ISOTOPIC BIOSIGNATURES IN MARS ANALOGUE ENVIRONMENTS

SEARCHING FOR BIOSIGNATURES IN MARS ANALOGUE SITES: AN ANALYSIS OF MICROBIAL CARBON CYCLING AND BIOSIGNATURES WITHIN ACID-SULFATE AND HYPERSALINE LAKE ENVIRONMENTS

By

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ABSTRACT

By studying signatures of life within extreme environments on Earth we better understand signatures of life that may be found within the geologic record on Earth as well as elsewhere in the universe. This thesis research utilized phospholipid fatty acid analysis (PLFA) in combination with carbon isotopic analysis ($\delta^{13}C$) to understand microbial carbon cycling and biosignatures within two Mars analogue environments.

The Golden Deposit was an acid sulfate environment that was an analogue to the acid-sulfate conditions present on early Mars. Mixing of inflowing surface water and upwelling acidic groundwater on the deposit caused variations in water chemistry. Such variations resulted in differences in the microbial communities and carbon isotopic compositions within the Golden Deposit. The most acidic groundwater seep on the deposit had a distinct geochemistry, which selected for a unique microbial community and distinct carbon isotopic signatures. Variations detected within the small spatial area of the Golden Deposit indicated that biosignatures preserved on Mars might be highly variable making the unambiguous identification of past life on the planet more difficult.

Three saline lakes of the Cariboo Plateau were used for this study and were analogues to environmental conditions present on early Earth as well as the alkaline, evaporitic conditions present on early Mars. All three lakes contained benthic microbial mat communities dominated by cyanobacteria. Photosynthetic influences by cyanobacteria resulted in an isotopic enrichment of the DIC pool. Enriched lake DIC values were preserved in precipitated carbonates ($\delta^{13}C_{carb}$) and the $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ was similarly preserved in $\Delta^{13}C_{carb\text{-}TOC}$ values and were biosignatures of photosynthetic activity. The preservation of these biosignatures suggested that concentrations of CO_2 on early Earth did not necessarily exceed modern levels and that measuring the carbon isotopic compositions of carbon pools within saline, carbonate-rich deposits on Mars could lead to the identification of past life on the planet.

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CHAPTER 1
INTRODUCTION TO RESEARCH AND FUNDAMENTAL CONCEPTS

1.1 INTRODUCTION

For many centuries, scientists have speculated the existence of life on Mars. The idea that Mars could support life increased in popularity in 1877 when the Italian astronomer Giovanni Schiaparelli identified 'canali', meaning channels, on the surface of the planet (Rothschild, 1990; Zahnle, 2001). Such features were misinterpreted to be artificially built canals and were believed to be a sign of the presence of intelligent life on the planet (Zahnle, 2001). Our views of Mars changed considerably after the Mariner probes and Viking lander experiments in the 1960s and 1970s found a heavily cratered planet, with no liquid water, and no signs of life (McKay, 1997; Zahnle, 2001). Mars today is a cold, desert environment with a thin atmosphere and strong oxidizing conditions that are unlikely to support life (Southam et al., 2007). However, the search for signs of life continue as past environmental conditions on Mars were much more habitable and similar to conditions when life first originated on Earth (Kanavarioti and Mancinelli, 1990; McKay, 1997; Tokano, 2005). Extreme environments on Earth today serve as terrestrial analogues to conditions that were present early in Mars' history. Such environments of study include but are not limited to: evaporitic and hypersaline environments, endolithic communities in desert rocks, preserved organisms in permafrost, and acid rock drainage environments (Rothschild, 1990; Navarro-González et al., 2003; Amils et al., 2007; Gilichinsky et al., 2007). Today, research is focused on understanding microbial processes and signatures of life that are preserved within these analogue environments. Scientific exploration within such environments allows us to better identify the signatures of life that may be preserved in the geologic record on Mars and brings us one step closer to answering one of the most basic human questions: are we alone in the universe?

1.2 ENVIRONMENTS ON EARLY MARS THAT MAY HAVE SUPPORTED LIFE

The environment thought to exist on early Mars, with active fluvial, glacial, and volcanic activity, is comparable to conditions that were present on early Earth (Bibring et al, 2006; Southam et al., 2007). Precambrian stromatolites are one of the earliest evidences of life on Earth and are thought to indicate that much life existed as microbial mat communities that were influenced by evaporitic conditions (Grotzinger and Knoll, 1999). Early Mars is believed to have been an aqueous, alkaline, evaporitic environment that would have resulted in the concentration of salts in residual waters (Tokano, 2005; Osterloo et al., 2008). The recent identification of chloride-bearing salt deposits in craters and sinuous channels globally distributed on Mars suggest that such deposits formed through the ponding and evaporation of brines (Osterloo et al., 2008). Furthermore, the detection of carbonates in a rock unit in the Nili Fossae region of Mars supports the existence of past neutral to alkaline waters on the planet and indicates that remnants of the saline, alkaline conditions present on early Mars are still preserved on the planet today (Ehlmann et al., 2008). Microbial fossils, biomarkers, and viable organisms have been found in many ancient salt deposits on Earth indicating that such environments are capable of preserving biomolecules and signatures of life (Farmer and Des Marais, 1999; Stan-Lotter et al., 2002; Mormile et al., 2003; Schubert et al., 2009). Therefore, such deposits on Mars are favourable environments for the search for signatures of past life on the planet.

After a period of heavy bombardment by meteorites on the planet, the climate on Mars shifted to a saline, aqueous, acid-sulfate environment (Bibring et al., 2006). Sulfate-rich sedimentary rocks containing iron and sulfur-rich minerals, such as jarosite and hematite, have been detected on the surface by Mars rovers. Such mineralogy is indicative of deposition in acidic sulfate-rich liquid water followed by evaporation and desiccation (Squyres et al., 2004a; Squyres et al., 2004b). Therefore, remnants of the aqueous, acid-sulfate environment are preserved on Mars today. Soluble ferric iron solutions and iron minerals on Earth

have been found to protect cellular destruction by UV radiation and could have acted as a protective agent against the strong UV radiation present on the surface of Mars (Phoenix et al., 2001; Gomez et al., 2007). Therefore, it is conceivable that life could have evolved during the aqueous, acid-sulfate period on Mars, biosignatures of which may still be preserved on the planet today.

1.3 EXTREME ENVIRONMENTS ON EARTH AS ANALOGUES TO MARS

By studying life in extreme environments on Earth that are analogues to other planets we can learn the physical and chemical limits of habitability allowing us to better understand the signatures of life that may be found elsewhere in the universe (Javaux, 2006). Such studies will also contribute to our understanding of both ancient and modern microbial processes operating on Earth. This thesis research is focused on understanding microbial carbon cycling and biosignatures within two Mars analogue sites that are representative of the dominant environments that were present in Mars' past. The two Mars analogue environments of interest are acid-sulfate and saline, alkaline lake environments.

1.3.1 Acid Sulfate Analogue Environment

The first environment of study is the Golden Deposit, located east of Norman Wells in the Northwest Territories of Canada. It is a naturally occurring acid rock drainage system, which is precipitating jarosite and goethite out of acidic iron and sulfur-bearing groundwater seeps. It represents an analogue to the acid-sulfate conditions that dominated the surface of Mars late in its aqueous history, evidence of which is preserved as iron and sulfur mineral deposits found on the planet today.

The most commonly studied Mars analogue site is the Rio Tinto basin in Spain (Amils et al., 2007). The Rio Tinto is an acidic river with a mean pH of ~2.2, has high concentrations of metals and a diversity of biologic activity (López-Archilla and Amils, 2001). The Rio Tinto is considered an analogue to Mars due to its iron and sulfur-based mineralogy of jarosite and hematite, minerals of which are also

found on the surface of Mars (Fernández-Remolar et al., 2005). However, the Rio Tinto River is located in a warm climate, while the aqueous acid-sulfate period on Mars is believed to have occurred while the planet was relatively cold and arid (Gaidos and Marion, 2003; Fairén, 2010). Furthermore, the Rio Tinto River has been influenced by thousands of years of human mining activity negating its applications as a Mars analogue environment (Davis et al., 2000). In contrast, the Golden Deposit is a naturally occurring acid-sulfate environment, which is isolated in the cold, arid environment of northern Canada. The mineralogy of the Golden Deposit and its location in a cold, permafrost environment makes it a better analogue to the conditions present during the formation of such deposits on Mars (Michel and Van Everdingen, 1987; Squyres et al., 2004a; Battler et al., unpublished).

1.3.2 Saline, Alkaline Lake Analogue Environments

The second environment of study is the saline lakes of the Cariboo Plateau located in the southern Interior of British Columbia, Canada. The Cariboo Plateau lakes chosen for this study are saline, alkaline, and carbonate-rich lakes that are dominated by benthic microbial mat communities. They represent an analogue to the alkaline, evaporitic conditions that dominated the surface of Mars early in its aqueous history, evidence of which is preserved in chloride and carbonate deposits detected on the surface of the planet today.

Studies of saline environments have primarily focused on non-lithifying microbial mats of the salt ponds of Guererro Negro and Solar Lake. High rates of photosynthesis are leading to CO_2 limitation within these locations and resulting in ^{13}C-enriched organic isotopic signatures inconsistent with the early Earth geologic record (Schidlowski et al., 1984; Des Marais et al., 1989; Schouten et al., 2001). However, the $\delta^{13}C$ of organic matter within the Cariboo Lakes do not display significant ^{13}C-enrichment and show isotopic values of organic and inorganic carbon in the range of what has been found in the geologic record during the period of stromatolite formation (Schidlowski, 2001; Brady et al., 2009). Therefore, the

Cariboo Lakes are a better terrestrial analogue to early Earth conditions and, by extension, a better analogue to environmental conditions that may have been present during the formation of life on early Mars.

1.3.3 Research Approach

This thesis research utilized phospholipid fatty acid (PLFA) and carbon isotopic analysis ($\delta^{13}C$) of PLFA, organic and inorganic carbon to understand microbial carbon cycling and the preservation of biomarkers within these two Mars analogue environments. By performing such analyses on the unique analogue environments of the Golden Deposit and the Cariboo Lakes, we gain a better understanding of the microbial processes that could have been present on early Mars and the signatures of life that may be preserved within its geologic record. The indisputable identification of past life on Mars will no doubt pose challenges and thus it is critically important to further our knowledge of microbial processes and the preservation and interpretation of biosignatures within Mars analogue environments.

The remainder of Chapter 1 will provide background information on the concepts and environments that are the focus of this research. This chapter will explore the use of PLFA and isotopes to identify microbial communities and microbial carbon cycling within environmental samples. It will also provide background information on the microbial communities and cycling of nutrients that are often found within acid-sulfate environments and microbial mat-dominated saline lakes.

1.4 LIPIDS AND BIOMARKERS

The tree of life on Earth spans three domains: Bacteria, Archaea and Eukarya. Organisms of all three domains are connected in that they each contain a lipid membrane, which separates the cytoplasmic cell from the external environment

(Alberts et al., 2002). However, the lipid membrane composition of organisms can vary significantly and therefore lipids can be used as biomarkers for specific organisms (Eglinton et al., 1964; Green and Scow, 2000). Furthermore, the isotopic signature of cellular components and carbon compounds in the environment can be diagnostic of biological processes as microbial processes influence the isotopic signatures of organic and inorganic carbon reservoirs on Earth (O'Leary, 1988). Used in combination, lipid and isotope analyses can be useful tools to elucidate microbial processes and identify signatures of life in Earth's many diverse environments (Boschker and Middelburg, 2002). Utilization of these techniques within extreme environments on Earth may help us identify signatures of life that exist within similar environments on Mars.

The term 'lipid' refers to a large number of compounds that may or may not be relatable in structure and function and, as a result, there is no widely accepted definition for the term. However, most sources define lipids as compounds that are more soluble in organic solvents than in water (Dowhan and Bogdanov, 2002). Some examples of lipid groups include phospholipid fatty acids (PLFA), hopanoids, waxes, sterols and phospholipid ether lipids (PLEL). The type and amount of lipids found in cellular membranes varies largely among organisms, and some can be used as specific biological markers or 'biomarkers' of microbial groups (Eglinton et al., 1964). Biomarkers are specific to the organism of interest and concentrations of a given biomarker should be relatively constant in that organism (Boschker and Middelburg, 2002).

1.4.1 Phospholipid Fatty Acid (PLFA) Biomarkers

Although the diversity and function of lipids is wide, a primary role of lipids is in the formation of cellular membranes usually composed of a glycerol-based phospholipid bilayer (Figure 1.1) (Dowhan and Bogdanov, 2002). Phospholipids are the primary building blocks of cellular membranes and the most common of the lipids (Dowhan and Bogdanov, 2002). Phospholipids are also turned over rapidly

upon cell death making them good indicators of the active bacterial and eukaryotic community (Boschker and Middelburg, 2002). The amount of viable biomass can be calculated from concentrations of PLFA using known conversions factors such as that found in Balkwill et al. (1988). However, some caution must be used in interpreting such results as different organisms can have different biovolumes that can vary with nutritional status (Guckert et al., 1985; White et al., 1995).

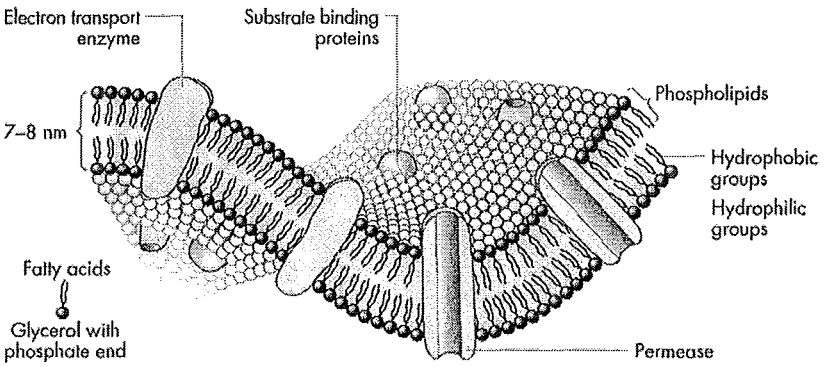

Figure 1.1: Cellular membrane made of a phospholipid bilayer (Konhauser, 2006).

Phospholipids are amphiphillic molecules that have a hydrophilic head group and hydrophobic fatty acid tails (Watson, 2006). Phospholipid fatty acids composed of glycerol that are ester linked to long chain fatty acids are found in the domains Bacteria and Eukarya (Boschker and Middelburg, 2002; Dowhan and Bogdanov, 2002). Fatty acids can be saturated, mono- or poly- unsaturated, branched, or cyclopropyl (Green and Scow, 2000). Depending on environmental stresses, bacteria can also change the lipid composition of their membranes in what is called 'homeoviscous adaptation'. Such adaptations are meant to maintain the cellular membrane in a liquid crystalline state allowing the cell to survive changes in environmental conditions (Van de Vossenberg et al., 1998; Hanford and Peeples, 2002). Nevertheless, some fatty acids may be diagnostic for certain groups of Bacteria and Eukarya. For example, fatty acids containing cyclopropyl units are more abundant in anaerobic than aerobic bacteria (Fang and Barcelona, 1998).

Methanotrophs have diagnostic fatty acids of 16 or 18 carbon chain lengths (Bowman et al., 1991) and sulfate reducers from the genus *Desulfobacter* can be identified in environmental samples by the biomarker 10me16:0 in the absence of 10me18:0 (Wakeham, 1995). Eukaryotes have membranes composed predominantly of polyunsaturated fatty acid chains and rarely have branched or cyclopropyl fatty acid chains (Madigan et al., 1997). However, polyunsaturates are also often found in cyanobacteria and therefore may be used as a biomarker for cyanobacteria in environments where eukaryotic activity is minimal (Kenyon, 1972; Jahnke et al., 2004).

Table 1.1: Common lipid biomarkers of Bacteria, Archaea, and Eukaryotes

Biomarker Class	Source Organisms	Potential Biomarkers
PLFA	Bacteria	i14:0, i15:0, a15:0, 15:0, 16:1w5, i17:0, 17:0 18:1w7c, cy19:0
	Gram + Bacteria	Branched PLFAs
	Gram - Bacteria	Monoenoic PLFAs
	Sulphate reducers	i17:1, 10Me16:0
	Desulfobacter	10Me16:0, cy18:0(w7,8)
	Desulfovibrio	i17:1w7c, i15:1w7c, i19:1w7c
	Desulfobulbus	17:1w6, 15:1
	Type I Methanotrophs	16:1w8c, 16:1w6c
	Type II Methanotrophs	18:1w8c or t, 18:1w6c
	Algae	20:5w3, 18:3w3
	Fungi	18:2w6c, 18:3w6c, 18:3w3c
	Actinomycetes	10Me17:0, 10Me18:0
	Protozoa	20:2w6, 20:3w6, 20:4w6
	Higher Plants, mosses	20:0, 21:0, 23:0, 24:0, 25:0, 26:0 (common, not diagnostic)
Ether Lipids	Methanogens	hydroxyarchaeol
	Crenarchaeota	cyclic tetra-ethers
	Archaea	archaeol
Sources: (Boschker and Middelburg, 2002; Green and Scow, 2000; Sundh et al., 1997)		

Phospholipid isoprenoid ether lipids (PLEL) consist of a glycerol that is ether linked to isoprenoid carbon skeletons (Dowhan and Bogdanov, 2002; Pancost and Sinninghe Damste, 2003). Archaeal PLEL are mainly composed of archaeol (diethers) with varying isoprenoid chain lengths and caldarchaeol (tetraethers) (Gattinger et al., 2002). Archaeol is the simplest of the archaeal lipids and can be used as a biomarker for the domain Archaea (Pancost and Sinninghe Damste, 2003). Hydroxyarchaeol, an ether lipid similar to archaeol but consisting of an extra

hydroxyl group, has been found almost exclusively in methanogens and may be used as a biomarker for this organism (Pancost and Sinninghe Damste, 2003). There are many other diagnostic lipids: see Table 1.1 for a compilation of biomarkers from literature.

Further insight into the microbial community composition and carbon cycling processes within an environment can be provided through carbon isotopic analysis. Carbon isotopic analysis can be performed on PLFA, organic carbon, inorganic carbon, and carbonates within a given environment. Microbial influences on the organic and inorganic carbon pools and controls on lipid isotopic composition are discussed below.

