics 10, 421.

Cerqueira, N.M.F.S.A., Oliveira, E.F., Gesto, D.S., Santos-Martins, D., Moreira, C., Moorthy, H.N., Ramos, M.J., Fernandes, P.A., 2016. Cholesterol Biosynthesis: A Mechanistic Overview. Biochemistry 55, 5483–5506.

Chan, D.I., Vogel, H.J., 2010. Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochemical Journal 430, 1–19.

Chintalapati, S., Kiran, M.D., Shivaji, S., 2004. Role of membrane lipid fatty acids in cold adaptation. Cellular and Molecular Biology 50, 631–642.

Choi, K.-H.H.K., Heath, R.J., Rock, C.O., 2000. β -Ketoacyl-Acyl Carrier Protein Synthase III (FabH) Is a Determining Factor in Branched-Chain Fatty Acid Biosynthesis ɴᴇKetoacyl-Acyl Carrier Protein Synthase III (FabH) Is a Determining Factor in Branched-Chain Fatty Acid Biosynthesis. Journal of Bacteriology 182, 365–370.

Cooper, G.M., Hausman, R.E., 2007. The Cell: A Molecular Approach 2nd Edition, Sinauer Associates.

Corey, E.J., Matsuda, S.P., Bartel, B., 1994. Molecular cloning, characterization, and overexpression of ERG7, the Saccharomyces cerevisiae gene encoding lanosterol synthase. Proceeding of the National Academy of Science of the United States 91, 2211–2215.

Cronan, J.E., Silbert, D.F., Wulff, D.L., 1975. Mapping of the fabA locus for unsaturated fatty acid biosynthesis in Escherichia coli. Journal of Bacteriology 112, 206–211.

Cronan, J.E., Waldrop, G.L., 2002. Multi-subunit acetyl-CoA carboxylases 41, 407–435.

Demel, R.A., Kruyff, D.B., 1976. The Function of Sterols in Membranes. Biochimica et Biophysica Acta 157, 109–132.

Denich, T.J., Beaudette, L.A., Lee, H., Trevors, J.T., 2003. Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes, Journal of Microbiological Methods. doi:10.1016/S0167-7012(02)00155-0

Doran, T.P., Fritsen, C.H., Murray, A.E., Kenig, F., Mckay, C.P., Kyne, J.D., 2008. Entry approach into pristine ice-sealed lakes - Lake Vida, East Antarctica, a model ecosystem. Limnology and Oceanography: Methods 6, 542–547.

Doun, S.S., Burgner, J.W., Briggs, S.D., Rodwell, V.W., 2005. Enterococcus faecalis phosphomevalonate kinase . Protein Science 14, 1134–1139.

Eisenreich, W., Bacher, A., Arigoni, D., Rohdich, F., 2004. Biosynthesis of isoprenoids via the non-mevalonate pathway. Cellular and Molecular Life Sciences 61, 1401–1426.

Falcone, D.L., Gibson, S., Lemieux, B., Somerville, C., 2002. Identification of a Gene that Complements an Arabidopsis Mutant Deficient in Chloroplast [omega]6 Desaturase Activity. Plant Physiology 106, 1453–1459.

Ferguson, J.J., Rudney, H., 1959. The Biosynthesis of β-Hydroxy-β -methylglutaryl Coenzyme A in Yeast: Identification and purification of the hydroxymethylglutaryl coenzyme-condensing enzyme. The Journal of Biological Chemistry 234, 1072–1075.

Fukunaga, N., Russell, N., 1990. Membrane lipid composition and glucose uptake in two psychrotolerant bacteria from Antarctica. Journal of General Microbiology 136, 1669–1673.

Gehring, U., Lynen, F., 1972. 13. Thiolase, in: The Enzymes. pp. 391–405.

Goodwin, T.W., 1979. Biosynthesis of terpenoids. Annual Review of Plant Physiology and Plant Molecular Biology 30, 369–404.

Heath, R.J., Rock, C.O., 1996. Roles of the FabA and FabZ beta-Hydroxyacyl-Acyl Carrier Protein Dehydratases in. The Jounral of Biological Chemistry 271, 27795–27801.

Heintz, R., Benveniste, P., 1974. Plant Sterol Metabolism: Enzymatic Cleavage of the 9B, 19B-cyclopropane ring of cyclopropyl sterols in bramble tissue culture. The Journal of Biological Chemistry 249, 4267–4274.

Huntemann, M., Ivanova, N.N., Mavromatis, K., James Tripp, H., Paez-Espino, D., Palaniappan, K., Szeto, E., Pillay, M., Chen, I.M.A., Pati, A., Nielsen, T., Markowitz, V.M., Kyrpides, N.C., 2015. The standard operating procedure of the DOE-JGI Microbial Genome Annotation Pipeline (MGAP v.4). Standards in Genomic Sciences 10, 4–9.

Hyatt, D., Chen, G.L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11(1), 119.

Jackowski, S., Rock, C.O., 1987. Acetoacetyl-acyl carrier protein synthase, a potential regulator of fatty acid biosynthesis in bacteria. Journal of Biological Chemistry 262, 7927–7931.

Jahnke, L., Klein, H.P., 1983. Oxygen requirements for formation and activity of the squalene epoxidase in Saccharomyces cerevisiae. Journal of Bacteriology 155, 488–492.

Jenni, S., Leibundgut, M., Maier, T., Ban, N., 2006. Architecture of a Fungal Fatty Acid Synthase at 5 A˚ Resolution. Science 311, 1263–1268.

Jennings, S.M., Tsay, Y.H., Fisch, T.M., Robinson, G.W., 2006. Molecular cloning and characterization of the yeast gene for squalene synthetase. Proceedings of the National Academy of Sciences 88, 6038–6042.

Kaneda, T., 1991. Iso-and Anteiso-Fatty Acids in Bacteria: Biosynthesis, Function, and Taxonomic Significant. Microbiological Reviews 55, 288–302.

Klein, W., Weber, M.H.W., Marahiel, M., 1999. Cold Shock Response of Bacillus subtilis : Isoleucine-Dependent Switch in the Fatty Acid Branching Pattern for Membrane Adaptation to Low Temperatures †. Journal of Bacteriology 181, 5341–5349.

Kuhn, E., Ichimura, A.S., Peng, V., Fritsen, C.H., Trubl, G., Doran, P.T., Murray, A.E., 2014. Brine Assemblages of Ultrasmall Microbial Cells within the Ice Cover of Lake Vida, Antarctica. Applied and Environmental Microbiology 80, 3687–3698.

Kuzuyama, T., Seto, H., 2003. Diversity of the biosynthesis of the isoprene units. Natural Product Reports 20, 171–183.

Lange, B.M., Rujan, T., Martin, W., Croteau, R., 2000. Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes. Proceedings of the National Academy of Sciences 97, 13172–13177.

Lois, L.M., Campos, N., Putra, S.R., Danielsen, K., Rohmer, M., 1998. Cloning and characterization of a gene from Escherichia coli encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamin, and pyridoxol biosynthesis. Proceeding of the National Academy of Science of the United States 95, 2105–2110.

Los, D.A., Murata, N., 1998. Structure and expression of fatty acid desaturases. Biochimica et Biophysica Acta 1394, 3–15.

Lütke-Brinkhaus, F., Kleinig, H., 1987. Formation of isopentenyl diphosphate via mevalonate does not occur within etioplasts and etiochloroplasts of mustard (Sinapis alba L.) seedlings. Planta 171, 406–411.

Lüttgen, H., Rohdich, F., Herz, S., Wungsintaweekul, J., Hecht, S., Schuhr, C.A., Fellermeier, M., Sagner, S., Zenk, M.H., Bacher, A., Eisenreich, W., 2000. Biosynthesis of terpenoids: YchB protein of Escherichia coli phosphorylates the 2-hydroxy group of 4-diphosphocytidyl-2C-methyl-d-erythritol. Proceedings of the National Academy of Sciences 97, 1062 LP – 1067.