1.5 CARBON ISOTOPES AND CONTROLS ON LIPID ISOTOPIC COMPOSITION

Isotopes are atoms of the same element that have varying atomic masses due to variations in the number of neutrons in the nucleus (Faure, 1986). Carbon has three main isotopes: ^{12}C, ^{13}C, and ^{14}C of which ^{12}C and ^{13}C are stable isotopes and ^{14}C is radioactive with a half-life of 5730 years (Brocks and Pearson, 2005). ^{12}C is the most abundant of the carbon isotopes (98.9 %) followed by ^{13}C (1.1 %) and ^{14}C (10^{-10} %) (Brocks and Pearson, 2005). The abundance of stable isotopes is referenced to the heavy isotope and samples are always referenced to an internationally accepted standard, which for carbon is the carbonate rock Vienna Pee Dee Belemnite (VPDB) (Sessions, 2006). The relative $\delta^{13}C$ of a given sample is calculated as follows:

$$\delta^{13}C_{sample} = \frac{(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{std}) \times 1000\ ‰}{^{13}C/^{12}C_{std}} \qquad (1.1)$$

There are many factors that influence the isotopic composition of carbon-based compounds. The source of substrate carbon, biological mechanism of carbon assimilation, and controls on carbon composition of lipids will be discussed below.

1.5.1 Source of Substrate Carbon

The primary controls on the isotopic composition of microbial biomass and microbial lipids are the original isotopic composition of the carbon source and the biological process by which the carbon source is assimilated by the organism (Pancost and Sinninghe Damste, 2003). Different organisms have different carbon sources and assimilatory pathways, and these can play an important role in the resulting isotopic composition of microbial biomass and the products of their metabolic reactions (Figure 1.2). For example, photoautotrophs assimilate inorganic carbon sources such as CO_2 or HCO_3^- and produce organic matter whereas heterotrophs use organic substrates such as carbohydrates and short-chain acids (ex. acetate) to produce a variety of compounds including inorganic carbon (Pancost and Sinninghe Damste, 2003).

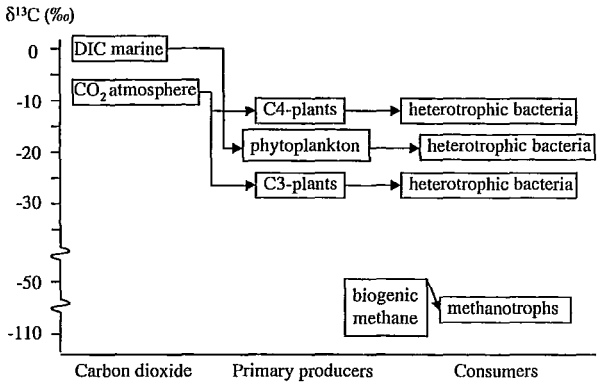

Figure 1.2: Isotopic signatures of DIC, CO_2, primary producers and consumers (Boschker and Middelburg, 2002).

The species of inorganic carbon is an important component when considering the carbon source for autotrophic microorganisms because there are significant isotope effects ($\Delta^{13}C$ of 4-10 ‰) associated with equilibrating between CO_2 and HCO_3^- (Equation 1.2) (Szaran, 1997; Brocks and Pearson, 2005; Conrad, 2005).

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+ \quad (1.2)$$

$\delta^{13}C$ values of HCO_3^- typically range from 1 to -1 ‰ and $CO_{2(aq)}$ values typically range from -6 to -10 ‰, however, these values can be significantly more depleted in environments where much of the dissolved inorganic carbon (DIC) is derived from highly depleted methane (Whiticar, 1999; Pancost and Sinninghe Damste, 2003). In contrast, utilization of DIC in closed or semi-closed systems can cause the

$\delta^{13}C$ values of the residual DIC to become enriched in ^{13}C (Whiticar, 1999; Pancost and Sinninghe Damste, 2003). The speciation of inorganic carbon is dependent on the pH of the system, whereas the isotopic distribution is primarily a function of the temperature in a given environment (Brocks and Pearson, 2005).

1.5.2 Biological Mechanism of Carbon Assimilation

The pathways by which microorganisms assimilate carbon can also cause kinetic isotope effects and cause further changes to the isotopic signature of carbon end products, which are then used by other microorganisms. Kinetic isotope effects (KIE) are associated with biological reactions and uptake of substrate carbon. This is governed at the atomic level by the fact that bonds containing ^{13}C require more energy to break than bonds containing ^{12}C and as a result microbial processes discriminate against ^{13}C (Brocks and Pearson, 2005). When carbon is taken up from the environment and fixed into microbial biomass specific biological enzymes and metabolic pathways control the KIE and they can be quite variable (Hayes, 2001; Brocks and Pearson, 2005).

There are many different pathways of inorganic carbon fixation by autotrophs and isotopic fractionations associated with different enzymatic steps in their metabolic processes. As a result, microbial biomass can be quite depleted relative to the substrate carbon (Hayes, 2001). Perhaps the most well known fractionation that occurs during carbon fixation occurs using the Calvin Cycle or C3 pathway (O'Leary, 1988). This pathway reduces CO_2 using the enzyme Rubisco that eventually leads to the production of isotopically depleted organic matter of ~ -22 to -35 ‰ (Hayes, 2001; Pancost and Sinninghe Damste, 2003). Organic matter is then used by heterotrophs in respiratory metabolism, however, the respiratory metabolism used by most heterotrophs results in very little carbon isotopic fractionation (Brocks and Pearson, 2005). Heterotrophic bacteria display the 'you are what you eat' principal in that their products generally show little ($\Delta^{13}C \approx$ 1-2 ‰) fractionation relative to the isotopic signature of their substrate carbon (Boschker and Middelburg, 2002).

1.5.3 Carbon Isotopic Composition of Lipids

Carbon that is used for metabolic processing by autotrophic and heterotrophic microorganisms, and which may already be isotopically depleted, is also used to produce lipids. However, pathways of lipid synthesis can cause further isotopic depletion in resulting carbon that is incorporated into microbial lipids. The pathway of lipid synthesis for the production of *n*-alkyl fatty acids is discussed briefly below.

Acetyl Co-A is an important molecule in biological metabolism and catalyzes the first step in the synthesis of *n*-alkyl fatty acids (Heath et al., 2002). Degradation of carbohydrates produces pyruvate, which is then decarboxylated into Acetyl Co-A by the enzyme pyruvate dehydrogenase (Hayes, 2001). This step, along with competition for the use of pyruvate in other biological processes, is associated with KIE and is considered to be the cause of the ^{13}C depletion in fatty acids (Hayes, 2001; Heath et al., 2002). Other factors may also play a role in the isotopic signature of fatty acids including other sources of Acetyl Co-A and reaction competition for Acetyl Co-A. The Acetyl Co-A pathway for lipid synthesis occurs in all domains of life and the isotopic fractionations that occur through this pathway of fatty acid synthesis result in an approximate 4-6‰ depletion of lipids relative to the microbial biomass (Blair et al., 1985; Hayes, 1993; Hayes, 2001; Boschker and Middelburg, 2002).

1.6 MICROBIAL CYCLING IN ACID-SULFATE ENVIRONMENTS

Acid-sulfate environments occur globally through both natural processes as well as anthropogenic mining activities (Baker and Banfield, 2003; Johnson and Hallberg, 2003). The oxidation of sulfide minerals through exposure to water and oxygen results in the formation of acidic water and the precipitation of iron and sulfur minerals, such as jarosite and hematite, from solution (Johnson and Hallberg, 2003). While the oxidation of sulfide minerals can occur abiotically, microbial activity within acid sulfate environments can catalyze the oxidation of sulfides and

greatly increase rates of acid generation (Nordstrom and Southam, 1997; Baker and Banfield, 2003). Pyrite (FeS_2) is the most abundant sulfide mineral on Earth and, therefore, plays an important role in the formation of acid sulfate environments (Johnson and Hallberg, 2003). The steps involved in the dissolution of pyrite and formation of acidic waters are listed below:

$$14Fe^{2+} + 3.5O_2 + 14H^+ \rightarrow 14Fe^{3+} + 7H_2O \quad (1.3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (1.4)$$

FOR THE TOTAL REACTION:

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1.5)$$

The dominant pathway for the oxidation of pyrite is for the initial oxidation of ferrous (Fe^{2+}) to ferric iron (Fe^{3+}) by oxygen (Equation 1.3) as Fe^{3+} is a better sulfide oxidant than oxygen (Baker and Banfield, 2003). Subsequently, sulfide is oxidized by Fe^{3+} (Equation 1.4) for the total reaction shown in Equation 1.5. The resulting H^+ that is formed from the above reactions decreases the pH of the system thereby creating the acidic conditions found in these environments (Baker and Banfield, 2003). The oxidation of Fe^{2+} to Fe^{3+} by O_2 in acidic environments is kinetically slow and can therefore limit pyrite dissolution. However, iron-oxidizing bacteria can catalyze this oxidation and can therefore greatly influence the dissolution of pyrite and the subsequent formation of acidic waters (Nordstrom and Southam, 1997; Baker and Banfield, 2003). The acidic conditions (often pH <2) that form through the dissolution of pyrite select for microbial communities able to utilize the iron and sulfur substrates available, as well as able to withstand the acidic conditions. As a result, the microbial community within acid-sulfate environments is dominated by acidophilic iron and sulfur utilizing organisms.

Most acid sulfate environments are characterized by a low diversity of organisms likely due to the limited substrates available as energy sources for microbial metabolisms. Acidophilic iron oxidizing organisms such as *Acidithiobacillus ferrooxidans* (an iron and sulfur oxidizer) and *Leptospirrilum ferrooxidans* (an iron oxidizer) play a central role in the microbial community in

acid-sulfate environments as they are responsible for catalyzing the oxidation of Fe^{2+} to Fe^{3+} (Johnson and Hallberg, 2003). Sulfur oxidizing organisms such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* as well as acidophilic heterotrophs from the generas *Acidiphilium, Acidocella*, and *Ferromicrobium* are also common members of acid sulfate microbial communities (Nordstrum et al., 2000; Johnson et al., 2001; López-Archilla and Amils, 2001; Johnson and Hallberg, 2003). Photosynthetic eukaryotes, such as *Cyanidium caldarium*, may also have an important role in primary productivity in acidic environments that receive light (Johnson and Hallberg, 2003).

Different organisms may be present within an acidic environment depending on the pH, temperature, and metal concentrations. For example, *At. ferrooxidans* is more tolerant of low temperature environments and moderately acidic pH while *L. ferrooxidans* has a lower limit growth temperature of ~20°C and can tolerate pH conditions <1.3 (Rawlings et al., 1999; Johnson and Hallberg, 2003). Nevertheless, microbial communities within such environments are dominated by a few distinct taxa (Baker and Banfield, 2003). Microbial cycling of iron, sulfur, and carbon found in most acid-sulfate environments at various temperatures is depicted in Figure 1.3.

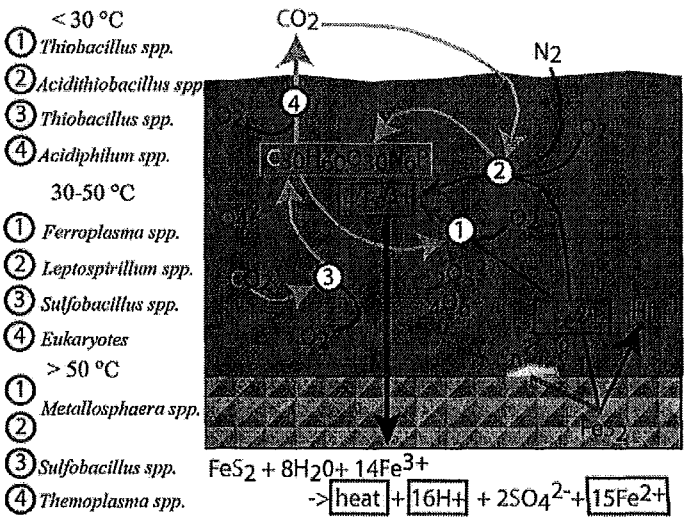

Figure 1.3: Microbial cycling of iron, sulfur, and carbon in acid rock drainage/acid mine drainage environments and dominant organisms at various temperatures (Baker and Banfield, 2003).

1.7 MICROBIAL CYCLING IN SALINE ENVIRONMENTS

Saline lakes and ephemeral ponds are dynamic environments due to seasonal changes in evaporation and precipitation, which influence salt concentrations and lake depths (Bauld, 1981). Microbial communities that dominate both permanent and transiently present saline bodies of water are usually arranged as microbial mat communities (Bauld, 1981). Microbial mats colonize benthic surfaces and are composed of a consortium of organisms that form laminated, cohesive structures that have varying preservation potential (Bauld, 1981; Jones, 1998). Microbial mat communities preserved as stromatolite structures in Precambrian carbonates provide evidence for the presence of life on Earth ~3.5 Ga (Grotzinger and Knoll, 1999; Riding, 2000). The decline in the abundance of stromatolites in the geologic record is hypothesized to be due to grazing after the emergence of metazoan heterotrophs (Schidlowski et al., 1984). Stresses by grazing within common aquatic habitats on Earth have led to a decline in the formation of stromatolite structures except for within extreme environments, such as hypersaline basins, which are unfavourable for predators and where grazing by other organisms is limited (Bauld, 1981).

The microbial mats of most saline lakes are dominated by cyanobacteria, which are autotrophic photosynthetic bacteria that couple light energy and CO_2 fixation to produce organic matter (Dupraz and Visscher, 2005). Cyanobacteria are the primary producers of microbial mat communities and are responsible for the high rates of photosynthetic activity and primary productivity associated with saline lakes (Grant et al., 1990; Jones, 1998). Other members of the microbial mat community include: anoxygenic phototrophs, aerobic heterotrophic bacteria, fermenters, anaerobic heterotrophs (predominantly sulfate reducing bacteria), and sulfide oxidizing bacteria (Figure 1.4) (Dupraz and Visscher, 2005).

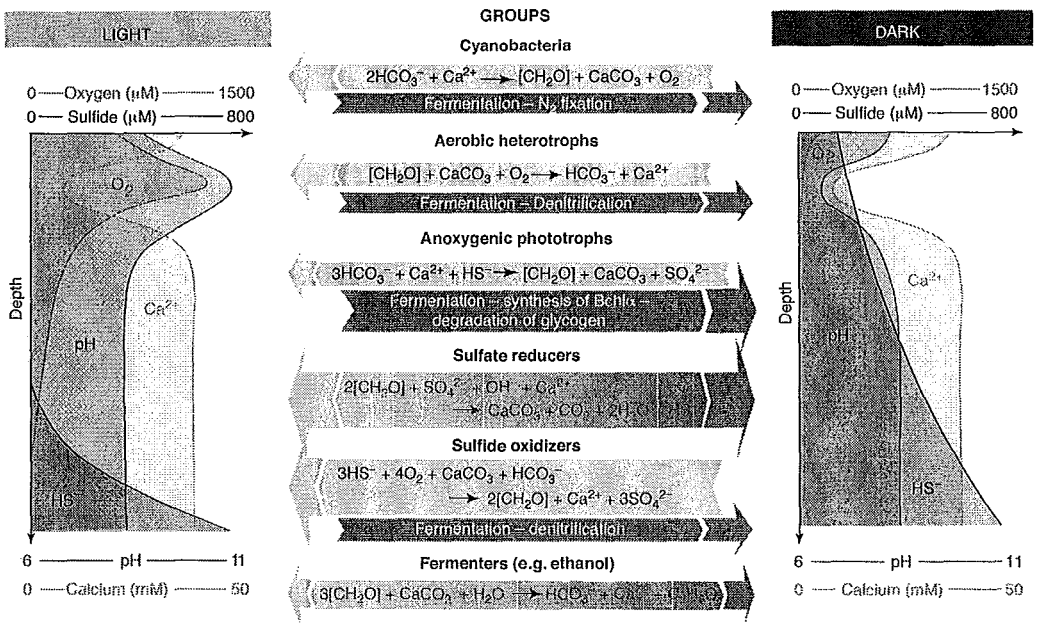

Figure 1.4: Microbial processes and geochemical gradients within microbial mat dominated environments (Dupraz and Visscher, 2005).

Cyanobacteria are believed to have a major role in the precipitation of carbonates and lithification of microbial mats via two main mechanisms. The first is through the trapping and binding of sediments by mucus of filamentous and coccoid cyanobacteria (Merz, 1992). The second mechanism is via photosynthetic uptake of CO_2, which results in shifts in concentration gradients of carbonate and bicarbonate, influencing local pH and facilitating the precipitation of carbonates from solution (Thompson et al., 1997; Altermann et al., 2006). The utilization of dead cyanobacteria material by heterotrophs deeper within microbial mats is also believed to influence the lithification of microbial mats (see Altermann et al., 2006). Sulfate reducing bacteria are also believed to have an important role in the lithification of microbial mats, as crustal regions within microbial mats are often associated with the area of highest sulfate reduction (Lyons et al., 1984; Walter et al., 1993; Visscher et al., 2000). Sulfate reduction can also influence the local pH within microbial mat microenvironments thereby increasing carbonate saturation

and the subsequent precipitation of carbonate minerals from solution (Visscher et al., 2000; Dupraz and Visscher, 2005).

1.7.1 Autotrophic Effects on Isotopic Signatures

Microbial carbon cycling within microbial mats also influences the isotopic signature of the organic and inorganic carbon pools, as well as precipitated carbonates. The preferential utilization of ^{12}C over ^{13}C during photosynthesis by cyanobacteria results in a ^{13}C enrichment of the remaining inorganic carbon pool (O'Leary, 1988). Such photosynthetically induced changes in geochemistry can result in system-wide effects or they may be restricted to local geochemical changes within microbial mat microenvironments (Hollander and McKenzie, 1991; Thompson et al., 1997; Andres et al., 2006; Brady et al., 2010). Nevertheless, the precipitation of carbonates facilitated by cyanobacterial activity can therefore lead to the precipitation of ^{13}C-enriched carbonates from solution (O'Leary, 1988; Hollander and McKenzie, 1991; Merz, 1992; Brady et al., 2010). If diffusion of inorganic carbon is slow within microbial mat microenvironments, photosynthesis by cyanobacteria can result in further enrichment of locally precipitated carbonates compared to carbonates that precipitate from ambient water (Rosen et al., 1995; Sumner, 2001). Carbonate precipitation would have to occur within the microbial mat during periods of rapid CO_2 fixation by cyanobacteria in order to preserve these microbial biosignatures (Sumner, 2001). High rates of photosynthesis and CO_2 utilization by cyanobacteria within hypersaline systems can also result in CO_2 limitation within these environments (Schidlowski, 1984; O'Leary, 1988; Schouten et al., 2001). Decreases in concentration of available CO_2 results in less discrimination between ^{12}C and ^{13}C which can result in the production of isotopically heavy (^{13}C-rich) organic matter, up to 5 ‰ in some hypersaline systems (Schidlowski, 1984; Des Marais et al., 1989; Hollander and McKenzie, 1991; Schouten et al., 2001).