Markowitz, V.M., Chen, I.M.A., Chu, K., Szeto, E., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., Pagani, I., Tringe, S., Huntemann, M., Billis, K., Varghese, N., Tennessen, K., Mavromatis, K., Pati, A., Ivanova, N.N., Kyrpides, N.C., 2014. IMG/M 4 version of the integrated metagenome comparative analysis system. Nucleic Acids Research 42, 568–573.

Morgulis, A., Gertz, E.M., Schäffer, A.A., Agarwala, R., 2006. A Fast and Symmetric DUST Implementation to Mask Low-Complexity DNA Sequences. Journal of Computational Biology 13, 1028–1040.

Morikawa, T., Mizutani, M., Aoki, N., Watanabe, B., Saga, H., Saito, S., Oikawa, A., Suzuki, H., Sakurai, N., Shibata, D., Wadano, A., Sakata, K., Ohtaa, D., 2006. Cytochrome P450 CYP710A Encodes the Sterol C-22 Desaturase in Arabidopsis and Tomato. The Plant Cell 18, 1008–1022.

Mthakathi, N.T., Kgosiemang, I.K.R., Chen, W., Mohlatsane, M.E., Mojahi, T.J., Yu, J.H., Mashele, S.S., Syed, K., 2015. Cytochrome P450 monooxygenase analysis in free-living and symbiotic microalgae Coccomyxa sp. C-169 and Chlorella sp. NC64A. Algae 30, 233–239.

Muehlbacher, M., Poulter, C.D., 1985. Isopentenyl Diphosphate:Dimethylallyl Diphosphate Isomerase. Irreversible Inhibition of the Enzyme by Active-Site-Directed Covalent Attachment. Journal of the American Chemical Society 107, 8307–8308.

Murray, A.E., Kenig, F., Fritsen, C.H., Mckay, C.P., Cawley, K.M., Edwards, R., Kuhn, E., Mcknight, D.M., Ostrom, N.E., Peng, V., Ponce, A., Priscu, J.C., Samarkin, V., Townsend, A.T., Wagh, P., Young, S.A., To, P., Doran, T.P., 2012. Microbial life at – 13 ° C in the brine

of an ice-sealed Antarctic lake. Proceedings of the National Academy of Sciences 109, 20626–20631.

Mykytczuk, N.C., Foote, S.J., Omelon, C.R., Southam, G., Greer, C.W., Whyte, L.G., 2013. Bacterial growth at −15 °C; molecular insights from the permafrost bacterium Planococcus halocryophilus Or1. The ISME Journal 78, 1211–1226.

Nes, W.D., 2011. Biosynthesis of Cholesterol and Other Sterols. Chemical Reviews 111, 6423–6451.

Nichols, D.S., Nichols, P.D., Mcmeekin, T.A., 1993. Polyunsaturated fatty acids in Antarctic bacteria. Antarctic Science 5, 149–160.

Nichols, D.S., Russell, N.J., 1996. Fatty acid adaptation in an Antarctic bacterium - changes in primer utilization. Microbiology 142, 747–754.

Nishihara, M., Koga, Y., 1987. Extraction and composition of polar lipids from the archaebacterium, Methanobacterium thermoautotrophicum: Effective extraction of tetraether lipids by an acidified solvent. Journal of Biochemistry 101, 997–1005.

Noguchi, H., Park, J., Takagi, T., 2006. MetaGene: Prokaryotic gene finding from environmental genome shotgun sequences. Nucleic Acids Research 34, 5623–5630.

Ourisson, G., Nakatani, Y., 1994. The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Chemistry and Biology 1, 11–23.

Ourisson, G., Rohmer, M., Poralla, K., 1987. Prokyaotic Hopanoids and Other Polyterpenoid Sterol Surrogates. Annual Reviews of Microbiology 41, 301–33.

Pascal, S., Taton, M., Rahier, A., 1993. Plant sterol biosynthesis. Identification and characterization of two distinct microsomal oxidative enzymatic systems involved in sterol C4- demethylation. Journal of Biological Chemistry 268, 11639–11654.

Pearson, A., 2013. Lipidomics for Geochemistry, 2nd ed, Treatise on Geochemistry: Second Edition. Elsevier Ltd. doi:10.1016/B978-0-08-095975-7.01022-6

Perzl, M., Reipen, I.G., Schmitz, S., Poralla, K., Sahm, H., Sprenger, G.A., Kannenberg, E.L., 1998. Cloning of conserved genes from Zymomonas mobilis and Bradyrhizobium japonicum that function in the biosynthesis of hopanoid lipids. Biochimica et Biophysica Acta - Lipids and Lipid Metabolism 1393, 108–118.

Piironen, V., Lindsay, D.G., Miettinen, T.A., Toivo, J., Lampi, A.M., 2000. Plant sterols: Biosynthesis, biological function and their importance to human nutrition. Journal of the Science of Food and Agriculture 80, 939–966.

Poralla, K., 1994. On the possible role of a repetitive amino acid motif in evolution of triterpenoid cyclases. Bioorganic & Medicinal Chemistry Letters 4, 285–290.

Prescott, D.J., Vagelos, P.R., 1972. Acyl Carrier Protein, in: Advances in Enzymology - and Related Areas of Molecular Biology. pp. 269–311.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth , maintenance , and survival. Proceedings of the National Academy of Sciences 101, 4631–4636.

Reddy, A.S., Nuccio, M.L., Gross, L.M., Thomas, T.L., 1993. Isolation of a Δ6-desaturase gene from the cyanobacterium Synechocystis sp. strain PCC 6803 by gain-of-function expression in Anabaena sp.strain PCC 7120. Plant Molecular Biology 22, 293–300.

Rho, M., Tang, H., Ye, Y., 2010. FragGeneScan: Predicting genes in short and error-prone reads. Nucleic Acids Research 38, 1–12.

Rock, C.O., Jackowski, S., 2002. Forty Years of Bacterial Fatty Acid Synthesis. Biochemical and Biophysical Research Communications 292, 1155–1166.

Rohdich, F., Hecht, S., Gartner, K., Adam, P., Krieger, C., Amslinger, S., Arigoni, D., Bacher, A., Eisenreich, W., 2002. Studies on the nonmevalonate terpene biosynthetic pathway: Metabolic role of IspH (LytB) protein. Proceedings of the National Academy of Sciences 99, 1158–1163.

Rohdich, F., Wungsintaweekul, J., Fellermeier, M., Sagner, S., Herz, S., Kis, K., Eisenreich, W., Bacher, A., Zenk, M.H., 1999. Cytidine 5†-triphosphate-dependent biosynthesis of isoprenoids: YgbP protein of Escherichia coli catalyzes the formation of 4-diphosphocytidyl-2-C-methylerythritol Felix. Proceeding of the National Academy of Science of the United States 96, 11758–11763.

Rohmer, M., 2002. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Natural Product Reports 16, 565–574.

Rohmer, M., Anding, C., Ourisson, G., 1980. Non-specific Biosynthesis of Hopane Triterpenes by a Cell-Free System from Acetobacter pasteurianum. European Journal of Biochemistry 112, 541–547.

Rohmer, M., Bouvier, P., Ourisson, G., 1979. Molecular evolution of biomembranes: Structural equivalents and phylogenetic precursors of sterols. Proceeding of the National Academy of Science of the United States 76, 847–851.

Rohmer, M., Sutter, B., Sahmb, H., 1989. Bacterial Sterol Surrogates. Biosynthesis of the Side-chain of Bacteriohopanetetrol and of a Carbocyclic Pseudopentose from W-Labelled Glucose in. Journal of the Chemical Society, Chemical Communications 1471–1472.

Russell, N.J., 1997. Psychrophilic Bacteria-Molecular Adaptations of Membrane Lipids. Comparative biochemistry and physiology 118, 489–493.

Saar, J., Kader, J.C., Poralla, K., Ourisson, G., 1991. Purification and some properties of the squalene-tetrahymanol cyclase from Tetrahymena thermophila. BBA - General Subjects 1075, 93–101.