1.7.2 Heterotrophic Effects on Isotopic Signatures

In contrast, heterotrophic utilization of ^{13}C depleted organic matter results in the production of ^{13}C-depleted inorganic carbon and can result in the precipitation of isotopically depleted carbonates (Blair et al., 1985; Andres et al., 2006; Breitbart et al., 2009). Therefore, precipitation of carbonates during periods where heterotrophic activity dominates can dilute the isotopic signatures produced by cyanobacteria (Sumner, 2001). As heterotrophic sulfate reducers have an important role in carbonate precipitation, precipitation in sulfate-rich waters will likely dilute the influences of the autotrophic cyanobacteria on the isotopic composition of precipitated carbonates (Sumner, 2001).

1.8 THESIS STRUCTURE

Chapter 1 provided an overview of the analogue environments of interest in this thesis research and their relevance to environments present on early Mars. It also provided background information on fundamental concepts and a general overview of the geochemical and microbial processes that occur in acid-sulfate environments and saline lakes.

Chapter 2 and Chapter 3 are written in paper format. Chapter 2 will focus on microbial carbon cycling in the Golden Deposit, Northwest Territories, Canada. The Golden Deposit is an area of natural acid rock drainage, which is precipitating out of sulfur and iron bearing groundwater seeps. Mixing of groundwater and surface water is resulting in geochemical variability on the Golden Deposit and is influencing the microbial communities found on the deposit. The influence of the geochemistry on the spatial variability of the microbial community composition and isotopic signatures within the Golden Deposit will be explored.

Chapter 3 will focus on microbial carbon cycling and biosignatures within the saline lakes of the Cariboo Plateau, British Columbia, Canada. Three saline lakes were chosen for this study based on variations in surface and porewater chemistries. Each lake contains benthic, microbial mat communities which are

dominated by cyanobacteria. Photosynthesis by cyanobacteria influences the isotopic signatures of organic and inorganic carbon pools within each lake. The influence of the cyanobacteria-dominated microbial mats on the carbon isotopic signatures of organic and inorganic carbon and the preservation of biosignatures in the sediments of each lake will be explored.

Chapter 4 contains the conclusions of this thesis. This chapter will provide an overview of the research completed within this thesis and identify the importance of this research in furthering our knowledge on Mars analogue environments. It will also identify future avenues of research that will aid in the current knowledge on biosignatures in Mars analogue sites.

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CHAPTER 2

MICROBIAL CARBON CYCLING IN THE GOLDEN DEPOSIT, N.W.T., CANADA: AN ANALOGUE TO SULFATE DEPOSITS ON MARS

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ABSTRACT

The Golden Deposit represents an analogue to acidic sulfate-rich conditions that existed during the formation of jarosite and hematite deposits found on the surface of Mars today. The peat surrounding the Golden Deposit supported a high number and diversity of organisms and appeared to influence the microbial community on the deposit as demonstrated by the similar microbial distributions and the consistent $\delta^{13}C_{TOC}$ (~ -26 ‰) both on and off the deposit. The absence of methanogenic and methanotrophic biosignatures, but presence of sulfate reducer biomarkers (10me16:0), indicated that the high S and SO_4^{2-} of groundwater flowing through the region was allowing sulfate reducers to outcompete methanogens in these soils. The water chemistry of the Golden Deposit exhibited a large degree of variability caused by the mixing of surface water and upwelling acidic groundwater and was influencing the microbial communities on the deposit. Such variability was reflected in the distribution of PLFA and differences in $\Delta^{13}C_{TOC\text{-}PLFA}$, which ranged from 0.6 to 6.8 ‰. The most acidic site on the deposit likely contained organisms such as *At. ferrooxidans* and *Desulfobacter* as demonstrated by the high proportions of monounsaturates, in particular 16:1Δ9*cis*, and the presence of 10me16:0. The variability in geochemistry and microbial communities and their associated isotopic signatures within the small spatial area of the Golden Deposit indicate that careful interpretation will be required for preserved microbial and isotopic signatures on Mars.

2.1 INTRODUCTION

The surface of Mars is a dry, cold, and seemingly inhospitable place for life to survive (Southam et al., 2007). However, iron and sulfur minerals, including hematite and jarosite, have recently been detected on the surface of Mars and indicate past deposition involving acidic waters (Squyres et al., 2004a; Squyres et al., 2004b). Extreme acidic environments on Earth, such as the Rio Tinto Basin in Spain, have been found to harbour a large diversity of organisms and indicate that similar environments in Mars' past could have supported life (López-Archilla et al., 2001; Amils et al., 2006; Bibring et al., 2006). The Golden Deposit in the Canadian Arctic is a jarositic mineral deposit, which is being precipitated out of acidic iron-bearing groundwater seeps and is surrounded by peat and permafrost (Michel and van Everdingen, 1987). The mineralogy of the site and its location in a cold, permafrost environment makes it an analogue to the conditions present during the formation of such deposits on Mars (Michel and van Everdingen, 1987; Squyres et al., 2004a; Battler et al., unpublished).

As the fundamental basis of life on Earth, carbon is an attractive element for the foundation of life should it exist elsewhere in the universe (Pace, 2001; Summons et al., 2008). By studying microbial carbon cycling, microbial biomarkers, and their associated carbon isotopic signatures ($\delta^{13}C$) within Mars analogue sites, such as the acid sulfate environment of the Golden Deposit, we gain a better understanding of carbon cycling processes in such chemically harsh environments and identify target signatures of life to search for in similar deposits on Mars.

2.1.1 Potential for Life on Mars

The environment thought to exist on early Mars, with active fluvial, glacial, and volcanic activity, is comparable to conditions when life first originated on Earth suggesting that Mars could also have supported life (Bibring et al, 2006; Southam et al., 2007). The rover Opportunity's landing at Meridiani Planum on Mars found sulfate-rich sedimentary rocks that indicate local deposition in acidic sulfate-rich

liquid water followed by evaporation and desiccation (Squyres et al., 2004a; Squyres et al., 2004b). It is hypothesized that after a period of heavy bombardment by meteorites on the planet, the local climate shifted from an alkaline, aqueous environment to a saline, aqueous, acid-sulfate environment (Bibring et al., 2006). Acid-sulfate systems on Mars are similar to acid mine drainage/acid rock drainage (AMD/ARD) environments on Earth today, such as the Rio Tinto Basin in Spain, which are able to support a wide diversity of life (Gonzalez-Toril et al., 2003). Therefore such environments on Mars could have supported life, biosignatures of which may still be preserved in its subsurface where they are protected from the strong oxidizing conditions on Mars today (Klein et al., 1976; Gonzalez-Toril et al., 2003; Squyres et al., 2004b; Amils et al., 2006).

Currently, life on Earth is the only form available for us to study. By studying life in extreme environments that are analogues to other planets we can learn the physical and chemical limits of habitability allowing us to better understand the signatures of life that may be found elsewhere in the universe (Javaux, 2006). Extreme environments currently present on Earth include both physical and geochemical extremes such as the cold, dry conditions of the McMurdo Dry Valleys, Antarctica, and acidic sulfate and iron mineral deposits such as the Golden Deposit, N.W.T., Canada and the Rio Tinto River, Spain (Michel and van Everdingen, 1987; Rothschild and Mancinelli, 2001; Amils et al., 2007; Gilichinsky et al., 2007). By studying Mars analogue sites on Earth, we gain a better understanding of microbial cycling within such environments and the biological signs of life or 'biosignatures' that life may leave behind in such environments.

2.1.2 Microbial Cycling in Acid-Sulfate Environments

Pyrite is an important source of energy for sulfur- and iron-oxidizing microorganisms on Earth. The products of their metabolisms can result in the production of hematite and jarosite, which could suggest that these minerals on Mars have a biological origin (Baker and Banfield, 2003; Amils et al., 2006). The

presence of high UV radiation on the planet could pose a challenge for the origin of life; however, soluble ferric iron solutions and iron minerals can shield cells and act as a protective agent against UV radiation (Phoenix et al., 2001; Gomez et al., 2007).

Natural ARD environments can be produced by chemical weathering of sulfide-rich rocks such as pyrite ($Fe^{(II)}S_2$) and can result in the subsequent precipitation of iron and sulfur minerals including hematite ($Fe^{(III)}_2O_3$) and jarosite ($KFe^{(III)}_3(OH)_6(SO_4)_2(OH)_6$) out of solution (Johnson and Hallberg, 2003). Pyrite dissolution is caused by the oxidation of ferrous (Fe^{2+}) to ferric iron (Fe^{3+}) by oxygen (Equation 2.1) and the subsequent oxidation of sulfide by Fe^{3+} (Equation 2.2) (Baker and Banfield, 2003) for the total reaction shown in Equation 2.3.

$$14Fe^{2+} + 3.5O_2 + 14H^+ \rightarrow 14Fe^{3+} + 7H_2O \quad (2.1)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (2.2)$$

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (2.3)$$

The resulting H^+ that is formed from the above reactions decreases the pH of the system thereby creating the acidic conditions found in these environments (Baker and Banfield, 2003). The oxidation of Fe^{2+} to Fe^{3+} by O_2 in acidic environments is kinetically slow and can therefore limit pyrite dissolution; however, iron-oxidizing bacteria can catalyze this oxidation and can therefore greatly influence the dissolution of pyrite and the subsequent formation of acidic waters (Nordstrom and Southam, 1997; Baker and Banfield, 2003).

The most common organisms encountered in AMD/ARD environments are the lithoautotrophs *Acidithiobacillus ferrooxidans* (an iron and sulfur oxidizer), *Leptospirrilum ferrooxidans* (an iron oxidizer), and *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* (sulfur oxidizers) along with acidophilic heterotrophs from the generas *Acidiphilium, Acidocella,* and *Ferromicrobium* (Nordstrum et al., 2000; Johnson et al., 2001; López-Archilla and Amils, 2001; Johnson and Hallberg, 2003). In most AMD/ARD environments either *At. ferrooxidans or L. ferrooxidans* is the dominant iron-oxidizing organism depending on redox potential, pH, and temperature (Rawlings et al., 1999). *At. ferrooxidans* is more tolerant of low

temperature environments and moderately acidic pH while *L. ferrooxidans* has a lower limit growth temperature of ~20°C and can tolerate pH conditions <1.3 (Rawlings et al., 1999; Johnson and Hallberg, 2003).

2.1.3 Microbial Lipid and Isotopic Biosignatures

The distribution and isotopic signature of lipids in an environment can be indicative of specific microbial groups. Therefore, the combination of lipid and isotope analyses can be used to elucidate microbial processes (Boschker and Middelburg, 2002). Phospholipid fatty acids (PLFA) are the primary building blocks of cellular membranes and are useful indicators of the active bacterial and eukaryotic community as they rapidly turnover upon cell death (Boschker and Middelburg, 2002; Dowhan and Bogdanov, 2002). Fatty acids can be saturated, mono- or poly- unsaturated, branched, or cyclopropyl and can vary significantly among different groups of organisms (Green and Scow, 2000). This makes PLFA useful tools as biological markers or 'biomarkers' of microbial groups (Eglinton et al., 1964). For example, cyclopropyl fatty acids are more abundant in anaerobic than aerobic bacteria (Fang and Barcelona, 1998) and methanotrophs have diagnostic monounsaturated fatty acids of 16 or 18 carbon chain lengths (Bowman et al., 1991). Eukaryotes have membranes composed predominantly of polyunsaturated fatty acid chains and rarely have branched or cyclopropyl fatty acid chains (Madigan et al., 1997) while sulfate reducers from the genus *Desulfobacter* can be identified in environmental samples by the biomarker 10me16:0 in the absence of 10me18:0 (Wakeham, 1995).

The primary controls on the isotopic composition of microbial biomass and microbial PLFA are the original isotopic composition of the carbon source and the biological process by which the carbon source is assimilated by the organism (Hayes, 2001; Pancost and Sinninghe Damste, 2003). The species of inorganic carbon is an important component when considering the carbon source for autotrophic microorganisms as there are significant isotope effects ($\Delta\delta^{13}C$ of 4-10

‰) associated with equilibrating between CO_2 and HCO_3^- (Equation 2.4) (Szaran, 1997; Brocks and Pearson, 2005; Conrad, 2005).

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+ \quad (2.4)$$

The speciation of inorganic carbon is dependent on the pH of the system, whereas the isotopic distribution is primarily a function of the temperature in the given environment (Brocks and Pearson, 2005). $\delta^{13}C$ values of $CO_{2(aq)}$ typically range from -6 to -10 ‰ while values of HCO_3^- typically range from 1 to -1 ‰ (Whiticar, 1999; Pancost and Sinninghe Damste, 2003). There are many different pathways of inorganic carbon fixation by autotrophs and isotopic fractionations associated with different enzymatic steps in their metabolic processes result in isotopically depleted biomass relative to the substrate carbon (Hayes, 2001). Similarly, the organic matter end products of autotrophy are depleted due to fractionation during metabolism. Isotopically depleted organic matter is then used by heterotrophs in respiratory metabolism to produce inorganic carbon with similar $\delta^{13}C$ values to substrate organic matter and results in a ^{13}C depletion of the inorganic carbon pool (Blair et al., 1985). The respiratory metabolism used by most heterotrophs results in very little carbon isotopic fractionation of microbial biomass relative to the organic carbon source ($\Delta^{13}C \approx 1$ ‰) (Blair et al., 1985; Hayes, 2001; Boschker and Middelburg, 2002). In general, the process of lipid synthesis is similar in both autotrophs and heterotrophs and as a result, most fatty acids are ~4-6 ‰ depleted relative to biomass (Hayes, 1993; Boschker and Middelburg, 2002).

Carbon is the fundamental basis of life on Earth and is one of the most abundant elements in the universe. Its ability to readily form chemical bonds with other elements and its chemical versatility makes it an attractive element as the foundation for life should it exist elsewhere in the universe (Pace, 2001; Summons et al., 2008). By studying microbial carbon cycling in Mars analogue sites we gain a better understanding of microbial cycling processes that may have existed in similar systems on Mars. Investigation of microbial lipid and isotopic signatures within

analogue sites can help us identify signatures of life that exist within these environments and may be preserved on Mars. If life on Mars evolved early in its history, it would have adapted to the strong acidic conditions present on the planet. The acidic conditions, mineralogy and water chemistry of the Golden Deposit represent an environment of similar state to what may have existed on Mars, and therefore can be used as an analogue for habitability in such environments. This study focused on understanding microbial carbon cycling, microbial biomarkers, and their associated carbon isotopic signatures ($\delta^{13}C$) within the Golden Deposit to gain a better understanding of carbon cycling processes in AMD/ARD environments and allow us to identify target signatures to search for life in similar deposits on Mars.

2.2 SAMPLING AND LABORATORY ANALYSIS

2.2.1 Study Site

The Golden Deposit is located 100 km east of Norman Wells, NWT, Canada (65°11'58" N, 124°38'15"W) and consists of a yellow-ochre which is precipitating out of acidic iron-bearing groundwater seeps (Michel and van Everdingen, 1987). It is approximately 140 m long and 50 m wide, is surrounded by muskeg-type (peat) vegetation and is underlain by permafrost (Battler et al., unpublished; Michel and van Everdingen, 1987). It is composed primarily of natrojarosite ($NaFe_3(SO_4)_2(OH)_6$), jarosite ($KFe_3(SO_4)_2(OH)_6$), some OH-rich jarosite ($(KH_3O)Fe_3(SO_4)_2(OH)_6$), and goethite ($FeO(OH)$) (Michel and van Everdingen, 1987; Battler et al., unpublished). Negative $\delta^{34}S$, near zero $\delta^{18}O$, and high trace metal concentrations indicate that aqueous sulfate is derived from the oxidation of sulfides from subsurface flow through dolomite and pyritiferous shales (Michel and van Everdingen, 1987). Approximately 44-49 % of the oxygen in the sulfate of the Golden Deposit is derived from water molecules and *Acidithiobacillus ferrooxidans* or similar bacteria are likely involved in the oxidation process (van Everdingen et al., 1985).

2.2.2 Field Sampling

The Golden Deposit was sampled in mid September 2008 and sample locations are shown in Figure 2.1a. SITE 5 was the inflow site of surface water from the surrounding environment and SITE 1 was the outflow site of the Golden Deposit which flowed into Pond 2. Sediment samples were collected for PLFA and $\delta^{13}C_{TOC}$ analysis from a dry crustal region not in contact with flowing water, a groundwater seep, and the water outflow site (SITE 5_{CRUST}, SITE 2, and SITE 1, respectively) in 500 mL precombusted glass jars and stored frozen until analysis. Two cores, CORE 1 and CORE 2, from the surrounding peat were taken at SITE 6 to depths of 42 cm and 25 cm, respectively, subsectioned on site and stored in Ziplock® or Whirlpack® bags. The bottom of CORE 2 appeared to be old, buried deposit dominated by jarosite consistent with mineralogical analyses that detected jarositic sediments under surrounding vegetation extending approximately 40 m outward from the Golden Deposit and to a depth of approximately 50 cm (Battler et al., unpublished).

Water samples were collected for water chemistry analysis from 5 sites on the deposit (SITEs 1 to 5), the outflow Pond 2 and a nearby lake, Airplane Lake, using precleaned polyethylene bottles. Samples were kept cool and in the dark following the protocol by the Environment Canada's Pacific Environmental Science Centre (PESC) in Vancouver, Canada. Water samples were collected for $\delta^{13}C_{DIC}$ from SITE 1, 2, 4 and 5 as well as Pond 2, Airplane Lake and CORE 1 in crimp sealed gas serum bottles with no headspace and fixed with mercuric chloride to prevent further microbial activity. Temperature was measured to the nearest 0.5 °C at each site using a handheld thermometer. Upon return to base camp, unfiltered samples were measured for pH with a Hannah pH meter that was calibrated to buffers daily. Conductivity levels were measured using a Yellow Springs Instrument temperature/conductivity/salinity meter. Samples were collected for methane gas analysis using a 60 cc. syringe and injected into crimp sealed pre-evacuated 160 mL Wheaton serum bottles that were fixed with mercuric chloride.

2.2.3 Laboratory Analysis

Organic and inorganic carbon contents of dry Golden Deposit sediment and core samples were determined using loss on ignition (LOI). Percent total organic carbon content (TOC) was determined by the mass difference of the original dry sample (105°C overnight) to that after combustion at 550°C for 4hrs. Percent inorganic carbon content was determined by the mass difference of the dry weight sample after combustion at 550°C to that after heating at 950°C for 2 hrs (Heiri et al., 2001). Percent error was < 2.5 %.

Golden Deposit samples were freeze-dried prior to extraction of phospholipid fatty acids (PLFA). PLFA were extracted using a modified Bligh and Dyer method (Bligh and Dyer, 1959) and fractioned into non-polar, neutral and polar lipids using silica gel chromatography. The polar fraction was subjected to mild alkaline methanolysis to convert PLFA into fatty acid methyl esters (FAMEs) (Guckert et al., 1985). Methanolyzed samples were fractionated using silica gel chromatography to remove any impurities. Microbial FAMEs were separated using gas chromatography mass spectrometry (GC/MS) on an Agilent GC/MS with DB-XLB capillary column (30 m x 0.25 mm I.D. x 0.25 μm film thickness) using a temperature program of 40°C (1 min.), 20°C/min to 130°C, 4°C/min to 160°C, 8°C/min to 300°C (5 min). PLFA were identified based on retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc., Pleasant Gap, Pennsylvania, USA, and PUFA-3 Menhaden Oil, Supelco Analytical, Oakville, ON, Canada) and characteristic ion fragments. Monoenoic double-bond positions and geometry were determined by derivitization with dimethyl disulfide and subsequent analysis by GC/MS (Nichols et al., 1986). The presence of archaeal diether lipids was determined by derivitization of polar lipids using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and subsequent analysis by GC/MS (Teixidor and Grimalt, 1992). Cell density was calculated from the total PLFA using the conversion factor $2x10^4$ cells/pmol PLFA from Balkwill et al. (1988).

Fatty acid nomenclature is designated as follows; A:BΔC, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position (Δ) of the double bond from the carboxyl end of the molecule. The geometry of the bond is indicated as 'c' for *cis* and 't' for *trans*. The prefix 'br' indicates a branched fatty acid whose branch position is unknown. Known branch positions are denoted as the branch location followed by the total number of carbon atoms. *Iso-* and *anteiso-* branching are denoted by the prefixes 'i' or 'a', respectively and cyclopropyl fatty acids are denoted by the prefix 'cy'.

Samples collected for methane gas analysis were run via manual injections on a split/splitless injector Agilent GC-MS with a GS-Q capillary column (30 m x 0.32 mm I.D.) with the temperature held isothermal at 26°C. No methane gas was detected in any peat water samples.

2.2.4 Isotopic Analysis

The carbon isotopic composition of the PLFA ($\delta^{13}C_{PLFA}$), total organic carbon ($\delta^{13}C_{TOC}$), and dissolved inorganic carbon ($\delta^{13}C_{DIC}$), are reported using standard delta notation ($\delta^{13}C$) and referenced to the internationally accepted standard carbonate rock Vienna Pee Dee Belemnite (VPDB) (Sessions, 2006). The relative $\delta^{13}C$ of a given sample is calculated as follows:

$$\delta^{13}C_{sample} = \frac{(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{std}) \times 1000‰}{^{13}C/^{12}C_{std}} \qquad (2.5)$$

Aliquots of microbial FAMEs were injected into a split/splitless injector set to splitless mode at 300°C prior to separation using a GC/MS on an Agilent GC/MS with DB-XLB capillary column (30 m x 0.32 mm I.D. x 0.25 μm film thickness) and a temperature program of 80°C (1 min.), 4°C/min to 280°C, 10°C/min to 320°C (20 min). Individual FAMEs were eluted from the column via a combustion oven set at 960°C, were combusted to CO_2 and analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS).

The methanol added to the FAMEs during mild alkaline methanolysis was isotopically characterized and $\delta^{13}C_{PLFA}$ values were corrected using the following equation:

$$\delta^{13}C_{PLFA} = [(N+1)* \delta^{13}C_{measured} - \delta^{13}C_{MeOH}]/N \quad (2.6)$$

where N is the number of carbon atoms. Individual samples were analyzed in triplicate and precision is reported as one standard deviation (s.d.).

$\delta^{13}C_{TOC}$ of Golden Deposit and core samples was determined by treating dry samples with 1 M HCl to remove carbonate. Isotopic values were determined on an EA-Delta XL at McMaster University and triplicate analyses gave a precision of ± 0.4 ‰ (1 σ) for the $\delta^{13}C_{TOC}$. $\delta^{13}C_{DIC}$ of the water samples was determined by acidification and conversion to CO_2 and analyzed by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa (St-Jean, 2003). Analytical precision was ± 0.2 ‰.

The equilibrium concentration of dissolved CO_2 was calculated according to Henry's law:

$$[CO_2] = K_h * pCO_2 \quad (2.7)$$

where K_h is Henry's law constant for CO_2 that varies with temperature and salinity (mol L^{-1} bar^{-1}) and pCO_2 represents the partial pressure of atmospheric CO_2 (bar) (Deines et al., 1974). The concentrations of various carbonate species were determined using the measured pH and temperatures of the waters and the expected $\delta^{13}C_{DIC}$ (without microbial influence) was determined based on known fractionation factors for carbonate species relative to gaseous CO_2 (Deines et al., 1974) (see Appendix A).

2.3 RESULTS

2.3.1 Golden Deposit Sample Description

The Golden Deposit appeared golden yellow with no vegetation on its surface but surrounded by organic-rich soils (peat) and muskeg-type vegetation (Figure 2.1b). The surface of the deposit had multiple streams of water that divided the

deposit into small sections and multiple 'holes' on the surface were identified as groundwater seeps (Figure 2.1c). SITEs 2 and 3 were both located close to groundwater seeps and samples from SITE 1, 2, and 5_{CRUST} appeared a golden yellow colour composed of fine-grained material. SITEs 1 and 2 were taken from areas of water flow and thus appeared saturated with water, whereas SITE 5_{CRUST} was taken from an exposed area of the deposit where there was no water contact and thus appeared dry and densely packed. Both peat cores appeared as very dark, organic-rich soil (Figure 2.1d) and were topped with muskeg-type vegetation and had a high concentration of roots near the surface. CORE 1 appeared as the very dark, organic-rich soil for the entire length of the core (~42 cm). CORE 2 appeared as organic-rich soil at the top of the core, but became more yellow with depth, the bottom of which appeared to be the same material as the exposed Golden Deposit and is believed to be an older buried part of the deposit.

2.3.2 Golden Deposit Water Chemistry, Organic Carbon Content and Cell Density

The field and water chemistry data for the Golden Deposit are listed in Table 2.1. The geochemistry of the Golden Deposit was quite variable with SITEs 2 and 3 being the most extreme. The pH ranged from 2.4 at SITE 2 to 6.3 at SITE 5 and 6.8 at the nearby Airplane Lake. Specific conductance was highest at SITEs 2 and 3 (6300 and 4260 µS/cm, respectively) compared to other sites that were sampled on and off the deposit, which ranged from 178 to 1320 µS/cm. The DIC at all sites on the deposit was less than 0.8 mg/L whereas Pond 2 and Airplane Lake had DIC concentrations of 5.0 and 11.8 mg/L, respectively. The DOC was lowest at SITE 2 (6.2 mg/L) and highest at SITE 4 and Airplane Lake (~23 mg/L). In general, SITEs 2 and 3 had the highest concentrations of SO_4^{2-}, Al, Ca, Fe, Mg, Mn, Na and S.

The organic carbon content and calculated cell density showed similar trends in both peat cores as shown in Figure 2.2. CORE 1 had a higher organic carbon content (83-94 %) than CORE 2 (7-78 %) and both cores showed a decreasing trend

in organic carbon content with depth. CORE 1 had a higher cell density than CORE 2 and both cores displayed a decreasing trend in cell density with depth. The Golden Deposit samples had much lower organic carbon contents (12-24 %) and cell densities than both of the peat cores with the exception of the bottom of CORE 2, which appeared to be an older buried part of the deposit. Within the deposit, SITE 1 had the highest organic carbon content (25 %), while SITEs 2 and 5_{CRUST} had similar but lower values (~12 %). Cell densities did not follow the same trend as organic carbon content with SITEs 1 and 2 having the highest cell densities and SITE 5_{CRUST} having the lowest cell density.

2.3.3 Phospholipid Fatty Acid Analysis

The phospholipid fatty acids of the Golden Deposit and both peat cores are listed in Tables 2.3 to 2.5 and the measured isotopic compositions are listed in Tables 2.6 to 2.8. The distribution of microbial PLFA groups is shown in Figure 2.3. Both peat cores had a large number (>70) of different phospholipid fatty acids with distributions of select PLFA changing downcore. Both cores had high proportions of 16:0, a common fatty acid found in most organisms (Boschker and Middelburg, 2002), as well as *i* and *a* 15:0, indicative of gram-positive bacteria (Kaneda, 1991; White et al., 1996), and polyunsaturates such as 18:2Δ9,12 and long chain (>20) saturates and unsaturates which are common in eukaryotes (Madigan et al., 1997). Both cores had a large number of monounsaturates such as 18:1Δ11 *cis* and *trans*, common in gram-negative and proteobacteria (Wilkinson, 1988; Hedrick et al., 2005) and branched fatty acids, common in Gram-positive bacteria (Harwood and Russell, 1984). Both cores had the sulfate reducer biomarker 10me16:0 (Wakeham, 1995) which increased with depth and an absence of methane gas, methanogen and methanotroph biosignatures. The mol % of cy17:0 and cy19:0, which are more abundant in anaerobic than aerobic bacteria, also increased with depth (Fang and Barcelona, 1998). In terms of microbial groupings the top of both cores had a

greater mol % of saturates and less terminally branched fatty acids than the rest of the cores and relatively consistent PLFA distributions below 7.5 to 10 cm.

The number of PLFA present at the Golden Deposit sites was much lower (<35 different PLFA) than that of the peat cores and was quite variable within the deposit. SITE 2 had the highest concentration of PLFA followed by SITE 1 and SITE 5_{CRUST} with the PLFA distribution at SITE 1 most closely resembling that of the peat cores (Figure 2.3). The distribution of PLFA at SITE 2 was significantly different from SITE 1, SITE 5_{CRUST} and both peat cores with high proportions of monounsaturates, consisting predominantly of 16:1Δ9*cis* and 18:1Δ9*cis* and *trans*, and low quantities of mid-chain branched, terminally branched, and saturated fatty acids. All of the sites had the sulfate reducer biomarker 10me16:0 with the highest concentrations found at SITE 1. SITE 1 and Site 5_{CRUST} also had much higher mol % of cy17:0 and cy19:0 which are common markers of anaerobic organisms (Fang and Barcelona, 1998).

The $\delta^{13}C_{PLFA}$ of CORE 1 ranged from -27.7 ‰ to -37.0 ‰ with a $\delta^{13}C_{BULK\ AVG\ PLFA}$ of -31.6 ‰ (Table 2.7). The $\delta^{13}C_{PLFA}$ of CORE 2 ranged from -27.1 ‰ to -35.7 ‰ with a $\delta^{13}C_{BULK\ AVG\ PLFA}$ of -31.1 ‰, a distribution similar to CORE 1 (Table 2.8). Isotopic values of individual or integrated fatty acids in both cores showed no visible pattern of enrichment or depletion in the different sections of the core. Golden Deposit with-in sample variation of the $\delta^{13}C_{PLFA}$ was small (~3 ‰) compared to that of the peat cores (~9 ‰ variation), however, there was a large variation between the 3 sampled sites on the deposit (Table 2.6). The $\delta^{13}C_{BULK\ AVG\ PLFA}$ of SITE 2 was -32.7 ‰, which is an offset of approximately 5.5 ‰ from SITE 1 and SITE 5_{CRUST} whose $\delta^{13}C_{BULK\ AVG\ PLFA}$ were -27.2 ‰ and -26.9 ‰, respectively.

2.3.4 Golden Deposit Organic, Inorganic, and Bulk PLFA Isotopes

The measured atmospheric, organic and inorganic carbon, and bulk PLFA isotopes for the Golden Deposit are shown in Table 2.2. The measured $\delta^{13}C$ of the atmosphere by the Golden Deposit was -9.1 ‰. Based on pH, temperature, and

Henry's law for the solubility of gases, the dominant carbonate species at all sites on the deposit would be carbonic acid (H_2C0_3). Using known fractionation factors (Deines et al., 1974) the expected $\delta^{13}C_{DIC}$ for sites on and off the deposit ranged from -2.3 ‰ for Airplane Lake to -9.9 ‰ for SITEs 2 and 6 (Figure 2.5). However, the measured $\delta^{13}C_{DIC}$ for the water samples ranged from -6.5 ‰ for Airplane Lake to -20.7 ‰ for SITE 1, a depletion of 4 to 15 ‰ for the different sites. Airplane Lake, SITE 4, and Pond 2 had measured $\delta^{13}C_{DIC}$ values that were closest to the expected values while SITE 1, 2, and 5 were the most depleted in ^{13}C relative to the expected value.

The $\delta^{13}C_{TOC}$ values were depleted at the top of both peat cores with values of -28.3 ‰ and -27.2 ‰ for CORE 1 and CORE 2, respectively (Figure 2.4). Both cores became slightly enriched in ^{13}C and had isotopic values within error below 7.5-10 cm depth. SITEs 1, 2, and 5_{CRUST} had $\delta^{13}C_{TOC}$ of -26.6, -25.9, and -25.0 ‰, respectively, and were within error of the $\delta^{13}C_{TOC}$ of the peat cores below 7.5-10 cm depth. The $\delta^{13}C_{TOC}$ for all sites on the deposit were more depleted than the $\delta^{13}C_{DIC}$ (Figure 2.6) with a $\Delta^{13}C_{DIC\text{-}TOC}$ of approximately 6-8.5 ‰ indicative of heterotrophic activity. The $\delta^{13}C_{BULK\ AVG\ PLFA}$ was calculated from the measurable $\delta^{13}C$ values of the individual PLFA. SITE 5_{CRUST} and SITE 1 $\delta^{13}C_{BULK\ AVG\ PLFA}$ were depleted relative to the $\delta^{13}C_{TOC}$ ($\Delta^{13}C_{TOC\text{-}PLFA}$ 0.6 to 1.9 ‰). The $\delta^{13}C_{BULK\ AVG\ PLFA}$ of the peat cores were also depleted (~2 to 5.5 ‰) relative to the $\delta^{13}C_{TOC}$. However, SITE 2 PLFA exhibited a much greater depletion of ~7 ‰ from the $\delta^{13}C_{TOC}$ value.

2.4 DISCUSSION

2.4.1 Geochemistry, Microbial Distribution and Carbon Cycling within the Golden Deposit

Based on the geochemistry of the groundwater seep at SITE 2, the groundwater flowing through the area is acidic (pH ~2.4) and has elevated concentrations of SO_4^{2-}, Al, Ca, Fe, Mg, Mn, Na and S. Limestone and dolomites are exposed 12 km west of the deposit and an extensive unit of pyrite-bearing marine

shale underlies the area surrounding the Golden Deposit (Yorath and Cook, 1981). Therefore, the high concentrations of metals, non-metals, and acidic pH are indicative of substantial alteration of recharge waters as a result of the dissolution of dolomite followed by interaction with pyrite during subsurface flow (Michel and van Everdingen, 1987). The muted geochemistry of Airplane Lake and intermediary water chemistries of several sites on the deposit (SITEs 1, 4, and 5) indicates that local surface water is influencing the water chemistry at the Golden Deposit. Therefore, the proximity of sample sites to groundwater seeps and surface water inflows is controlling the water chemistry at each location.

The variability in water chemistry is a driving force for the microbial distributions detected within the Golden Deposit. The high concentrations of S and SO_4^{2-} in the upwelling groundwaters provides a substrate for sulfate reducers and accounts for the widespread occurrence of the *Desulfobacter* biomarker, 10me16:0, on the deposit. This indicates that sulfate reducers are an important component of the microbial community in this environment. The distributions of PLFA are distinctly different between the most acidic SITE 2 and less acidic sites on the deposit. SITE 2 has the largest $\Delta\delta^{13}C_{TOC\text{-}PLFA}$ (~7.0 ‰) compared to the other sites on the deposit and both peat cores. Furthermore, the distinct PLFA distribution and the depleted $\delta^{13}C_{PLFA}$ (-32.7 ‰) could indicate a distinct microbial community at this location due to the acidic pH and low temperature and is discussed further below.

The influence of surface water flows on SITE 1 and SITE 5_{CRUST} is illustrated by the less extreme geochemical conditions and less acidic pH and is selecting for a different microbial community with a greater diversity of organisms than SITE 2. This is evident from the similarity in PLFA distributions and $\delta^{13}C_{BULK\ AVG\ PLFA}$ (~ -27 ‰), and greater proportions of mid-chain and terminally branched fatty acids at both locations. However, the limited availability of water and access to nutrients is resulting in a smaller microbial community at SITE 5_{CRUST} with eukaryotes being an insignificant component of the community, evident by the low cell densities and lack

of polyunsaturated fatty acids detected at this location (Balkwill et al., 1988; Madigan et al., 1997).

The depleted $\delta^{13}C_{DIC}$ relative to the expected $\delta^{13}C_{DIC}$ at all sampled sites on the Golden Deposit indicates microbial respiration inputs to the DIC pool as heterotrophic metabolism of ^{13}C depleted organic matter results in inputs of ^{13}C depleted inorganic carbon to the DIC pool (Blair et al., 1985). Higher concentrations of substrate DOC in Golden Deposit waters at SITE 1 and 5 supports greater heterotrophic activity and is demonstrated by the largest $\Delta^{13}C$ between measured and expected $\delta^{13}C_{DIC}$ (~10-14 ‰) compared to other locations on the deposit. This is further supported by the greater presence of heterotrophic markers such as *iso* and *anteiso* and mid-branched PLFA (Dowling et al., 1986; Vestal and White, 1989; Parkes et al., 1993; Londry et al., 2004) at SITE 1 and 5_{CRUST} and the similarity in $\Delta^{13}C_{TOC\text{-}BULK\ AVG\ PLFA}$ (~ 1-2 ‰) of SITE 1 and 5_{CRUST} in comparison to that of SITE 2 ($\Delta^{13}C_{TOC\text{-}BULK\ AVG\ PLFA}$ = ~ 7.0 ‰). The variability in water chemistry is ultimately resulting in differences in the microbial community and $\delta^{13}C_{DIC}$ within the small spatial area of the Golden Deposit.