Sahm, H., Rohmer, M., Bringer-Meyer, S., Sprenger, G.A., Welle, R., 2008. Biochemistry and Physiology of Hopanoids in Bacteria. Advances in Microbial Physiology 35, 247–273.

Salas, J.J., Ohlrogge, J.B., 2002. Characterization of substrate specificity of plant FatA and FatB acyl-ACP thioesterases. Archives of Biochemistry and Biophysics 403, 25–34.

Sato, T., Kouda, M., Hoshino, T., 2004. Site-directed Mutagenesis Experiments on the Putative Deprotonation Site of Squalene-hopene Cyclase from Alicyclobacillus acidocaldarius. Bioscience, Biotechnology, and Biochemistry 68, 728–738.

Seemann, M., Bui, B.T.S., Wolff, M., Tritsch, D., Campos, N., Boronat, A., Marquet, A., Rohmer, M., 2002. Isoprenoid biosynthesis through the methylerythritol phosphate pathway: The (E)4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) is a [4Fe-4S] protein. Angewandte Chemie - International Edition 41, 4337–4339.

Serre, L., Verbree, E., Dauter, Z., Stuitje, A., Derewenda, Z., 1995. The Escherichia coli Malonyl-CoA:Acyl Carrier Protein Transacetylase at 1.5-A Resolution. The Journal of Biological Chemistry 270, 12961–12964.

Summons, R.E., Jahnke, L.L., Hope, J.M., Logan, G.A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature. doi:10.1038/23005

Takagi, M., Kuzuyama, T., Kaneda, K., Watanabe, H., 2000. Studies on the nonmevalonate pathway: formation of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate from 2-phospho-4-(cytidine 5?-diphospho)-2-C-methyl-D-erythritol. Tetrahedron Letters 41, 3395–3398.

Tchen, T.T., 1958. Mevalonic Kinase : Purification and Properties. Journal of Biological Chemistry 233, 1100–1104.

Tsay, J.T., Oh, W., Larson, T.J., Jackowski, S., Rock, C.O., 1992. Isolation and characterization of the β-ketoacyl-acyl carrier protein synthase III gene (fabH) from Escherichia coli K-12. Journal of Biological Chemistry 267, 6807–6814.

Turnbaugh, J.P., Gordon, L.J., 2008. An Invitation to the Marriage of Metagenomics and Metabolomics. Cell 134, 708–713.

Wang, G., Kuriki, T., Roy, K.L., Kaneda, T., 1993. The primary structure of branched-chain a-oxo acid dehydrogenase from Bacillus subtilis and its similarity to other a-oxo acid dehydrogenases. European Journal of Biochemistry 213, 1091–1099.

Welander, P. V., Coleman, M.L., Sessions, A.L., Summons, R.E., Newman, D.K., 2010. Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proceedings of the National Academy of Sciences 107(19), 8537-8542.

Welander, P. V., Hunter, R.C., Zhang, L., Sessions, A.L., Summons, R.E., Newman, D.K., 2009. Hopanoids play a role in membrane integrity and pH homeostasis in Rhodopseudomonas palustris TIE-1. Journal of Bacteriology 191, 6145–6156.

Welander, P. V., Summons, R.E., 2012. Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. Proceedings of the National Academy of Sciences 109, 12905–12910.

Wenk, M.R., 2005. The emerging field of lipidomics. Nature Reviews Drug Discovery 4, 594–610.

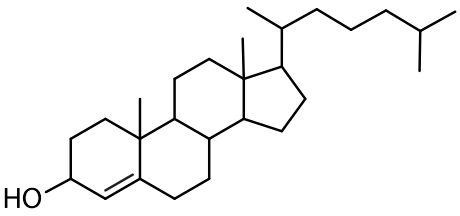
Wilding, E.I., Kim, D.-Y., Bryant, A.P., Gwynn, M.N., Lunsford, R.D., McDevitt, D., Myers J E, J., Rosenberg, M., Sylvester, D., Stauffacher, C.V., 2000. Essentiality, expression, and characterization of the class II 3-hydroxy-3-methylglutaryl coenzyme a reductase of Staphylococcus aureus. Journal of Bacteriology 182, 5147–5152.

Wright, L.D., 2003. Biosynthesis of Isoprenoid Compounds. Annual Review of Biochemistry 30, 525–548.

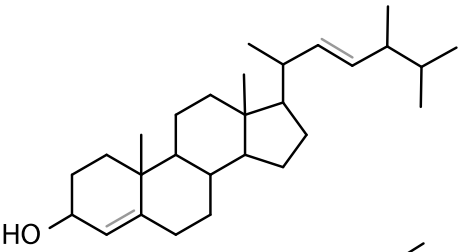
Zhang, Y.M., Rao, M.S., Heath, R.J., Price, A.C., Olson, A.J., Rock, C.O., White, S.W., 2001. Identification and Analysis of the Acyl Carrier Protein (ACP) Docking Site on β-Ketoacyl-ACP Synthase III. Journal of Biological Chemistry 276, 8231–8238.

APPENDIX IV

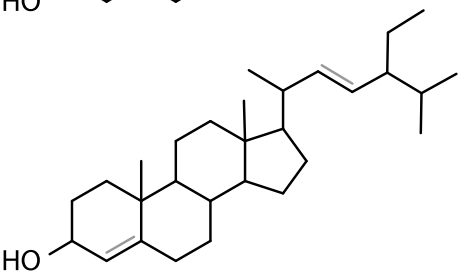
C_{27}- C_{29} sterol



I : cholest-5-en-3β-ol (cholesterol)

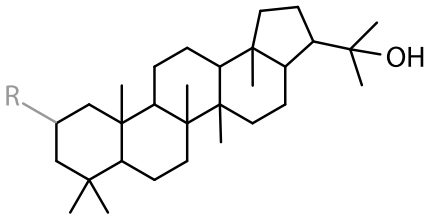


IIa : 24-methyl-cholest-5,22-dien-3β-ol (brassicasterol)
IIb : 24-methyl-cholest-5-en-3β-ol (campesterol)



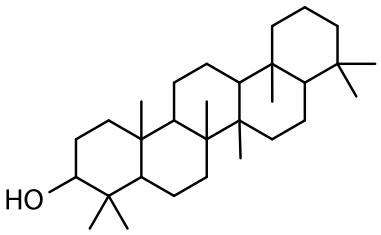
IIIa : 24-ethyl-cholest-5,22-dien-3β-ol (stigmasterol)
IIIb : 24-ethyl-cholest-5-en-3β-ol (sitosterol)

Hopanol



R = H **IVa** : hopan-22-ol (diplopterol)
R = CH_3 **IVb** : 2-methyl hopan-22-ol

Tetrahymanol



V: gammaceran-3β-ol (tetrahymanol)

APPENDIX IV (Cont.)