2.4.1.1 *Microbial Distribution at SITE 2*

SITE 2 is located by an acidic groundwater seep resulting in a distinct water chemistry compared to the rest of the deposit and surrounding peat. The extreme water chemistry is selecting for microbial life that can survive in such conditions. The presence, although small, of polyunsaturates at SITE 2 could indicate the presence of acidophilic eukaryotes such as *Cyanidium caldarium* and *Euglena mutabilis* (Madigan et al., 1997; Johnson and Hallberg, 2003). The depleted $\delta^{13}C_{DIC}$ relative to the expected $\delta^{13}C_{DIC}$ also indicates microbial respiration inputs to the DIC pool, as was described earlier, and could indicate the presence of acidophilic heterotrophs such as *Acidiphilium* and *Acidocella* (Johnson and Hallberg, 2003).

The abundance of SO_4^{2-} at SITE 2 and the presence of the *Desulfobacter* biomarker 10me16:0 in the absence of 10me18:0 (Wakeham, 1995) indicates the

presence of sulfate reducing bacteria in this environment. The low concentrations of 10me16:0 could be due to homeoviscous adaptation where bacteria alter their lipid composition depending on environmental stresses in order to maintain the cellular membrane in a liquid crystalline state (Van de Vossenberg et al., 1998; Hanford and Peeples, 2002). In cold environments, *Desulfobacter hydrogenophilus* was found to significantly decrease the concentrations of 10me16:0 fatty acids in their lipid membranes and increase the production of 16:1Δ9*cis* fatty acids (Wakeham, 1995; Könneke and Widdel, 2003). The low proportions (0.5 mol %) of 10me16:0 but high proportions (51.1 mol %) of 16:1Δ9*cis* could be due to homeoviscous adaptation and could indicate a large presence of sulfate reducers in this environment.

At. ferrooxidans or similar bacteria is likely the dominant iron oxidizer at the Golden Deposit due to the acidic conditions and cold temperatures of upwelling groundwater. In most AMD/ARD environments either *At. ferrooxidans or L. ferrooxidans* is the dominant iron-oxidizing organism depending on the redox potential, pH, and temperature of the environment (Rawlings et al., 1999; Johnson and Hallberg, 2003). *At. ferrooxidans* is more tolerant of low temperature environments and moderately acidic pH. In contrast, *L. ferrooxidans* has a lower limit growth temperature of ~20°C and can tolerate pH conditions <1.3 (Hallman et al., 1993; Rawlings et al., 1999; Johnson and Hallberg, 2003). The abundance of *At. ferrooxidans*-like organisms and scarcity of *L. ferrooxidans* in the subarctic King's Mine, Norway and gossan samples from the high arctic were attributed to the cold temperatures of these locations (Langdahl and Ingvorsen, 1997; Johnson et al., 2001). The cold temperatures of the Golden Deposit waters (~ 8°C in late summer) are far below the lower temperature growth limit of *L. ferrooxidans* and therefore *At. ferrooxidans* may be the dominant iron oxidizer in the deposit. *At. ferrooxidans* are autotrophic organisms that use the Calvin Benson cycle to fix CO_2 (Gale and Beck, 1967; Nemati et al., 1998) and the depletion of $\delta^{13}C_{PLFA}$ at SITE 2 relative to $\delta^{13}C_{TOC}$ and $\delta^{13}C_{DIC}$ is on the order of that measured by Cowie et al. (2009) for autotrophic

growth of *At. ferrooxidans* in laboratory cultures. PLFA analysis of cultured *At. ferrooxidans* and *L. ferrooxidans* reveals that ~45 % of the PLFA in *L. ferrooxidans* is 16:1Δ7 whereas this fatty acid is not present in *At. ferrooxidans*. The absence of 16:1Δ7 in the PLFA profile of SITE 2 as well as at SITE 1 and SITE 5_{CRUST} could indicate the dominance of *At. ferrooxidans* in the deposit. Furthermore, the high mol % of monounsaturates at SITE 2, which are common in proteobacteria such as *At. ferrooxidans* (Wilkinson, 1988; Hedrick et al., 2005), also indicates the dominance of this or similar organism at the Golden Deposit.

2.4.2 Microbial Cycling and Distribution in Peat and its Influence on the Golden Deposit

In contrast to the Golden Deposit a very large diversity of organisms exists in the peat surrounding the deposit with similar groups of organisms present in both cores. Despite differences in the appearances and organic carbon contents of the cores similar microbial distributions and isotopic compositions ($\delta^{13}C_{BULK\ AVG\ PLFA}$ ~ -27 ‰) were detected. The high cell density, variety of PLFA, and variability in $\delta^{13}C_{PLFA}$ (~ 9 ‰ variation) suggest that the organic-rich soils (55-95 % organic carbon by dry weight) are a favourable environment for a variety of microbes to survive.

Bacteria, gram- positive and negative, and eukaryotes are active members of the microbial community evident from the presence of branched, monounsaturated and polyunsaturated fatty acids (Harwood and Russell, 1984; Wilkinson, 1988; Madigan et al., 1997; Hedrick et al., 2005). The depleted $\delta^{13}C_{DIC}$ relative to the expected $\delta^{13}C_{DIC}$ indicates microbial respiration inputs to the DIC pool and is supported by the presence of heterotrophic markers such as *iso* and *anteiso* and mid-branched PLFA (Blair et al., 1985; Dowling et al., 1986; Vestal and White, 1989; Parkes et al., 1993; Londry et al., 2004). Cell density decreases with depth indicating a decrease in the microbial population, an effect also found in Boreal peatlands by Sundh et al. (1997) and attributed to changes from aerobic to anaerobic conditions with increasing depth. Both peat cores exhibit changes in

microbial distribution and enrichment in $\delta^{13}C_{TOC}$ downcore that could be caused by changes from an aerobic to an anaerobic environment and is supported by an increase in mol % of the anaerobic biomarkers cy17:0 and cy19:0 (Fang and Barcelona, 1998).

The absence of methane gas in peatland waters, as well as methanogenic and methanotrophic biosignatures in both cores, is an interesting observation as methanogenesis is an important and dominant process in most peatlands (Schlesinger, 1997; Schuur et al., 2008; Turetsky et al., 2008). The high proportions of 10me16:0, a sulfate reducer biomarker, could indicate a dominance of sulfur reducers which outcompete methanogens, an effect often found in marine environments where sulfur concentrations are high (Oremland, 1982; Botz et al., 1996; Summons et al., 1998). The high concentrations of S and SO_4^{2-} in the groundwater surrounding the Golden Deposit would support the more thermodynamically favourable redox reactions of sulfur reducers and thereby outcompete methanogenic processes in this environment (Thaur et al., 2008).

2.4.2.1 Influence of Surrounding Peat on the Golden Deposit

Surface waters flow onto the deposit from many small streams, with the greatest input being at SITE 5. These streams could serve as a transport mechanism for organic carbon and microbes from the surrounding peat into the Golden Deposit. The intermediary water chemistries and elevated DIC and DOC concentrations, compared to the acidic seep sites, are a clear indication of the mixing of surface and groundwater sources on the deposit. Despite differences in cell density and PLFA distributions on and off the deposit, the $\delta^{13}C_{TOC}$ of the Golden Deposit and surrounding peat is ~ -26.2 ± 1.2 ‰, which indicates that organic carbon from the surrounding peat is likely being transported to the Golden Deposit and could serve as a substrate for microbial respiration.

The older buried deposit encountered in CORE 2 (20-25 cm) may be most representative of the influence of the overlying peat on Golden Deposit sediment.

The intermediary water chemistry, similarity in PLFA distribution, organic carbon content, and $\delta^{13}C_{BULK\ AVG\ PLFA}$ of SITE 1 and, to a lesser extent, SITE 5_{CRUST} to that of the older buried deposit could reflect the interaction of these sites with surface water flows from the surrounding peat thereby resulting in similar communities inhabiting these locations. In contrast, the unique distribution of PLFA and $\delta^{13}C_{BULK\ AVG\ PLFA}$, much lower DOC and elevated metal and nonmetal concentrations of SITE 2 indicate that it is most influenced by the acidic groundwater chemistry.

2.4.3 Implications for the Identification of Biosignatures on Mars

The acidic conditions, mineralogy and water chemistry of the Golden Deposit represent an environment of similar state to what may have existed on Mars, and therefore can be used as an analogue for habitability in such environments. Iron is an attractive element for life because it is both a good electron donor and acceptor, has good buffer characteristics and can maintain a stable pH allowing for organisms to survive more easily in acidic conditions (Phoenix et al., 2001; Amils et al., 2007; Gomez et al., 2007). It is conceivable, although unlikely, that life may still exist on Mars where there is the possibility of a subsurface hydrosphere and protection from UV radiation by soluble ferric iron solutions and iron minerals (Malin and Edgett, 2000; Phoenix et al., 2001; Gomez et al., 2007).

Sulfuric and carbonic acid dominate Golden Deposit waters due to the acidic pH. Such acidic conditions limit the precipitation and preservation of carbonates (Brocks and Pearson, 2005) and accounts for the inability to measure an isotopic signal and % inorganic carbon content for carbonates in Golden Deposit samples. This is consistent with the lack of carbonates detected at Meridiani Planum, Mars as a result of its past acidic water bodies, but may not be true of all locations on Mars (Fairen et al., 2004; Amils et al., 2007). However, the depleted $\delta^{13}C_{DIC}$ relative to the expected $\delta^{13}C_{DIC}$ of Golden Deposit waters was a clear indication of the influence of microbial respiration on the DIC pool, where sites with greater heterotrophic activity had the largest depletions. If subsurface water exists on Mars, as is

suggested by Malin and Edgett (2000), offsets of expected $\delta^{13}C_{DIC}$, calculated from the $\delta^{13}C_{CO2\ ATM}$, to measured $\delta^{13}C_{DIC}$ of subsurface water could indicate past or current microbial activity. However, these results will require careful interpretation in combination with other signatures of life as solar wind stripping has changed the isotopic composition of atmospheric CO_2 on Mars over time (Krasnopolsky et al. 1996; van Zuilen, 2008). Volcanic and geologic activity are believed to have ended on Mars approximately 3.5 billion years ago, leaving any preserved organic matter isotopically unaltered. Organic matter would have been stripped from the surface due to the strong oxidizing conditions on Mars today, but may still be present in the subsurface (Klein et al., 1976; van Zuilen, 2008). Measuring the isotopic composition of subsurface organic matter ($\delta^{13}C_{TOC}$) on Mars and its offset from other known carbon pools ($\delta^{13}C_{CO2\ ATM}$ and $\delta^{13}C_{DIC}$) could lead to the identification of current or past life on the planet.

The similarity in PLFA distribution and isotopic signatures of SITE 1 and 5_{CRUST} on the Golden Deposit to that of the peat indicates that these sites are largely influenced by surface water flows originating in the surrounding peat environment. Such transport of organic carbon and nutrients would have been minimal on the surface of Mars (Knoll et al., 2005), and thus SITE 1 and 5_{CRUST} may not be good analogues for conditions of habitability of its surface. However, the geochemistry and PLFA distribution at SITE 2 indicate minimal influence of the surrounding peat environment to the microbial community and isotopic signatures and thereby it may serve as a good analogue to signatures that may be present on Mars. The cold, acidic conditions, and high concentrations of metals and nonmetals at SITE 2 selects for microbial communities able to withstand such geochemical conditions. As discussed above, SITE 2 is likely dominated by the iron oxidizer *At. ferrooxidans* and sulfate reducing bacteria from the genus *Desulfobacter* demonstrated by the high proportions of monounsaturates, particularly 16:1Δ9*cis*, and the presence of 10me16:0. However, such interpretations must be made carefully as homeoviscous adaptation due to environmental stresses can alter expected PLFA profiles. If life

originated on Mars during the aqueous period of jarosite and hematite formation such as that found at Meridiani Planum, *At. ferrooxidans*- and *Desulfobacter*–like organisms may have been dominant on Mars as the cold, acidic, and high Fe and S conditions would select for such organisms. Furthermore, the existence of acidic-sulfate rich waters in Mars' past also could have suppressed methanogenesis resulting in a lack of preserved archaeal biosignatures on the planet.

If Mars contained both surface and groundwater flows, as is suggested by Malin and Edgett (2000), then the surface of Mars may have exhibited a large degree of geochemical and microbial variability such as that seen at the Golden Deposit. Such variability in microbial communities is reflected in the Golden Deposit in the differences in PLFA profiles and $\Delta^{13}C_{TOC\text{-}PLFA}$. Sites with less extreme geochemical conditions due to the mixing of water sources, such as SITE 1 on the deposit, would have been able to support a greater diversity of organisms and may have been more favourable environments to support life. Limited access to water and nutrients, such as that seen at SITE 5_{CRUST}, would severely restrict the growth of microbes and indicates that such locations on Mars would not have been able to support a large microbial community. The most acidic sites on Mars, such as SITE 2, would have supported a lower diversity of organisms likely dominated by iron and sulfur utilizing organisms. As is demonstrated at the Golden Deposit, geochemical variability could have resulted in large differences in microbial communities and isotopic compositions within small spatial areas if life existed during the period of aqueous acid sulfate conditions on Mars. This would make measurements of preserved microbial biosignatures more difficult and will require careful interpretation before it is possible to identify the past or current existence of life on the planet.

2.5 CONCLUSIONS

The Golden Deposit exhibits a large degree of geochemical variability caused by the mixing of surface water and upwelling acidic groundwater that is the result of

subsurface flow through dolomite and pyrite. Many sites on the deposit exhibit intermediary water chemistries due to the mixing of these different water sources and as a result the microbial communities inhabiting different locations on the deposit and their isotopic signatures can be highly variable. The peat surrounding the Golden Deposit supports a high number and diversity of organisms and appears to influence the microbial community and organic carbon source to sites on the deposit with mixed water chemistries. The most acidic site on the deposit (SITE 2) is most representative of the acidic groundwater chemistry, and is resulting in distinct isotopic signatures and PLFA profiles dominated by organisms such as *At. ferrooxidans* and *Desulfobacter*.

The Golden Deposit represents an analogue to acidic sulfate-rich conditions that existed on Mars during the period of jarosite and hematite formation found on the surface today. The dominance of sulfate reducers in the surrounding peat indicates that acidic, sulfate-rich waters in Mars' past could have resulted in a lack of preserved archaeal biosignatures on the planet. If Mars contained both surface and groundwater flows, then its surface may have exhibited a large degree of geochemical and microbial variability within a small spatial area as is demonstrated in the Golden Deposit. Despite variability in PLFA and isotopic signatures within the deposit, such analyses are clear indications that life can survive in such environments. By continuing to study Mars analogue sites we can further our knowledge of microbial processes, biomarkers, and isotopic signatures that are present or preserved in such environments. Such analyses will allow us to better identify and interpret signatures of life that may exist on Mars.

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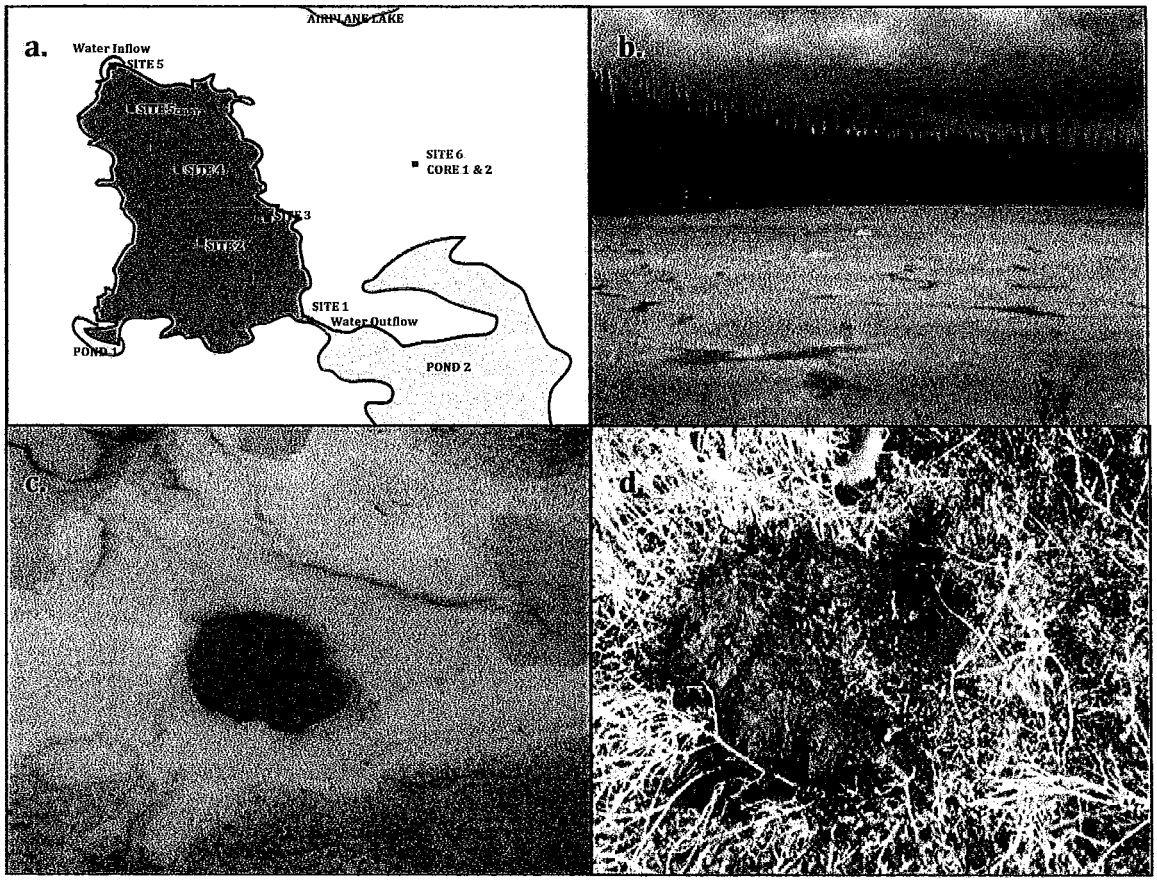

Figure 2.1: The Golden Deposit, N.W.T, Canada a. Sampled sites on the deposit b. The Golden Deposit surrounded by muskeg-type vegetation c. Acidic groundwater seep (SITE 2) d. organic-rich peat soils of CORE 1

Table 2.1: Water chemistry data for the Golden Deposit.