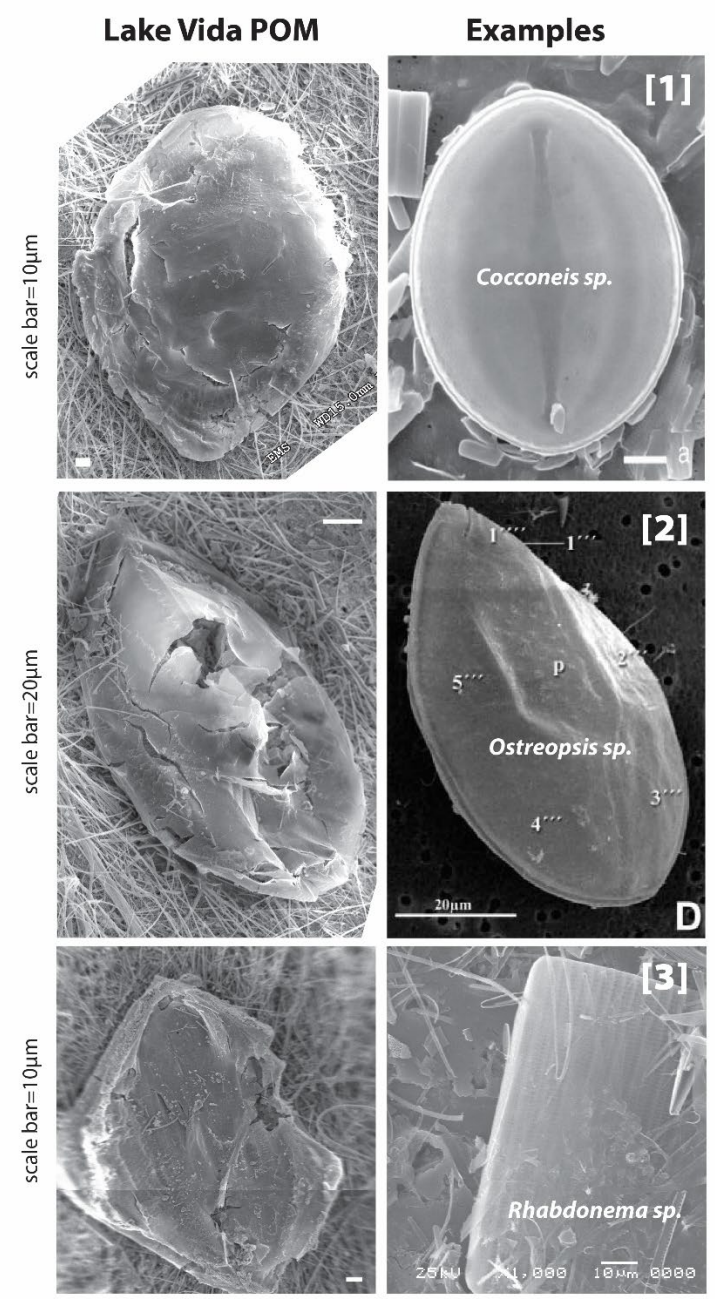


Image source:

[1] https://taxonomic.aad.gov.au/keys/diatoms/key/Antarctic%20Marine%20Diatoms/Media/Html/Cocconeis_type_A.htm

[2] Vila, M., Garcés, E., & Masó, M. (2001). Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean. Aquatic microbial ecology, 26(1), 51-60.

[3] http://fcelter.fiu.edu/data/database/diatom/

APPENDIX IV (cont.)

Lake Vida Brine Metagenome Statistics obtained from IMG/M

Assembled Metagenome		
	Number	**% of Assembled**
Number of sequences	422906	100.00%
Number of bases	292700959	100.00%
GC count	126909444	43.36%
CRISPR Count	598	-
Genes		
Protein coding genes	703717	99.52%
with Product Name	155507	21.99%
with COG	168204	23.79%
with Pfam	169205	23.93%
with KO	96897	13.70%
with Enzyme	53925	7.63%
with MetaCyc	30029	4.25%
with KEGG	51291	7.25%
COG Clusters	4359	94.13%
Pfam Clusters	5411	33.18%
Biosynthetic Clusters	202	
Genes in Biosynthetic Clusters	1106	

CHAPTER V

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Understanding the biogeochemical processes involved in the production, transformation, and preservation of organic biosignatures in cold, subzero, hypersaline conditions is critical to the search for extant life on other planets. Lake Vida, Antarctica, is a unique environment that provides an important opportunity for studying the fate of biosignatures in an isolated cryogenic ecosystem. The findings of this study shed light on the importance of recognizing legacy signatures in a slow-growing, cold-limited environment where organic matter transformation occur extremely slowly and does not significantly affect the composition of the standing crop of organic biosignatures at a given time.

5.1 Synthesis and recommendations

The first goal of this study was to examine biosignatures of Lake Vida. Based on the results obtained in this study, we determined that the biosignatures of Lake Vida can range from paleometabolites of surface-derived organisms (Chapter II and III), to large intact degraded microbes of legacy communities (Chapter IV). We also observed organic sulfones that may represent a pool of legacy metabolites, or abiotic transformation products of biogenically-derived organic sulfur compounds (Chapter III).

The second goal of this study was to investigate the presence of contemporary biosignatures, and thus, the occurrence of extant life, in cryogenic environments. The findings of this work indicate that varying levels of legacy signatures can be retained in different fractions of the organic matter present in the Lake Vida brine system. Results from Chapter II and Chapter III show that the dissolved organic matter fraction of Lake Vida brine contains an abundance of legacy biosignatures that represent a previous ecological condition, more indicative of photic and oxic environments, rather than the current aphotic and anoxic state (Figure 1). For example, solvent-extractable fraction of the environmental metabolome of LVBr contains chlorophyll- and carotenoid-derived compounds, which were most likely biosynthesized in an ancient Lake Vida system supported by sunlight and was open to the atmosphere. The transformation of the precursor compounds to those observed in the environmental metabolomics study of the Lake Vida brine likely also took place in the previous

ecosystem as some diagenetic products are a result of photo- or auto-oxidation (Chapter II). However, we cannot definitively rule out the possibility that the current extant microbial community are degrading those compounds to their current form.

Nevertheless, the particulate organic matter of Lake Vida brine may present an avenue for observing contemporary biosignatures belonging to the extant microbial community. The results presented in Chapter IV show that particulate organic matter may also contain legacy organic matter. The detection of large eukaryotic detritus, and sterol lipids in Lake Vida brine particulate organic matter (POM) suggest that there has not been sufficient time between the shift from the previous ecosystem to the current ecosystem for the legacy organic matter to degrade. Though the modern microbial community is active, the abundant legacy signatures affect our ability to interpret signatures of the extant microbial community in Lake Vida brine, and is not likely a trivial problem in the analysis of other slow-growing, cold-limited ecosystem, on Earth and in icy extraterrestrial environments.

One major strategy that we implemented to elucidate the origin of Lake Vida organic biosignatures was to perform a system-level comparison between the observed lipid biosignatures and the genetic information required to produce them. We pursued this approach by performing a targeted metagenomics analysis, which allowed us to determine whether the Lake Vida brine microbial assemblage harbors the genes necessary to produce the enzymes that catalyze the pathways used to make lipids in the lipidome. As a results, we ruled out the possibility that the current microbial community produce some of the lipids detected.

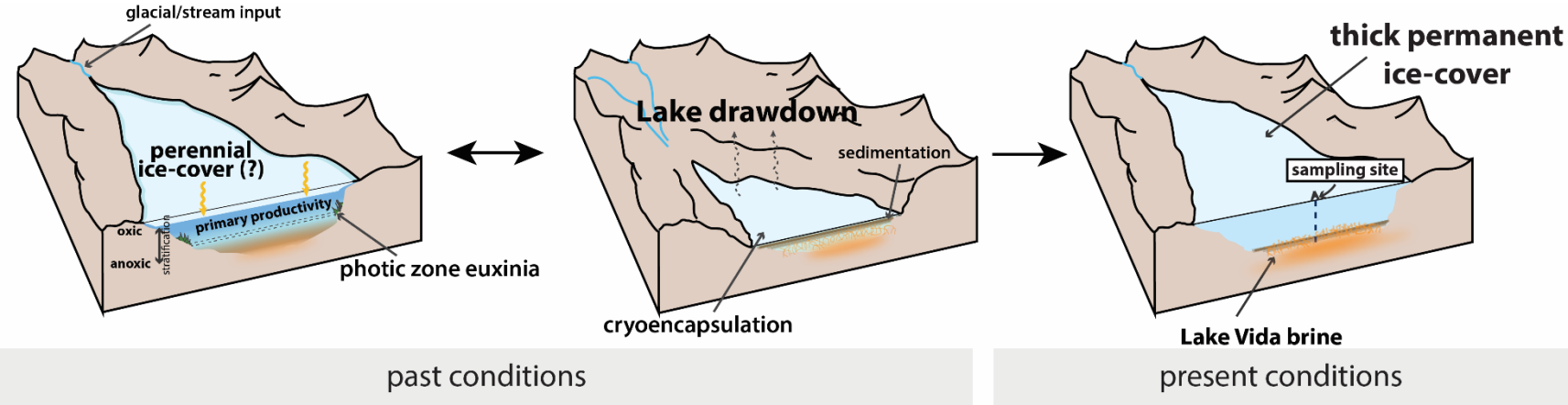


Figure 1: Schematic representation of the Lake Vida brine past environmental conditions and its transition to the current cryoencapsulated state.

While there is evidence to support the possibility that the Lake Vida brine microbial assemblage could produce the fatty acids observed in the lipidome, the same cannot be concluded for sterols as the metagenome does not contain the genes necessary (missing genetic instruction for making enzymes) to carry out the biosynthesis of the sterols. Ultimately, we suggest that in slow-growing, metabolically-limited ecosystems, multiple lines of corroborating evidence are essential to presume a biosignature as contemporary.

The third goal of this study was to determine if there are strategies that can be implemented to facilitate the detection and identification of organic biosignatures in cold-limited environments using modern space-capable technology. In this study, we utilized an analytical instrument, the gas chromatography-mass spectrometer, that is typically used in planetary missions (i.e., Cassini-Huygens, Viking Landers, Curiosity Rover) (Biemann et al., 1977; Niemann et al., 2010; Mahaffy et al., 2012). For example, the Sample Analysis at Mars (SAM) onboard the Curiosity Rover is an instrument suite that harbors GC-MS capable of separating and detecting organic molecules in Mars samples (Mahaffy et al., 2012). Our investigations, thus, may be used as a guide for sample target selection and data interpretation, especially in putative brines that may be present on the surface or in the subsurface of Mars. Similarly, a GC-MS has also been proposed as an organic analyzer for the Europa Lander mission (Managadze et al., 2017). Furthermore, Lake Vida brine has been used as an analytical analog for the detection and quantification of organic matter in a brine that is rich in oxychlorines (ClO_4^- = 49 $\mu g \cdot L^{-1}$, ClO_3^- =11 $\mu g \cdot L^{-1}$) (Kenig et al., 2016). Currently, we are

exploring methods that would allow for the removal of these oxidative salts in brines to facilitate volatile organic matter detection in Lake Vida and in extraterrestrial samples.

Because it is of interest to astrobiologists to search for the evidence of extant life in an analog environment, we suggest that future researchers should carefully consider the target investigation in the context of growth rates and organic matter cycling. One of the major findings of our study incentivizes the need to obtain supporting evidence for the biosynthesis of organic biosignatures with the genetic capacity of the extant microbial community to generate those molecules. Doing so would prevent the preemptive conclusions that any observed biosignatures belong to the current extant community, which are adapted to modern conditions rather than previous conditions. This is an important caveat as we begin to understand the context in which biosignatures are generated and transformed in analog environments. Thus, we suggest that researchers not only study the organic biosignatures obtain from a system, but also its genetic makeup.

In planetary missions aimed at detecting extant life, we propose that serious considerations should be given to instruments capable of *in situ* nucleic acid sequencing. Such technology is already in development. For example, an automated nucleic acid extraction and nanopore sequencing instrument has already been proposed as an *in situ* life detection instrument for Mars (Mojarro et al., 2017). Nanopore sequencing has been proven successful in the analysis of DNA from low biomass samples obtained from Antarctica (Johnson et al., 2017), and has been shown to be tolerant to harsh planetary conditions (Sutton et al., 2019).

5.2 Future research directions

Like most dissertations, however, the findings of this study also raised several questions that prompt future investigations. For example, what are the physical and chemical constraints on the growth rates, and consequently, organic matter cycling rates, of various extreme planetary analog conditions such as Lake Vida? Considerable efforts have been undertaken to investigate the rate of organic matter degradation in subseafloor marine sediments (Arndt et al., 2013). Intact lipid compositions of microbial cells in the deep seafloor environments have been shown to contain a substantial fraction of fossilized component (Schouten et al., 2010). This led researchers to re-estimate microbial biomass turnover time using sensitive amino acid racemization techniques, which was determined to be on the timescales of hundreds of thousands of years (Lomstein et al., 2012; Jørgensen and Marshall, 2015). However, research on the contribution of legacy lipids to environments in the cryosphere is much more scarce, especially in the context of astrobiology. We advocate for future researchers to contextualize their studies of Antarctic organic biosignatures in tandem with *in situ* growth rate estimates. Doing so would allow for a more robust examination of the provenance of the organic signatures in question.

Another major question remains on the abundance and distribution of cold-limited, low energy environments in the solar system. It has very likely that other icy environments of

our neighboring planet, Mars, and the Jovian satellites (i.e., Europa, Enceladus) harbor extremely cold-limited systems. For example, the temperature of the ocean of Europa may be -20°C, and with pressures comparable to the deep ocean trenches on earth (~11 km depth), indicating that this pressure and temperature can support life (Priscu and Hand, 2014). Likewise, the subsurface of Mars can also be hospitable to life at extremely low temperatures due to the presence of various salts with low eutectic values (Cull et al., 2010; Gough et al., 2016). If life currently exists in these cryogenic habitats, the metabolic rates of the biological community would be exceedingly slow, leading to the accumulation of organic matter over long timescales. Missions to the icy environments of the solar system elicit a need for multiple approaches to study organic biosignatures. However, more remains to be seen with the ubiquity of these types of conditions that straddle between the metabolically favorable environments and the lowest temperature limit for life.

The research undertaken in this dissertation provokes an important thought on the persistence of legacy organic biosignatures, on earth and in other icy extraterrestrial environments. Lake Vida brine is one of the few unique places on earth that can provide insight into what life could be like in the conditions of other icy worlds. Elucidating the organic biosignatures in Lake Vida brine not only enhanced our understanding of life in extreme conditions, but it also improved our contextual approach to the search for life elsewhere. Ultimately, we can use the results from this study to further implement better strategies for the detection and interpretation of extant life outside of earth.

Work Cited

Arndt, S., Jørgensen, B.B., LaRowe, D.E., Middelburg, J.J., Pancost, R.D., Regnier, P., 2013. Quantifying the degradation of organic matter in marine sediments: A review and synthesis. Earth-Science Reviews 123, 53–86.

Biemann, K., Oro, J., Toulmin, P., Orgel, L.E., Nier, A.O., Anderson, D.M., Simmonds, P.G., Flory, D., Diaz, A. V, Rushneck, D.R., Biller, J.E., Lafleur, A.L., 1977. The search for organic substances and inorganic volatile compounds in the surface of Mars. Journal of Geophysical Research 82, 4641–4658.

Cull, S.C., Arvidson, R.E., Catalano, J.G., Ming, D.W., Morris, R. V, Mellon, M.T., Lemmon, M., 2010. Concentrated perchlorate at the Mars Phoenix landing site: Evidence for thin film liquid water on Mars. Geophysical Research Letters 37, L22203–L22203.

Gough, R. V., Chevrier, V.F., Tolbert, M.A., 2016. Formation of liquid water at low temperatures via the deliquescence of calcium chloride: Implications for Antarctica and Mars. Planetary and Space Science 131, 79–87.

Johnson, S.S., Zaikova, E., Goerlitz, D.S., Bai, Y., Tighe, S.W., 2017. Real-Time DNA Sequencing in the Antarctic Dry Valleys Using the Oxford Nanopore Sequencer. Journal of Biomolecular Techniques 28, 2–7.

Jørgensen, B.B., Marshall, I.P.G., 2015. Slow Microbial Life in the Seabed. Annual Review of Marine Science 8, 311–332.

Kenig, F., Chou, L., McKay, C.P., Jackson, W.A., Doran, P.T., Murray, A.E., Fritsen, C.H., 2016. Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments. Journal of Geophysical Research-Planets 121, 1190–1203.

Lomstein, B.A., Langerhuus, A.T., Jørgensen, B.B., Spivack, A.J., 2012. Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. Nature 484, 101–104.

Mahaffy, Paul R, et a., 2012. The Sample Analysis at Mars Investigation and Instrument Suite. Space Science Reviews 170, 401–478.