SAMPLE	pH	T (°C)	Specific Conductance (µs/cm)	DIC (mg/L)	DOC (mg/L)	SO_4 (mg/L)	Al (mg/L)	Ca (mg/L)	Fe (mg/L)	Mg (mg/L)	Mn (mg/L)	Na (mg/L)	S (mg/L)
POND 2	5.9	8.6	1320.0	5.0	9.8	743.0	0.2	91.4	8.5	84.4	0.7	80.7	239.0
SITE 1	4.0	6.5	616.0	< 0.5	17.2	305.0	1.9	29.5	7.1	40.1	0.6	19.2	100.0
SITE 2	2.4	7.3	6300.0	0.6	6.2	4170.0	1.1	343.0	309.0	475.0	4.2	426.0	1596.0
SITE 3	3.1	8.5	4260.0	0.6	13.6	2770.0	16.9	227.0	11.3	373.0	4.0	322.0	1030.0
SITE 4	5.8	8.1	354.0	< 0.5	22.9	148.0	1.4	20.0	5.8	26.2	0.4	7.4	57.8
SITE 5	6.3	3.9	274.0	0.8	11.7	111.0	0.5	14.9	18.4	12.1	0.1	2.9	27.8
SITE 6	6.0	8.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Airplane Lake	6.8	6.9	178.0	11.8	23.2	37.0	<0.05	21.7	0.1	11.3	0.0	1.9	13.0

n.d., not determined

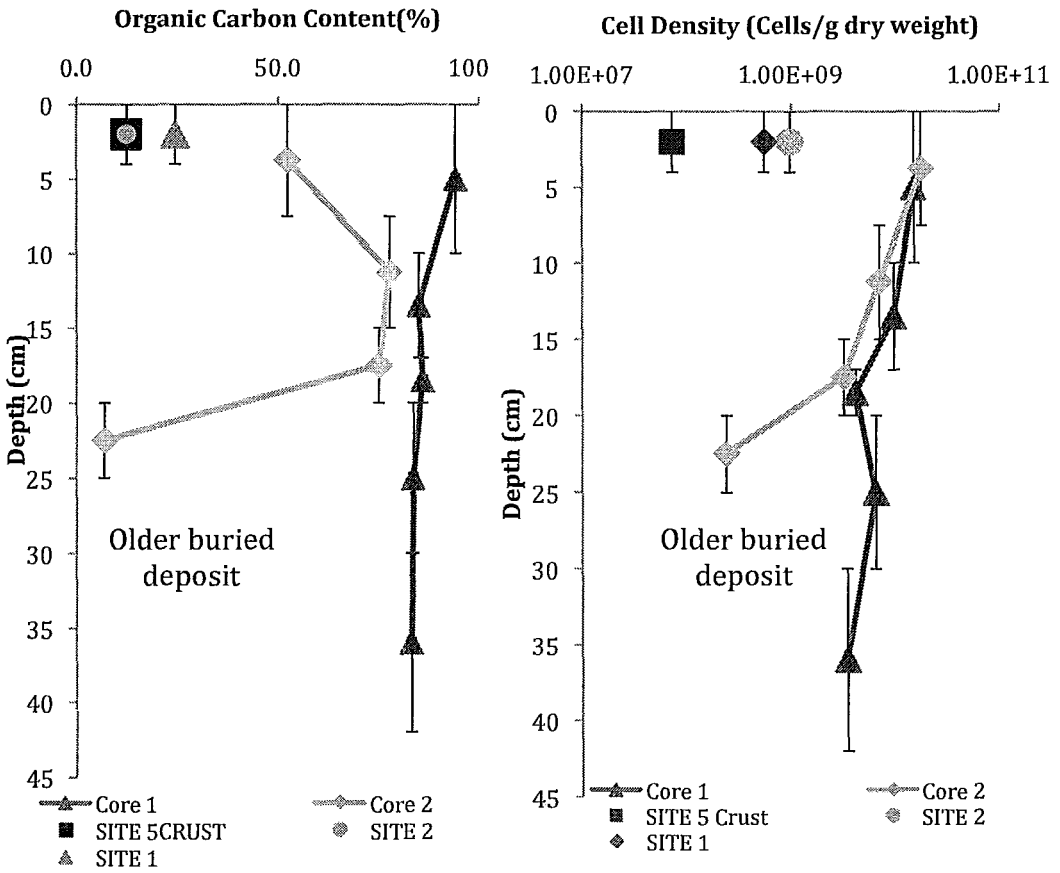

Figure 2.2: a) Organic carbon content of the Golden Deposit showing decreasing organic content in both peat cores and lower organic carbon content in sites on the deposit, but on the order of the older buried deposit **b)** Cell densities of the Golden Deposit showing a decreasing trend in both peat cores and lower cell densities of sites on the deposit, but on the order of that found in the older buried deposit.

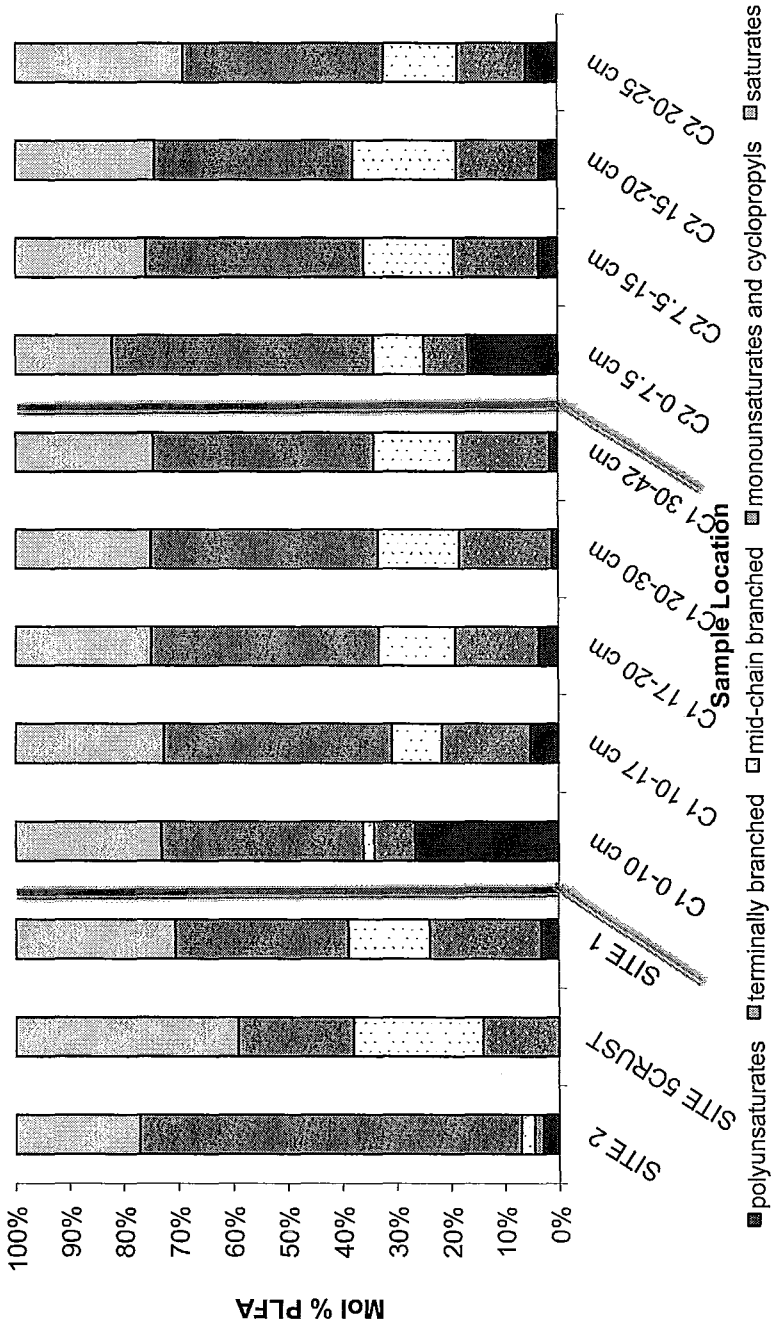

Figure 2.3: PLFA distributions of Golden Deposit samples and peat cores in mol %. The distribution of the peat cores changes below 7.5-10 cm due to changes from aerobic to anaerobic conditions. SITE 5crust and SITE 1 have similar distributions to the bottom (below 7.5-10 cm) peat cores. SITE 2 has a unique distribution with high proportions of monounsaturates due to the presence of a distinct microbial community.

Table 2.2: $\delta^{13}C$ values of atmospheric CO_2, DIC, TOC, and bulk avg PLFA. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB.

Location	$\delta^{13}C_{CO2\ ATM}$ (‰)	$\delta^{13}C_{DIC}$ (‰)	$\delta^{13}C_{TOC}$ (‰)	$\delta^{13}C_{BULK\ AVG\ PLFA}$ (‰)	$\Delta^{13}C_{DIC-TOC}$ (‰)	$\Delta^{13}C_{TOC-PLFA}$ (‰)
Pond 2		-11.6				
SITE 1		-20.7	-26.6	-27.2	5.9	0.6
SITE 2		-17.9	-25.9	-32.7	8.0	6.8
SITE 4		-13.9				
SITE 5		-20.2				
SITE 5_{CRUST}			-25.0 ±	-26.9		1.9
Airplane	-9.1	-6.5				
CORE 1 Avg.		-18.5	-27.1	-31.6	8.6	4.5
C1 0-10 cm			-28.3 ±	-33.1		4.8
C1 10-17 cm			-27.0 ±	-31.2		4.2
C1 17-20 cm			-26.4	-30.8		4.4
C1 20-30 cm			-26.5 ±	-31.2		4.7
C1 30-42 cm			-26.7 ±	-31.7		5.0
CORE 2 Avg.			-26.7	-31.0		4.3
C2 0-7.5 cm			-27.2 ±	-29.5		2.3
C2 7.5-15			-25.9	-31.5		5.6
C2 15-20 cm			-26.7	-32.1		5.4
C2 20-25 cm			-26.4 ±	-30.9		4.5

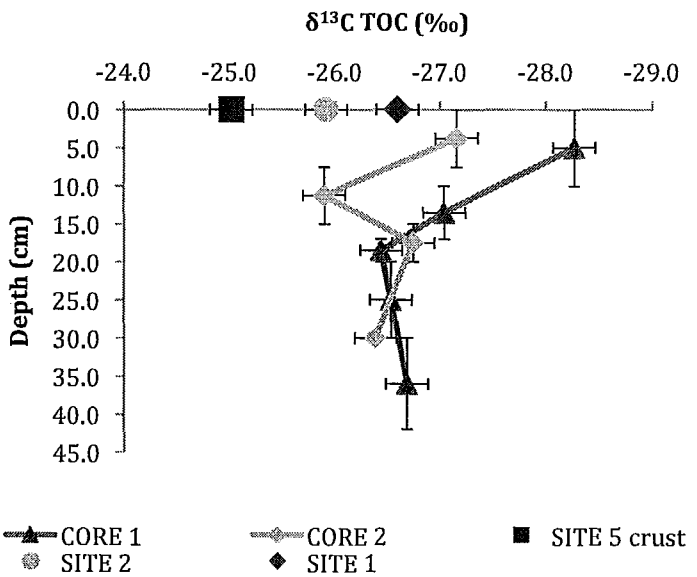

Figure 2.4: $\delta^{13}C_{TOC}$ of peat cores and Golden Deposit samples. $\delta^{13}C_{TOC}$ of peat cores generally decreases with depth and sites on the deposit have similar $\delta^{13}C_{TOC}$ values as the peat cores.

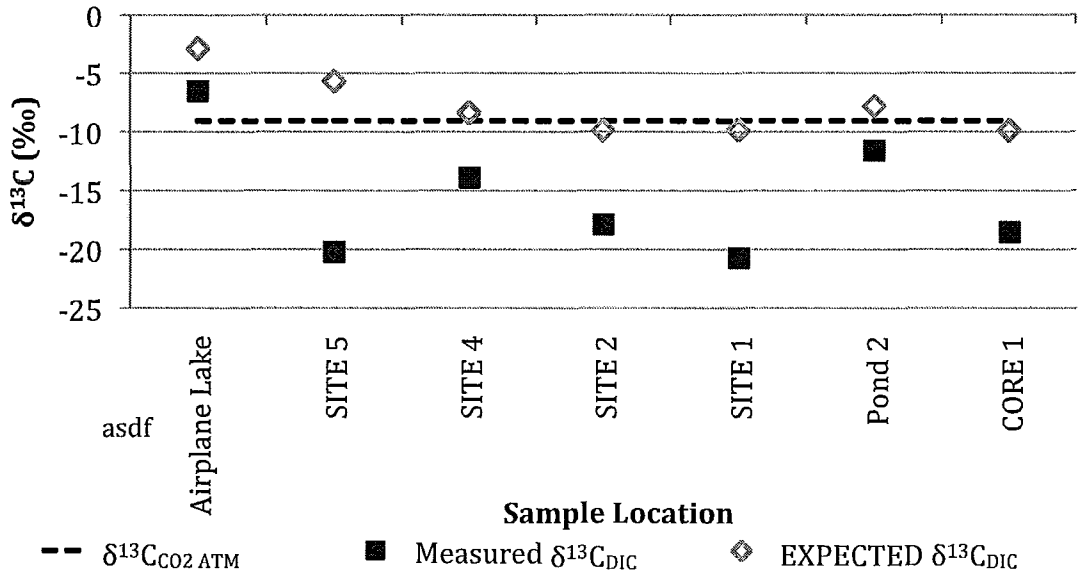

Figure 2.5: Expected and measured $\delta^{13}C_{DIC}$ of water samples from sites on and off the deposit. All sites had more depleted measured $\delta^{13}C_{DIC\ values}$ than expected $\delta^{13}C_{DIC}$ values indicating heterotrophic inputs of isotopically depleted DIC to the DIC pool. Error for $\delta^{13}C_{DIC}$ is 0.2 ‰.

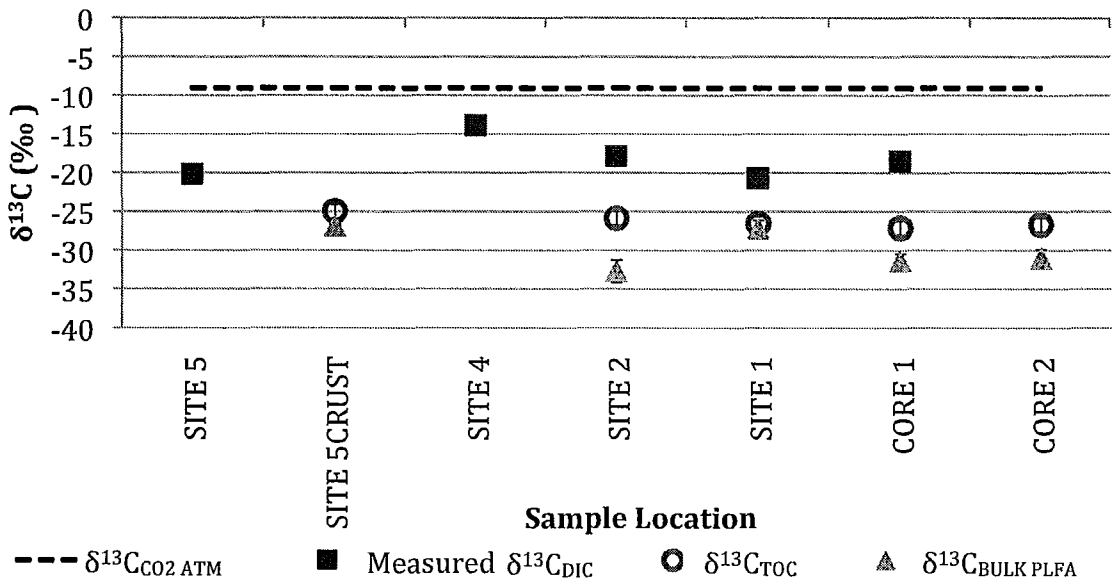

Figure 2.6: $\delta^{13}C$ values of atmospheric CO_2, DIC, TOC, and bulk avg PLFA. $\delta^{13}C_{TOC}$ values indicate that the peat is a source of organic carbon to the deposit. The depleted $\delta^{13}C_{BULK\ PLFA}$ at SITE 2 relative to $\delta^{13}C_{TOC}$ and $\delta^{13}C_{DIC}$ is due to the presence of a distinct microbial community. Error for $\delta^{13}C_{DIC}$ and $\delta^{13}C_{TOC}$, is on average 0.2 ‰.

Table 2.3: PLFA profile of Golden Deposit samples expressed in ug/g and mol % for each site.