Managadze, G.G., Safronova, A.A., Luchnikov, K.A., Vorobyova, E.A., Duxbury, N.S., Wurz, P., Managadze, N.G., Chumikov, A.E., Khamizov, R.K., 2017. A New Method and Mass-Spectrometric Instrument for Extraterrestrial Microbial Life Detection Using the Elemental Composition Analyses of Martian Regolith and Permafrost/Ice. Astrobiology 17, 448–458.

Mojarro, A., Ruvkun, G., Zuber, M.T., Carr, C.E., 2017. Nucleic Acid Extraction from Synthetic Mars Analog Soils for in situ Life Detection. Astrobiology 17, 747–760.

Niemann, H.B., Atreya, S.K., Demick, J.E., Gautier, D., Haberman, J.A., Harpold, D.N., Kasprzak, W.T., Lunine, J.I., Owen, T.C., Raulin, F., 2010. Composition of Titan's lower atmosphere and simple surface volatiles as measured by the Cassini-Huygens probe gas chromatograph mass spectrometer experiment. Journal of Geophysical Research: Planets 115, 1–22.

Priscu, J.C., Hand, K.P., 2014. Microbial Habitability of Icy Worlds. Microbe Magazine 7, 167–172.

Schouten, S., Middelburg, J.J., Hopmans, E.C., Sinninghe Damste, J.S., 2010. Fossilization and degradation of intact polar lipids in deep subsurface sediments: A theoretical approach. Geochimica et Cosmochimica Acta 74, 3806–3814.

Sutton, M.A., Burton, A.S., Zaikova, E., Sutton, R.E., Brinckerhoff, W.B., Bevilacqua, J.G., Weng, M.M., Mumma, M.J., Johnson, S.S., 2019. Radiation Tolerance of Nanopore Sequencing Technology for Life Detection on Mars and Europa. Scientific Reports 9, 1–10.

APPENDIX A

Copyright statement for *Organic Geochemistry* from Elsevier:

APPENDIX B

Table 1: Lake Vida Ice Core Sediments analytical information

Sample	Depth	Depth in cm	TOC %	Mass of Dry Seds (g)	Vial	Vial + Sample	Sample (g)	Sample (mg)	Mass per g of sediment (mg/g)	Mass per g of sediment (μg/g)
A	21.67	2167	0.3701	9.36	6.0452	6.0459	0.0007	0.7	0.07	74.79
B	21.7	2170	0.3538	28.18	6.0341	6.0343	0.0002	0.2	0.01	7.10
D	21.74	2174	0.4108	30.26	6.0414	6.0418	0.0004	0.4	0.01	13.22
F	21.82	2182	0.3834	36.15	6.0798	6.0803	0.0005	0.5	0.01	13.83
H	22.905	2291	0.3379	27.25	6.0762	6.0766	0.0004	0.4	0.01	14.68
J	22.952	2295	0.4923	28.19	6.0658	6.0673	0.0015	1.5	0.05	53.21
L	23.005	2301	0.3060	2.05	6.0897	6.0902	0.0005	0	0.00	0.00
N	25.62	2562	0.4518	31.98	6.1308	6.131	0.0002	0.2	0.01	6.25
P	25.67	2567	0.3011	40.99	6.0544	6.0558	0.0014	1.4	0.03	34.15
R	26.315	2632	0.4815	34.12	6.0457	6.0475	0.0018	1.8	0.05	52.75
T	26.365	2637	0.39	34.39	6.0627	6.065	0.0023	2.3	0.07	66.88
V	26.415	2642	?	37.32	6.1162	6.1174	0.0012	2.8	0.08	75.03

APPENDIX B (Cont.)

Table 2: Lake Vida Ice Core Sediments analytical information

Sample	% Aliquot	Mass of sample in aliquot (mg)	Mass of added internal standard (μg)	Mass of i.s. per inj (ng)	Volume added to vial (μl)	Dilution factor	Volume injected	GC Run #
A	30%	0.21	1.875	44.24779	21.1875	10ug/uL	0.5	2019114
B	50%	0.10	0.893	44.2548	10.0893	10ug/uL	0.5	2019115
D	50%	0.20	1.7857	44.24744	20.17857	10ug/uL	0.5	2019116
F	50%	0.25	2.232	44.24498	25.2232	10ug/uL	0.5	2019117
H	50%	0.20	1.7857	44.24744	20.17857	10ug/uL	0.5	2019118
J	20%	0.30	2.6786	44.24826	30.26786	10ug/uL	0.5	2019119
L	50%	0.25	2.232	44.24498	25.2232	10ug/uL	0.5	2019124
N	50%	0.10	0.893	44.2548	20.1786	5ug/uL	1	2019125
P	25%	0.35	3.2499	46.00001	35.32499	10ug/uL	0.5	2019126
R	25%	0.45	4.0185	44.2548	45.40185	10ug/uL	0.5	2019127
T	20%	0.46	4.1078	44.2548	46.41078	10ug/uL	0.5	2019128
V	13%	0.32	2.8576	44.2548	32.28576	10ug/uL	0.5	2019138

APPENDIX C

BLAST+ script example

Description: BLASTp was used to identify CDSs that did not have a KO in the IMG/M database. Amino acid sequences were obtained from KEGG using coding regions of previously studied genes (Welander et al., 2010, Welander and Summons et al., 2012, Banta et al., 2015)

Input to cmd (Windows 10):

```
blastp -query HOP_KO_AA_Bacteria -out HOP_AA_output_e-10 -db VidaMet -num_threads 4
-evalue 1e-10 -outfmt 7 &
```

Output to cmd (Windows 10):

```
# BLASTP 2.9.0+
# Query: rpa:RPA3740 K06045 squalene-hopene/tetraprenyl-beta-curcumene cyclase
[EC:5.4.99.17 4.2.1.129] | (GenBank) sqhC; squalene-hopene-cyclase (A)
# Database: VidaMet
# 0 hits found
# BLASTP 2.9.0+
# Query: unknown:00000 K00000 tetrahymanol synthase, unknown [EC:0.0.0.00] |
(RefSeq) Ths; Tetrahymanol synthase
# Database: VidaMet
# 0 hits found
# BLASTP 2.9.0+
# Query: rpa:RPA3748 K22705 hopanoid C-2 methylase [EC:2.1.1.-] | (GenBank)
Elongator protein 3/MiaB/NifB (A)
# Database: VidaMet
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, mismatches,
gap opens, q. start, q. end, s. start, s. end, evalue, bit score
# 3 hits found
rpa:RPA3748   JGIcombinedJ13537_102402711      45.263 95     50     1      147    241
       15     107    5.28e-24      99.0
rpa:RPA3748   JGIcombinedJ13537_100038592      24.225 355    245    8      40     389
       22     357    6.70e-21      97.4
rpa:RPA3748   JGIcombinedJ13537_100618261      30.542 203    135    5      181    381
       40     238    2.16e-16      82.4
# BLASTP 2.9.0+
# Query: mah:MEALZ_1315 K22704 hopanoid C-3 methylase [EC:2.1.1.-] | (GenBank)
putative methylthiotransferase; putative cobalamin binding domain protein (A)
# Database: VidaMet
# Fields: query acc.ver, subject acc.ver, % identity, alignment length, mismatches,
gap opens, q. start, q. end, s. start, s. end, evalue, bit score
# 1 hits found
mah:MEALZ_1315      JGIcombinedJ13537_100011535      26.748 329    200    16     25
       334    22     328    3.59e-14      77.4
# BLAST processed 4 queries
```

APPENDIX D

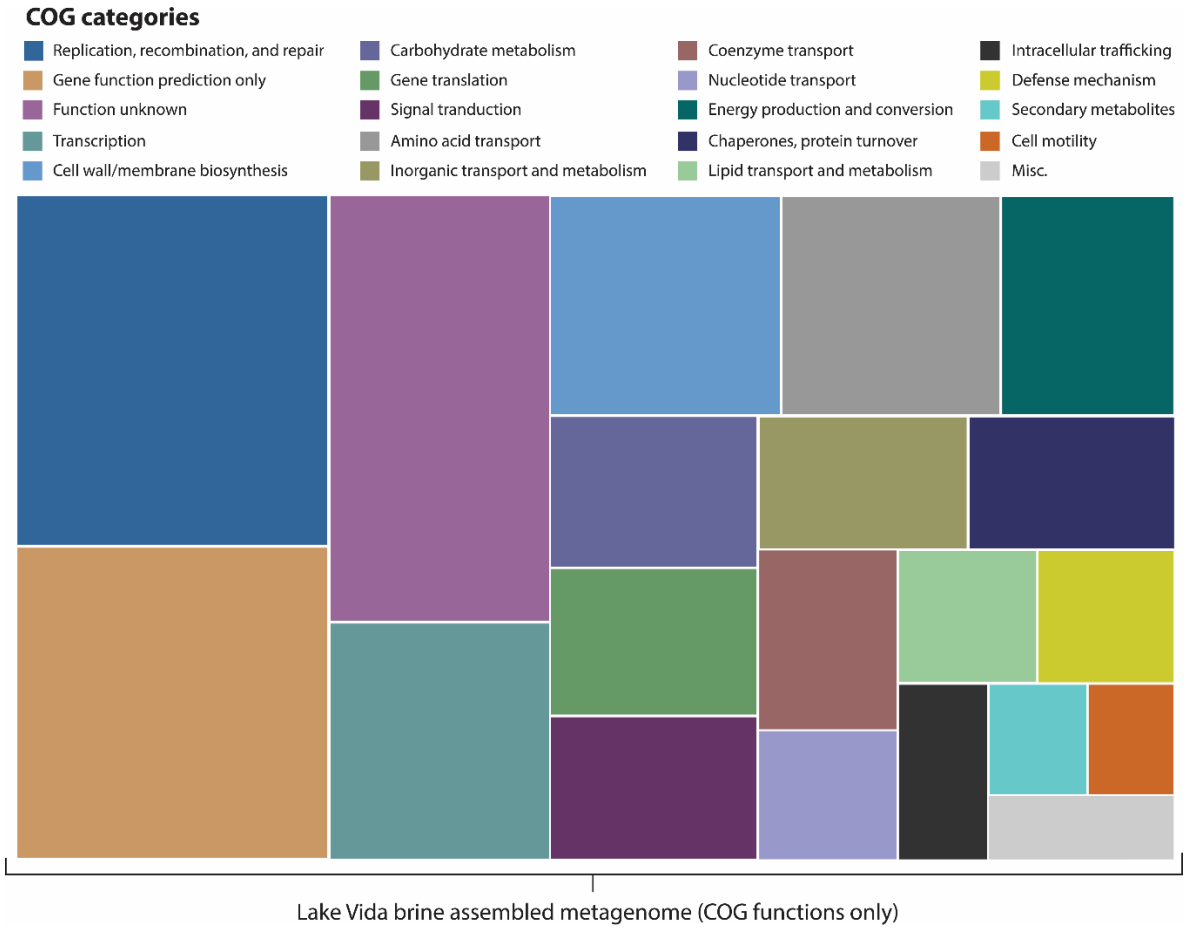


Figure 1: A treemap diagram of assigned COG categories showing their abundance and distribution in the Lake Vida brine assembled metagenome.

The Lake Vida brine combined assembly metagenome (>0.2 μm) contain 292,700,959 base pairs, and 707,118 total genes. Protein coding genes accounted for 99.52% (703,717) of the total metagenome, with 94.13% belonging to COG clusters with functional categories and about 75.77% that have known COG functions (Figure 1). Genes that were found to relate to a COG cluster were quantified based on their gene counts. These COG clusters include gene replication, recombination, and repair (15.78%), gene transcription (7.65%), biosynthesis of cell wall and cell membrane (7.23%), amino acid metabolism (including transport; 6.9%), energy production and conversion (5.5%), carbohydrate metabolism and transport (4.47 %), and other notable clusters that are important for growth and survival such as inorganic ion transport (4.06%) and lipid transport (2.64%), etc.

VITA

NAME Luoth Chou

RESEARCH INTEREST Astrobiology of icy worlds, organic biomarker detection and characterization, metabolomics and metagenomics of the cryosphere, mission design, instrument development (mass spectrometry), and project management.

EDUCATION

Ph.D. Earth & Environmental Sciences. University of Illinois at Chicago. 2019.
Dissertation: "The Organic Matter of Lake Vida, Antarctica: Biogeochemistry of an Icy Planetary World Analog"
Advisor: Dr. Fabien Kenig

B.S. Microbiology (Minor: Astronomy). University of Maryland at College Park. 2013.

RESEARCH EXPERIENCE

Graduate Researcher | University of Illinois at Chicago | **Fall 2013 - Present**
The metabolomics, metagenomics, and geochemistry of the brine of Lake Vida (Antarctica)
Advisor: Fabien Kenig Ph.D.

Undergraduate Research Intern | U.S. Food and Drug Administration | **June 2011 - May 2013**
The molecular genetics of *Listeria monocytogenes*, under cold and salinity stress
Advisor: Atin Datta Ph.D.

AWARDS + FELLOWSHIP

NASA Early Career Collaboration Award. Fall 2018.
NASA Earth and Space Science Fellowship. **$116,114.** Fall 2016 - Spring 2019
Chancellor's Graduate Research Fellowship. UIC. **$8,000**. Summer 2015-16
On To the Future Travel Award. Geological Society of America. **$500**. Fall 2015
LAS PhD Travel Award. University of Illinois at Chicago. **$250.** Fall 2015.
Bodmer Science International Travel Award. University of Illinois at Chicago. **$750.** Summer 2015
Outstanding Teaching Assistant Award. National Association of Geoscience Teachers. Spring 2015
Graduate Research Fellowship. Illinois Space Grant Consortium (ISGC). **$10,000.** Fall 2014
The Colonnade Society Undergraduate Scholarship. U of MD Alumni Association. **$2,000.** Fall 2012
The College of CMNS Scholarship. U of MD. **$2,000.** Spring 2012
The Sam Walton Scholarship. **$3,000.** Fall 2009

PROFESSIONAL TRAINING

NASA Jet Propulsion Laboratory (10 weeks remote, 1 week on site) | July 2017
Project Manager for JPL Planetary Science Summer Seminar. Mission design exercise for a Centaur Reconnaissance Mission.

NASA Review Panel | 2014, 2018(a), 2018(b)
Executive Secretary

The History And Philosophy Of Astrobiology Summer School (1 week) | Summer 2015
Ven Island, Sweden

Josep Comas I Solà International Summer School In Astrobiology (1 Week) | Summer 2014
Santander, Spain

Smithsonian Institution (12 Weeks) | Summer 2010
Summer Intern. National Science Resources Center (for K-12 Education).

TEACHING EXPERIENCE

Teaching Assistant. University of Illinois at Chicago. | Fall 2013 - Spring 2016
Global Environmental Change. Earth, Energy, and the Environment. Statistical Methods in Earth and Environmental Science

Teaching Assistant. Princeton University (John Hopkins Center for Talented Youth) | Summer 2013
Epidemiology.

Teaching Assistant. University of Maryland, College Park. | Fall 2012 , Spring 2013
Principles of Microbiology.

FIELD EXPERIENCE

Biosignatures And The Search For Life On Mars Summer School (2 weeks) | Summer 2016
Iceland (sample collection, analysis, geochemical characterization, ATP assay for biosignature detection)

The International Geobiology Course (5 weeks) | Summer 2015
California, Nevada, and Catalina Island (Sample preparation and field collection. Geochemical, petrographic, clumped isotope, and microbiological analyses, and paleoenvironmental reconstruction of Walker Lake, NV).

INVITED LECTURES

Astrobiology: in search of who's out there by looking at who we are. (2016) UIC Biology Colloqium

Environmental Geochemistry: Introduction to Organic Geochemistry. (2017) UIC Department of Earth and Environmental Sciences.