PLFA I.D.	SITE 1		SITE 5_{CRUST}		SITE 2	
	(ug/g)	(mol%)	(ug/g)	(mol%)	(ug/g)	(mol%)
12:0	0.07	1.26	0.03	4.22	0.04	0.42
i14:0	0.11	1.57	0.00	0.00	0.00	0.00
14:0	0.19	2.79	0.03	3.85	0.45	3.81
i15:0	0.56	7.94	0.03	3.35	0.05	0.38
a15:0	0.36	5.06	0.03	3.42	0.05	0.41
15:0	0.10	1.37	0.03	3.32	0.06	0.50
br15:0	0.09	1.19	0.04	3.80	0.07	0.56
i16:0	0.24	3.17	0.04	3.77	0.07	0.50
16:3Δ6	0.00	0.00	0.00	0.00	0.06	0.46
16:1Δ4	0.10	1.41	0.00	0.00	0.00	0.00
16:1Δ9cis	0.27	3.60	0.04	3.74	6.70	51.07
16:1Δ9trans	0.10	1.30	0.00	0.00	0.26	2.02
16:1Δ11	0.15	1.99	0.00	0.00	0.00	0.00
16:0	0.89	11.82	0.06	5.66	1.99	15.07
10me16:0	0.43	5.50	0.04	3.80	0.07	0.49
br16:0	0.14	1.76	0.04	3.71	0.05	0.37
br16:0	0.14	1.80	0.04	3.50	0.00	0.00
i17:0	0.23	2.88	0.04	3.54	0.05	0.34
cy17:0	0.28	3.59	0.04	3.55	0.00	0.00
17:0	0.11	1.43	0.04	3.51	0.05	0.34
br17:0	0.11	1.30	0.00	0.00	0.05	0.33
18:3Δ6	0.00	0.00	0.00	0.00	0.12	0.83
br17:0	0.17	2.02	0.05	4.53	0.10	0.67
18:2	0.11	1.33	0.00	0.00	0.00	0.00
br17:0	0.12	1.47	0.05	4.57	0.00	0.00
18:2Δ9,12	0.16	1.94	0.00	0.00	0.23	1.62
18:1Δ9cis	0.45	5.53	0.05	4.56	1.83	12.64
18:1Δ9trans	0.37	4.53	0.05	4.59	0.45	3.08
18:0	0.39	4.69	0.06	5.31	0.22	1.49
cy19:0	0.84	9.72	0.05	4.66	0.20	1.30
20:0	0.15	1.66	0.06	5.40	0.08	0.52
22:0	0.16	1.60	0.06	5.01	0.07	0.43
24:0	0.16	1.55	0.06	4.61	0.06	0.34
26:0	0.14	1.27	0.00	0.00	0.00	0.00

Table 2.4: PLFA profile of CORE 1 samples expressed in ug/g and mol % for each site.

PLFA ID	C1 0-10 cm		C1 10-17 cm		C1 17-20 cm		C1 20-30 cm		C1 30-42 cm	
	(ug/g)	(mol%)	(ug/g)	(mol%)	(ug/g)	(mol%)	(ug/g)	(mol%)	(ug/g)	(mol%)
br11:0	0.00	0.00	0.00	0.00	0.42	0.97	0.00	0.00	0.20	0.57
12:0	0.66	0.42	1.13	1.13	0.52	1.20	0.75	1.13	0.45	1.25
br12:0	0.00	0.00	0.49	0.46	0.48	1.04	0.63	0.89	0.23	0.82
br12:0	0.00	0.00	0.00	0.00	0.44	0.96	0.58	0.81	0.00	0.00
13:0	0.00	0.00	0.00	0.00	0.43	0.93	0.54	0.75	0.31	0.73
br12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.63
i or a 14:0	0.87	0.49	1.80	1.59	0.80	1.65	1.15	1.51	0.24	1.73
14:1	0.49	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14:0	2.07	1.16	3.68	3.25	0.90	1.85	1.57	2.07	1.22	3.01
15:1	0.78	0.42	0.51	0.43	0.00	0.00	0.00	0.00	0.22	0.52
15:1Δ4	0.00	0.00	0.56	0.47	0.46	0.90	0.61	0.77	0.28	0.65
15:1	0.00	0.00	1.02	0.86	0.51	1.00	0.68	0.85	0.00	0.00
br14:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.74
15:1	0.00	0.00	0.65	0.55	0.00	0.00	0.60	0.76	0.29	0.68
br14:0	0.00	0.00	0.56	0.47	0.46	0.89	0.65	0.82	0.35	0.82
i15:0	5.37	2.85	6.56	5.47	2.13	4.12	3.87	4.83	2.22	5.18
a-15:0	3.51	1.86	6.24	5.20	2.65	5.13	4.86	6.07	2.71	6.33
15:1Δ9	0.51	0.27	0.54	0.45	0.44	0.00	0.55	0.69	0.21	0.60
15:0	2.73	1.45	1.61	1.35	0.65	1.26	1.03	1.29	0.50	1.16
br15:0	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.71	0.24	0.53
16:1	0.00	0.00	0.56	0.45	0.51	0.00	0.64	0.75	0.28	0.63
16:1	0.00	0.00	0.38	0.30	0.00	0.00	0.58	0.70	0.28	0.59
16:1	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.72	0.26	0.59
16:1Δ4	1.19	0.60	0.75	0.60	0.00	0.00	0.63	0.75	0.26	0.58
br15:0	0.00	0.00	0.65	0.52	0.53	0.97	0.71	0.84	0.25	0.76
i16:0	1.87	0.94	2.37	1.88	0.93	1.71	1.41	1.67	0.74	1.63
16:1Δ5	1.32	0.67	1.55	1.24	0.61	1.13	0.84	1.01	0.43	0.96
16:1Δ7	1.35	0.69	0.90	0.72	0.54	1.00	0.80	0.95	0.41	0.91
16:1Δ9cis	8.76	4.44	9.07	7.23	2.04	3.77	4.03	4.81	2.68	5.97
16:1Δ9trans	0.00	0.00	1.11	0.88	0.83	1.54	1.16	1.38	0.66	1.47
16:1Δ11	3.95	2.00	3.62	2.88	0.73	1.36	1.05	1.25	0.51	1.13
16:0	32.14	16.17	19.45	15.39	5.16	9.49	8.36	9.89	4.56	10.09
br16:0	0.00	0.00	0.00	0.00	0.47	0.82	0.55	0.62	0.00	0.00
17:1	0.00	0.00	0.72	0.55	0.48	0.00	0.61	0.69	0.00	0.00
17:1Δ9cis	0.62	0.30	2.32	1.76	0.74	1.29	1.68	1.90	1.11	2.34
10me16:0	0.68	0.33	4.02	3.03	1.59	2.78	2.82	3.18	1.42	2.98
17:1Δ9trans	0.00	0.00	0.00	0.00	0.55	0.96	0.54	0.00	0.36	0.76
br16:0	1.34	0.64	0.86	0.65	0.54	0.95	0.69	0.78	0.39	0.81
i17:0	1.20	0.58	1.52	1.15	0.71	1.24	1.05	1.19	0.28	1.13

a17:0	1.79	0.86	1.49	1.12	0.80	1.40	1.13	1.27	0.59	1.25
17:1Δ11	2.46	1.18	0.97	0.74	0.55	0.98	0.73	0.82	0.41	0.86
cy17:0	2.23	1.07	4.11	3.11	2.53	4.46	3.99	4.52	2.19	4.63
17:0	1.94	0.93	1.20	0.91	0.68	1.20	0.98	1.10	0.45	0.96
br17:0	1.02	0.47	1.17	0.84	0.71	1.17	0.92	0.98	0.46	0.91
br17:0	0.00	0.00	0.77	0.55	0.67	1.12	0.89	0.95	0.47	0.95
18:3	1.43	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18:2	2.88	1.33	1.12	0.82	0.65	1.10	0.00	0.00	0.36	0.80
br17:0	0.00	0.00	0.66	0.47	0.00	0.00	0.00	0.00	0.00	0.00
18:2Δ9,12	53.21	24.58	6.02	4.38	1.41	2.38	1.01	1.10	0.39	0.78
18:1Δ9	19.85	9.11	4.79	3.46	1.77	2.96	2.02	2.18	0.85	1.72
18:1Δ11	22.41	10.28	13.64	9.85	4.95	8.29	5.90	6.36	3.08	6.22
18:1Δ13	1.64	0.75	0.78	0.57	1.60	2.68	1.83	1.98	0.99	2.00
18:0	4.27	1.94	0.00	0.00	1.18	1.96	1.76	1.89	1.04	2.08
19:1	0.00	0.00	0.99	0.68	0.94	1.50	1.10	1.14	0.54	1.04
br18:0	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.71	0.33	0.64
br18:0	1.37	0.59	1.42	0.97	0.75	1.19	0.94	0.96	0.45	0.86
br18:0	0.00	0.00	0.70	0.48	0.62	0.99	0.79	0.81	0.34	0.74
br18:0	0.00	0.00	0.00	0.00	0.60	0.00	0.70	0.72	0.34	0.65
br18:0	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.73	0.36	0.69
19:1	1.07	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
br18:0	0.00	0.00	1.18	0.81	0.00	0.00	0.00	0.00	0.56	1.07
cy19:0	7.37	3.23	5.82	4.01	4.38	7.01	4.88	5.03	2.76	5.32
19:0	0.00	0.00	0.00	0.00	0.60	0.95	0.71	0.73	0.00	0.63
20:0	2.76	1.15	1.87	1.23	0.94	1.44	1.39	1.37	0.91	1.66
21:0	1.23	0.49	0.86	0.54	0.00	0.00	0.00	0.00	0.54	0.00
22:0	3.06	1.17	1.96	1.18	0.90	1.27	1.32	1.19	0.79	1.33
23:0	1.28	0.47	0.95	0.55	0.77	1.04	1.03	0.89	0.45	0.70
24:1	1.05	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24:0	2.14	0.76	1.73	0.97	0.84	1.09	1.16	0.97	0.64	1.00
25:1	1.17	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25:0	1.06	0.36	0.74	0.40	0.00	0.00	0.00	0.00	0.00	0.00
26:1	1.12	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26:0	1.30	0.43	0.96	0.50	0.76	0.92	0.97	0.76	0.46	0.67

Table 2.5: PLFA profile of CORE 2 samples expressed in ug/g and mol % for each site.

PLFA I.D.	C2 0-7.5 cm		C2 7.5-15 cm		C2 15-20 cm		C2 20-25 cm	
	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)
12:0	0.59	0.31	0.65	0.89	0.44	1.32	0.04	1.61
i13:0	0.00	0.00	0.52	0.67	0.34	0.95	0.00	0.00
a13:0	0.00	0.00	0.48	0.62	0.32	0.91	0.00	0.00
13:0	0.00	0.00	0.45	0.58	0.31	0.88	0.00	0.00
i or a 14:0	2.09	0.99	1.09	1.33	0.61	1.62	0.04	1.53
14:0	2.16	1.02	1.74	2.11	0.90	2.37	0.07	2.35
15:1	0.81	0.37	0.48	0.56	0.00	0.00	0.00	0.00
15:1	0.65	0.29	0.51	0.59	0.32	0.80	0.00	0.00
br14:0	1.85	0.83	0.63	0.73	0.34	0.86	0.00	0.00
15:1Δ4cis	0.89	0.40	0.54	0.63	0.33	0.82	0.00	0.00
br14:0	0.83	0.37	0.55	0.63	0.33	0.84	0.00	0.00
i15:0	6.53	2.92	4.10	4.71	1.58	3.95	0.10	3.32
a15:0	5.33	2.39	4.65	5.34	2.10	5.27	0.12	3.94
15:1Δ9	0.61	0.27	0.54	0.63	0.33	0.83	0.04	1.25
15:0	1.58	0.71	0.94	1.08	0.48	1.20	0.05	1.61
br15:0	0.00	0.00	0.50	0.54	0.34	0.82	0.00	0.00
br15:0	0.00	0.00	0.00	0.00	0.37	0.88	0.00	0.00
16:1	0.00	0.00	0.00	0.00	0.34	0.82	0.00	0.00
16:1	0.65	0.28	0.49	0.54	0.33	0.80	0.00	0.00
16:1	0.00	0.00	0.46	0.50	0.33	0.79	0.00	0.00
16:1	1.15	0.49	0.53	0.58	0.35	0.84	0.00	0.00
br15:0	0.79	0.33	0.58	0.64	0.40	0.95	0.04	1.17
i16:0	2.66	1.13	1.42	1.55	0.66	1.56	0.06	1.72
16:1Δ4	3.08	1.32	1.13	1.24	0.46	1.09	0.05	1.64
16:1Δ7	1.70	0.73	0.77	0.85	0.41	0.98	0.05	1.59
16:1Δ9*cis*	17.60	7.53	5.43	5.95	1.50	3.60	0.13	4.19
16:1Δ9*trans*	1.02	0.44	0.90	0.98	0.50	1.20	0.05	1.59
16:1Δ11	7.91	3.38	2.09	2.29	0.57	1.36	0.07	2.22
16:0	28.15	11.95	9.85	10.72	4.14	9.84	0.25	7.88
17:1	0.00	0.00	0.48	0.49	0.00	0.00	0.00	0.00
17:1	0.00	0.00	0.46	0.48	0.00	0.00	0.00	0.00
br16:0	0.00	0.00	0.50	0.52	0.00	0.00	0.00	0.00
17:1	0.94	0.38	0.60	0.62	0.35	0.81	0.00	0.00
17:1Δ9*cis*	3.04	1.24	1.36	1.42	0.64	1.45	0.00	0.00
10me16:0	6.32	2.55	3.17	3.28	1.31	2.97	0.15	4.38
br16:0	0.00	0.00	0.84	0.87	0.39	0.88	0.00	0.00
17:1Δ9*trans*	0.80	0.32	0.00	0.00	0.00	0.00	0.00	0.00
br16:0	1.46	0.59	0.68	0.71	0.36	0.82	0.05	1.62
i17:0	1.83	0.74	1.04	1.08	0.52	1.18	0.05	1.60

br16:0	2.04	0.82	1.19	1.23	0.60	1.36	0.00	0.00
17:1Δ11	1.73	0.70	0.77	0.80	0.38	0.86	0.00	0.00
cy17:0	4.30	1.75	2.94	3.06	1.40	3.20	0.13	3.75
17:1	0.64	0.26	0.49	0.51	0.33	0.75	0.05	1.38
17:0	1.36	0.55	0.99	1.02	0.56	1.27	0.06	1.67
br17:0	0.88	0.34	0.62	0.62	0.44	0.94	0.00	0.00
br17:0	1.26	0.48	0.80	0.79	0.48	1.03	0.06	1.70
br17:0	0.89	0.34	0.66	0.65	0.46	0.99	0.06	1.69
18:2	3.17	1.24	0.82	0.82	0.44	0.96	0.06	1.67
br17:0	1.20	0.47	0.70	0.70	0.42	0.90	0.06	1.70
18:2Δ9,12	37.36	14.56	1.99	1.98	0.69	1.50	0.08	2.21
18:1Δ9	7.28	7.28	3.03	3.01	0.98	2.12	0.00	2.72
18:1Δ11*cis*	18.80	15.47	8.03	7.97	2.95	6.39	0.10	5.48
18:1Δ11*trans*	39.97	0.00	1.34	1.33	0.83	1.81	0.19	0.00
18:1Δ13	2.53	0.98	0.73	0.72	0.44	0.95	0.00	0.00
18:0	3.63	1.39	1.95	1.92	0.97	2.08	0.12	3.29
19:1	1.99	0.74	0.87	0.82	0.63	1.31	0.06	1.66
br18:0	0.00	0.00	0.59	0.56	0.41	0.84	0.00	0.00
br18:0	2.27	0.83	0.96	0.91	0.46	0.94	0.07	1.77
br18:0	1.07	0.39	0.73	0.69	0.42	0.87	0.00	0.00
br18:0	0.80	0.30	0.62	0.58	0.41	0.85	0.00	0.00
br18:0	0.00	0.00	0.64	0.60	0.42	0.86	0.07	1.85
19:1	0.85	0.31	0.89	0.00	0.00	0.00	0.00	0.00
cy19:0	8.52	3.15	4.26	4.03	2.07	4.28	0.16	4.39
19:0	0.75	0.28	0.62	0.58	0.42	0.87	0.00	0.00
20:2	1.13	0.40	0.00	0.00	0.00	0.00	0.00	0.00
br20:0	0.00	0.00	0.76	0.69	0.00	0.00	0.00	0.00
20:1Δ11	1.69	0.60	0.00	0.00	0.00	0.00	0.00	0.00
20:1	1.13	0.40	0.00	0.00	0.00	0.00	0.08	1.96
20:0	1.48	0.52	1.61	1.45	0.77	1.51	0.10	2.51
22:0	1.47	0.47	1.26	1.05	0.71	1.29	0.09	2.21
23:0	1.03	0.32	0.83	0.66	0.59	1.04	0.00	0.00
24:0	1.30	0.39	1.02	0.78	0.64	1.08	0.10	2.27
26:0	0.00	0.00	0.79	0.57	0.57	0.89	0.10	2.12
28:0	0.00	0.00	0.00	0.00	0.00	0.00	0.09	1.79

Table 2.6: $\delta^{13}C_{PLFA}$ for sites on the Golden Deposit. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB. Highlighted sections indicate integrated $\delta^{13}C$ of individual PLFA.

COMPOUND	$\delta^{13}C$ SITE 1	$\delta^{13}C$ SITE 5_{CRUST}	$\delta^{13}C$ SITE 2
14:0			-31.4 ± 0.2
i15:0	-26.8 ± 0.2		
a15:0			
i16:0	-27.08 ± 0.4		
16:1Δ9cis	-26.3 ± 0.2		-34.4 ± 0.1
16:1Δ11			
16:0	-29.4 ±0.1	-27.1 ± 0.3	
br16:0	-26.2 ±0.2		
br16:0			
br16:0	-26.1 ± 0.5		
i17:0	-26.4 ± 0.1		
cy17:0	-27.8 ± 0.1		
18:2Δ9,12			-33.4 ± 0.2
18:1Δ9	-27.4 ± 0.1		
18:1Δ11			
18:0	-27.6 ± 0.3	-26.7 ±0.5	
cy19:0	-28.7 ±0.4		-31.6 ± 0.5
AVG $\delta^{13}C_{PLFA}$	**-27.2**	**-26.9**	**-32.7**

Table 2.7: $\delta^{13}C_{PLFA}$ for CORE 1. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB. Highlighted sections indicate integrated $\delta^{13}C$ of individual PLFA.