TECHNICAL SKILLS

Chemistry
Gas chromatography (GC)-Mass spectrometry (MS)
GC-Flame Ionization Detector (GC-FID), Pyrolysis-GC
Solidphase microextraction (SPME)
Multidimensional GC×GC-TOF-MS
Ion exchange chromatography (IX)
Organic matter extraction (lipids, metabolites)

Software
ChemStation, ChromaTOF
Adobe Illustrator, InDesign, Photoshop
RStudio
Qiime, NCBI, BLAST+
Geographic Information System (ArcGIS)

Molecular Biology
Growth Cultures Assays, CFU Study
DNA Extraction, PCR
Spectrophotometry
DNA cloning
Bacterial transformation

OUTREACH ACTIVITIES

Visits to Chicago Montessori School (Chicago, IL). April 2016, 2017, and 2019.
Co-create lesson plans and in-class demonstrations of geology, and planetary science.

Visit to Brentano Math And Science Academy (Chicago, IL) to speak about Earth science. December 2018.

Saganet.Org. Monthly Newsletter Organizer and **Astrobiology "Expert"**. August 2018 - present.

Abgradcon Outreach Event. "An Evening of Wonder – Life and Art on Earth and Beyond"
Volunteered as an astrobiologist to speak with the general public at Georgia Tech University. June 2018.

Social Media Organizer and **Science Marshal**. Februrary to April, 2017.
March for Science at Chicago event on April 22nd, 2017 with 60,000+ attendees

UIC Today. April 2017. Interview for article on The March for Science. https://today.uic.edu/marching-for-science

Interview on The Show About Science. April 2017.
A children's podcast hosted by 6-years-old Nate Butkus. Episode title: Marching for Science and Extraterrestrials.

(Honest) Conversations With (Real) Scientists. Hosted by Jimmy Dagger and Joey Pasterski.
Invited to speak on panel about life in the universe and astrobiology. Cafe Mustache, Chicago. June 2017.

PEER-REVEIWED PUBLICATIONS

*under review, ‡in press, ^Δ^in preparation (manuscript available upon request)

ΔLuoth Chou, Murray A., Kenig F, Organic sulfones in the brine of Lake Vida (Antarctica).

ΔLuoth Chou, Murray A., Kenig F., Metagenomic Evidence for Lipid Legacy: the Brine of Lake Vida, Antarctica

ΔLuoth Chou, Griza R., Kenig F., Organic Geochemical Analysis of Lake Vida Ice Core Sediment Layers: A biogeochemical Record of Past Environmental Conditions

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1. Kenig F., **Luoth Chou.,** Wardrop D.J. Comment on "Evaluation of the Tenax trap in the Sample Analysis at Mars instrument suite on the Curiosity rover as a potential hydrocarbon source for chlorinated organics detected in Gale Crater" by Miller et al. 2015. JGR: Planets, 124(2). doi.org/10.1029/2018JE005606

2. Howell, S., **Luoth Chou** , Thompson M., Bouchard M., Cusson S., Marcus M., Smith H., Bhattaru S., Blalock J., Brueshaber S., Eggl S., Jawin E., Miller K., Rizzo M., Steakley K., Thomas N., Trent K., Ugelow M., Budney C. and Mitchell K. (2018) Camilla: A centaur reconnaissance and impact mission concept. Planetary and Space Science 164, 184-193. doi.org/10.1016/j.pss.2018.07.008

3. **Luoth Chou**, Kenig F., Murray A., Doran P., Fritsen C., (2018) Effects of legacy metabolites from previous ecosystems on the environmental metabolomics of the brine of Lake Vida, East Antarctica. Organic Geochemistry 122, 161-170. doi:10.1016/j.orggeochem.2018.05.002

4. Stelmach, K., Neveu, M., Vick-Majors, T., Mickol, R., **Luoth Chou,** Webster, K., Tilley, M., Zacchei, F., Escudero, C., Flores-Martinez, C., Labrado, A., Fernandez, E., (2018) Secondary Electrons as a Novel Energy Source: Implications for Life on Icy Worlds. Astrobiology 18 (1), 73-85. doi:10.1089/ast.2016.1510

5. Kenig F., **Luoth Chou,** McKay C., Jackson A. W., Doran P., Murray A., Fritsen C. (2016) Perchlorate and volatiles of the brine of Lake Vida (Antarctica): Implication for the in situ analysis of Mars sediments. Journal of Geophysical Research: Planets, 121(7), doi:10.1002/2015JE004964

6. L.S. Burall, A. C. Simpson, **Luoth Chou,** P. Laksanalamai, A. R. Datta, (2015) A novel gene, lstC, of Listeria monocytogenes is implicated in high salt tolerance, Food Microbiology 48, 72-82, doi:10.1016/j.fm.2014.12.008

CONFERENCES & ABSTRACTS

****oral presentation

****Luoth Chou**, A. E. Murray, F. Kenig (2019). The nature of legacy biosignatures in cold-limited, slow-growing ecosystems. Astrobiology Science Conference 2019. Bellevue, WA. Paper 411-8.

A. E. Murray, **Luoth Chou**, F. Kenig, C. H. Fritsen, P. T. Doran. (2018) Forecasting Habitability Through the Ice-Sealed Lens of Antarctica's Lake Vida. American Geophysical Union 2018, Washington, D. C.

Luoth Chou and F. Kenig (2018). Legacy metabolites and organic matter preservation in an Antarctic cryoencapsulated hypersaline brine. Astrobiology Graduate Conference 2018, Atlanta, GA.

M.C. Bouchard, S.M. Howell, **Luoth Chou,** et al. (2018). Flyby and Impact of Chariklo: A New Frontiers Class Centaur Reconnaissance Mission Concept from the 2017 NASA-JPL Planetary Science Summer Seminar. 49th Lunar and Planetary Science Conference. 2018. The Woodlands, Texas. LPI Contribution No. 2083, id.2087.

Luoth Chou, F. Kenig, W. A. Jackson (2017). Strategies for facilitating organic matter detection in subsurface perchlorate-rich brines of Earth and Mars. American Geophysical Union 2017, New Orleans, LA.

Luoth Chou, Howell S., et al. (2017) Centaur Reconnaissance Mission: a NASA JPL Planetary Science Summer Seminar mission design experience. American Geophysical Union 2017, New Orleans, LA.

**** Luoth Chou,** F. Kenig, W. A. Jackson (2017). Perchlorate Removal From the Brine of Lake Vida for Volatile Organic Matter Analysis in a Mars Analog. Astrobiology Science Conference 2017, Mesa, AZ.

**** Luoth Chou,** F. Kenig, A. E. Murray, C. H. Fritsen, P.T. Doran (2016). GC × GC-TOF MS of metabolites of Lake Vida brine (Antarctica). American Geophysical Union 2016, San Francisco, CA.

**** Luoth Chou,** et al. International Geobiology Course 2015. (2015). Coupled Stratigraphy, Petrography, and $\Delta 47$ of Ancient Walker Lake, Nevada Reveals Unique Analog for Studying Proterozoic Stromatolite Formation and Climatic Forcings. Geological Society of America 2015, Baltimore, MD.

L. A. Zinke, J. Buongiorno, **Luoth Chou.,** L. M. van Maldegem, et al. (2015). Couple geochemical and microbiological characterization of non--carbonate firmgrounds from a modern soda lake, Walker Lake, Nevada. Geological Society of America 2015, Baltimore, MD.

Luoth Chou, F. Kenig, A. E. Murray, P.T. Doran, C. H. Fritsen (2015). The Metabolomics of the Brine of Lake Vida (McMurdo Dry Valleys, Antarctica). American Geophysical Union 2015, San Francisco, CA.

Luoth Chou, F. Kenig, A. E. Murray, P.T. Doran, C. H. Fritsen (2015). The Lipidomics of the Brine of Lake Vida (McMurdo Dry Valleys, Antarctica). Astrobiology Science Conference 2015. Chicago, IL.

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