COMPOUND	$\delta^{13}C$ 0-10	$\delta^{13}C$ 10-17	$\delta^{13}C$ 17-20	$\delta^{13}C$ 20-30	$\delta^{13}C$ 30-42
12:0					-35.5 ± 0.5
I or A 14:0		-29.5 ± 0.2	-31.8 ± 0.2	-30.8 ± 0.2	-30.9 ± 0.3
14:0	-34.3 ± 0.2	-36.5 ± 0.2	-33.5 ± 0.3	-33.8 ± 0.2	-35.8 ± 0.5
i15:0	-28.0 ± 0.3	-27.7 ± 0.2	-28.8 ± 0.1	-28.0 ± 0.1	-28.6 ± 0.1
a15:0					
15:1Δ9					
15:0	-33.7 ± 0.1	-29.4 ± 0.4			
i16:0					
16:1Δ9*cis*					
16:1Δ11*cis*	-33.5 ± 0.2	-32.0 ± 0.1	-32.6 ± 0.2	-32.6 ± 0.3	-33.3 ± 0.9
16:1Δ11*trans*					
16:0					
br16:0			-30.4 ± 0.2	-30.6 ± 0.2	-31.3 ± 0.4
17:1Δ9*trans*					
10me16:0		-29.1 ± 0.2			
br16:0					
i17:0	-29.3 ± 0.1				
a17:0					
17:1		-29.3 ± 0.4	-29.2 ± 0.2	-29.1 ± 0.2	-29.4 ± 0.5
cy17:0					
17:0					
18:2Δ9,12					
18:1Δ9					
18:1Δ11	-32.2 ± 0.2	-30.4 ± 0.2	-29.4 ± 0.1	-29.4 ± 0.1	-29.7 ± 0.6
18:1Δ13					
18:0					
cy19:0	-32.9 ± 0.4			-30.7 ± 0.7	-30.6 ± 0.6
20:0	-35.7 ± 0.3	-34.4 ± 0.4		-32.5 ± 0.3	-31.2 ± 0.7
22:0	-34.7 ± 0.2	-32.7 ± 0.3		-32.2 ± 0.6	-32.5 ± 0.3
24:0	-37.0 ± 0.5			-33.4 ± 0.2	-33.5 ± 0.2
AVG $\delta^{13}C_{PLFA}$	**-33.1**	**-31.1**	**-30.8**	**-31.2**	**-31.9**

Table 2.8: $\delta^{13}C_{PLFA}$ for CORE 2. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB. Highlighted sections indicate integrated $\delta^{13}C$ of individual PLFA.

COMPOUND	$\delta^{13}C$ 0-7.5 cm	$\delta^{13}C$ 7.5-15 cm	$\delta^{13}C$ 15-20 cm	$\delta^{13}C$ 20-25 cm
I or a 14:0	-27.1 ± 1.0			
14:0	-31.7 ± 0.1	-35.7 ± 0.8		-31.9 ± 0.3
i15:0	-26.5 ± 0.1	-27.8 ± 0.1	-32.1 ± 0.6	-28.1 ± 0.1
a15:0				
i16:0				
16:1Δ9cis	-30.5 ± 0.2	-32.5 ± 0.2		-31.8 ± 0.8
16:1Δ11				
16:0			-32.3 ± 0.1	-32.3 ± 0.2
10me16:0		-28.25 ± 0.1	-33.1 ± 0.2	-29.0 ± 0.2
i17:0				
br16:0				
17:1Δ11	-27.7 ± 0.4			
cy17:0				-29.7 ± 1.0
17:1Δ				
17:0				
18:2Δ9,12				
18:1Δ9				
18:1Δ11	-30.3 ± 0.3	-31.1 ± 0.5	-31.7 ± 0.1	-29.1 ± 0.1
18:1Δ11t or 13				
18:0				
cy19:0	-30.3 ± 0.3	-33.8 ± 0.6	-31.1 ± 0.4	-29.7 ± 0.2
24:0				-32.6 ± 0.3
26:0				-32.4 ± 0.3
28:0				-33.9 ± 0.6
AVG $\delta^{13}C_{PLFA}$	**-29.5**	**-31.5**	**-32.1**	**-30.9**

CHAPTER 3

MICROBIAL CARBON CYCLING AND BIOSGINATURES WITHIN THREE SALINE LAKES OF THE CARIBOO PLATEAU, BRITISH COLUMBIA

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ABSTRACT

The saline lakes of the Cariboo Plateau provide an opportunity to investigate biosignatures of life that may be preserved within saline, carbonate rich deposits on Mars. They also provide insight into microbial processes operating under extreme environmental conditions and can be used to further our understanding of biosignatures preserved within the geologic record on Earth. The Cariboo lakes of this study were carbonate-rich, saline lakes that contained benthic microbial mat communities. Geochemical signatures of phospholipid fatty acid (PLFA) and carbon isotopic ($\delta^{13}C$) distributions of organic and inorganic carbon pools were consistent with these microbial mats being dominated by cyanobacteria. The isotopic composition of surface water DIC ($\delta^{13}C_{DIC}$) of all three lakes was expected to be ~-2.2 ‰ if in equilibrium with atmospheric CO_2. However, the measured $\delta^{13}C$ of surface water DIC was 0.0, 3.1, and -1.1 ± 0.2 ‰ for Probe Lake, Deer Lake, and Goodenough Lake, respectively. The enriched $\delta^{13}C_{DIC}$ (+ 1.3- 5.3 ‰) as well $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ of ~23 ‰ were indicative of photosynthetic influences by cyanobacteria on the isotopic composition of the organic and inorganic carbon pools. Similarities between the $\delta^{13}C_{DIC}$ and $\delta^{13}C_{carb}$ ($\Delta^{13}C_{DIC\text{-}carb}$ = 0.5, 0.02, and 0.07 ‰ for Probe Lake, Deer Lake and Goodeough Lake, respectively) in all three lakes reflected rapid precipitation of carbonates and the preservation of the photosynthetic biosignatures in $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ values. Photosynthetic biosignatures were preserved downcore within all three lakes and were not overprinted by heterotrophic activity. If life developed within saline, carbonate rich environments on Mars, biosignatures of photosynthetic activity may be detected through isotopic measurements of carbonates ($\delta^{13}C_{carb}$) and their offsets from the organic carbon pool ($\Delta^{13}C_{carb\text{-}TOC}$).

3.1 INTRODUCTION

The environment on early Mars is hypothesized to have been an alkaline, aqueous environment with evaporitic conditions causing the concentration and precipitation of mineral salts from solution (Bibring et al., 2006; Osterloo et al., 2008). The recent identification of chloride-bearing salt deposits in craters and sinuous channels globally distributed on Mars suggest that such deposits formed through the ponding and evaporation of brines (Osterloo et al., 2008). Furthermore, the detection of carbonates in a rock unit in the Nili Fossae region of Mars supports the existence of past neutral to alkaline waters and indicates that the acidic conditions that were believed to have been widespread on Mars did not affect all aqueous environments on the planet (Fairen et al., 2004; Ehlmann et al., 2008). The conditions present during the formation of such deposits on early Mars are hypothesized to be comparable to conditions when life first originated on Earth (Bibring et al, 2006; Southam et al., 2007). Microbial mats are believed to have been the earliest form of life on Earth, evidence of which is preserved as stromatolites in Precambrian carbonates (Grotzinger and Knoll, 1999). Saline, carbonate rich environments on Earth today are dominated by benthic microbial mats (Bauld, 1981; Jones et al., 1998; Dupraz and Visscher, 2005) and are considered analogues for the study of signatures of life preserved on early Earth and potentially on Mars. The lakes of the Cariboo Plateau, British Columbia are saline, alkaline lakes some of which are dominated by thickly developed benthic microbial mat communities (Renaut and Long, 1989; Schultze-Lam, 1996). Expanding our knowledge on microbially driven precipitation and preservation of carbonates within sediments of saline lakes will shed light on the preservation of microbial biosignatures in similar environments in the geologic record. By studying microbial influences on carbon cycling and carbonate precipitation within selected saline lakes of the Cariboo Plateau, we gain a better understanding of the biosignatures of life that may be preserved within saline, carbonate-rich deposits on Mars as well as gain insight into both modern and ancient microbial processes on Earth.

3.1.1 Saline Environments as Analogues for Habitability on Mars

Low atmospheric pressure and cold temperatures currently dominate the surface of Mars preventing the persistence of liquid water on its surface (Farmer and Des Marais, 1999). As a result, water detected on Mars has been in the form of water-ice clouds, frost, sublimated water vapour and a shallow ice-table at the Phoenix landing site near the arctic polar ice cap (Smith et al., 2009; Whiteway et al., 2009). Any liquid water that is present on the surface is likely transiently present as a saturated salt solution (Landis, 2001). Aqueous, evaporitic environmental conditions that dominated the surface of Mars early in its history were much more habitable, and remnants of such conditions are preserved in the form of carbonate and chloride-bearing salt deposits detectable on the surface of Mars today (Bibring et al., 2006; Ehlmann et al., 2008; Osterloo et al., 2008).

On Earth, saline carbonate rich environments often harbour extensive bacterial communities dominated by benthic microbial mats that trap sediment, influence local water chemistries and result in the precipitation of carbonates from solution (Bauld, 1981; Merz, 1992; Rosen et al., 1995; Jones et al., 1998; Dupraz and Visscher, 2005). Through microbial trapping and binding of sediment and influences on local water geochemistry, microbial mats are thought to be responsible for the formation of stromatolites found in Precambrian carbonates, which provide evidence for the earliest form of life on Earth (Grotzinger and Knoll, 1999). Microbial fossils, biomarkers and viable organisms have been found in many ancient salt deposits on Earth (Farmer and Des Marais, 1999; Stan-Lotter et al., 2002; Mormile et al., 2003; Schubert et al., 2009). Therefore, salt deposits on Mars are favourable environments for the search for biosignatures of past or present life on the planet. By studying the physical and chemical limits of habitability and the preservation of signatures of life in saline environments on Earth, we gain insight into microbial cycling processes and identify target biosignatures of life on early Earth as well as develop a better understanding of biosignatures to search for in similar deposits on Mars.

3.1.2 Microbial Communities and Lipid Biomarkers in Hypersaline Environments

Saline lakes and ephemeral ponds are dynamic environments due to seasonal changes in evaporation and precipitation, which influence salt concentrations and lake depths (Bauld, 1981). Organisms that live in such environments must handle osmotic stresses, cellular dehydration, desiccation and changing salt concentrations (Rothschild and Mancinelli, 2001). Most saline lake microbial communities are arranged as microbial mats, which are communities of microorganisms that form cohesive, often laminated structures through which microbes can completely cycle nutrients (Bauld, 1981; Jones, 1998). Microbial mat communities in hypersaline systems are often dominated by cyanobacteria, which are the autotrophic photosynthetic primary producers that support the other members of the microbial community (Jones et al., 1998). Microbial mats are composed primarily of six microbial groups that include oxygenic (cyanobacteria) and anoxygenic phototrophs, aerobic and anaerobic heterotrophs (including sulfate reducers), fermenters, and sulfide oxidizing bacteria (Dupraz and Visscher, 2005).

Phospholipid fatty acids (PLFA) are the primary building blocks of cellular membranes and are useful indicators of the active bacterial and eukaryotic community as they rapidly turnover upon cell death (Boschker and Middelburg, 2002; Dowhan and Bogdanov, 2002). Furthermore, they vary significantly among different groups of organisms and therefore PLFA can be useful tools as biological markers or 'biomarkers' of microbial groups (Eglinton et al., 1964). Saturated, monounsaturated, and terminally branched fatty acids are major components of cyanobacteria-dominated mats (Sakata et al., 1997; Schouten et al., 2001; Jahnke et al., 2004). Polyunsaturates are rare in most bacteria but are often found in cyanobacteria and therefore may be used as a biomarker for cyanobacteria in environments where eukaryotic activity is minimal (Kenyon, 1972; Jahnke et al., 2004). Sulfate reducing bacteria of the genus *Desulfobacter* can be identified in environmental samples by the biomarker 10me16:0 in the absence of 10me18:0

(Wakeham, 1995). Furthermore, archaea, including methanogens, can be identified by the diether archaeal biomarker archaeol in environmental samples (Pancost and Sinninghe Damste, 2003). The distribution of lipids and their relative concentrations can be used to determine the microbial composition of microbial mat communities within saline environments. Furthermore, microbial carbon cycling within microbial mats influences the carbon isotopic signatures of resulting carbon pools. Therefore, by studying microbial community composition and the preservation of isotopic signatures within carbonate-rich, saline environments we gain a better understanding of signatures of life that we may find in similar deposits on Mars.

3.1.3 Microbial Isotopic Biosignatures

Microbial mats are composed of both autotrophs and heterotrophs each of which influence the isotopic composition of organic and inorganic carbon pools. Within microbial mat environments there is the potential for biological influences to lead to an enrichment or depletion of the isotopic composition of precipitated carbonates from solution depending on the dominance of autotrophy vs. heterotrophy.

Autotrophs use inorganic carbon to produce organic matter, which is isotopically depleted due to fractionations that occur during microbial metabolism (O'Leary, 1988). Autotrophic cyanobacteria use the enzyme RUBISCO in photosynthesis and therefore resulting organic matter is approximately 22 ‰ depleted relative to the substrate CO_2 (Preuβ et al., 1989; Guy et al., 1993). In addition, cyanobacterial PLFA are often 7-9 ‰ depleted relative to biomass (Sakata et al., 2001; Jahnke et al., 2004). The preferential utilization of ^{12}C over ^{13}C during photosynthesis leaves the dissolved inorganic carbon (DIC) pool enriched in ^{13}C (O'Leary, 1988). Such enrichments can result in changes in the isotopic composition of the DIC pool system-wide and/or they may be restricted to local geochemical changes within microbial mat microenvironments (Hollander and McKenzie, 1991; Thompson et al., 1997; Andres et al., 2006; Brady et al., 2010). Photosynthetic

enrichments of DIC, either locally or system-wide, can result in the precipitation of carbonates that are enriched in ^{13}C (O'Leary, 1988; Hollander and McKenzie, 1991; Merz, 1992). If diffusion of DIC is slow within microbial mat systems, photosynthesis can greatly enrich the isotopic composition of local DIC and, if carbonate minerals precipitate within microbial mat microenvironments, carbonates that are precipitated within the mat will be enriched in ^{13}C relative to carbonates that precipitate from ambient water (Rosen et al., 1995; Sumner, 2001). In contrast, hypersaline environments can also become undersaturated with respect to CO_2 and high growth rates of bacteria in these environments can lead to CO_2 limitation (Schidlowski, 1984; O'Leary, 1988; Schouten et al., 2001). The decrease in CO_2 concentration results in less discrimination between ^{12}C and ^{13}C resulting in a decreased fractionation between the DIC and resulting organic carbon pool ($\Delta^{13}C_{DIC\text{-}TOC}$). This has lead to the production of isotopically heavy organic matter in many hypersaline environments (Schidlowski, 1984; Des Marais et al., 1989; Hollander and McKenzie, 1991; Schouten et al., 2001).

Organic matter produced through autotrophy is then used by heterotrophs in respiratory metabolism to produce inorganic carbon. Heterotrophic metabolism of organic carbon results in minimal depletion (1-2 ‰) of resulting inorganic carbon relative to source organic matter. Heterotrophic PLFA are also depleted in ^{13}C and exhibit an ~ 2-4 ‰ depletion relative to bulk biomass (Monson and Hayes, 1982; Blair et al., 1985; Hayes, 1993; Hayes, 2001; Boschker and Middelburg, 2002). In contrast to photosynthetic enrichment of the DIC pool, heterotrophs utilize isotopically depleted organic matter to produce isotopically depleted inorganic carbon (Blair et al., 1985). This can dilute the isotopically enriched signature produced through autotrophy and may result in the precipitation of isotopically depleted carbonates (Blair et al., 1985; Andres et al., 2006; Breitbart et al., 2009). Heterotrophic sulfate reducing bacteria likely have a prominent role in the precipitation of carbonates, as crustal regions in microbial mats are often associated with the area of highest sulfate reduction (Lyons et al., 1984; Walter et al., 1993;

Visscher et al., 2000). Sulfate reduction can influence the local pH within microbial mat microenvironments thereby increasing carbonate saturation and the subsequent precipitation of isotopically depleted carbonate minerals from solution (Visscher et al., 2000; Dupraz and Visscher, 2005). This supports observations of crust formation within microbial mats in zones associated with sulfate reduction and indicates that SRB are a key player in the lithification of modern microbial mats (Lyons et al., 1984; Walter et al., 1993; Jones et al., 1998; Visscher et al., 2000; Dupraz and Visscher 2005).

By studying microbial carbon cycling in Mars analogue sites we gain a better understanding of microbial cycling processes that may have existed in similar systems on Mars. Investigation of microbial lipid and isotopic signatures and their preservation within analogue sites can help us identify signatures of life that exist within these environments and may be preserved on Mars. The hypersaline lakes of the Cariboo Plateau, British Columbia provide an opportunity to investigate microbial carbon cycling and the preservation of microbial signatures within sediments and may shed light on the preservation of biosignatures within the early Earth record and salt and carbonate deposits on Mars. This study focused on understanding microbial carbon cycling and the preservation of biosignatures within three hypersaline lakes of the Cariboo Plateau in order to gain a better understanding of the preservation of microbial biosignatures and the implications for identifying signatures of life in the early Earth geologic record and in similar deposits on Mars.

3.2 SAMPLING AND LABORATORY ANALYSIS:

3.2.1 Study Site

The Cariboo Plateau is located in the southern Interior of British Columbia and contains hundreds of saline lakes that show diversity in form and chemical behaviour (Renaut and Long, 1989). The saline lakes occur in the intermontane

region that lies between the Columbia-Rocky Mountain chains and Coastal Ranges and are characterized by semi-arid to sub-humid climates (Renaut and Long, 1989; Renaut, 1990). The saline lakes vary from subsaline to hypersaline lakes with varying brine compositions depending on bedrock composition and carbonate precipitation (Renaut and Long, 1989). The saline lakes of this study were underlain by a mantle of glacial till and Miocene and Pliocene basalt flows (Renaut and Long, 1989; Renaut, 1990). Three lakes located in the Cariboo Plateau were chosen for this study based on surface and porewater chemistries determined by Slater (1997): Probe Lake, Deer Lake, and Goodenough Lake (GEL) (see Figure 3.1).

All three lakes were hypersaline, alkaline (pH ~ 10), sodium carbonate lakes poor in Ca and Mg with Na-CO_3-(SO_4)-Cl brine compositions (Renaut and Long, 1989; Slater, 1997). Probe Lake and Deer Lake had high concentrations of methane in surface waters (5-9μM) and low dissolved sulfate (1mM) while Goodenough Lake had high sulfate (36mM) and low methane (0.3μM) concentrations in its surface water (Slater, 1997). The lakes contained benthic microbial mat communities that were dominated by phototrophic coccoid and filamentous cyanobacteria in both the aerobic and anaerobic zones, accompanied by purple sulfur bacteria in the anaerobic zone (Renaut and Long, 1989; Schultze-Lam, 1996). PLFA of surface microbial mat communities and isotopic characterization of porewater methane and DIC had been determined previously by Brady et al. (2009).

3.2.2 Field Sampling

The Cariboo lakes were sampled in mid-July 2009. Parameters including temperature, salinity and pH were determined in the field using a hand-held YSI instrument (YSI Incorporated, Yellowsprings, OH). Surface water samples were collected for water chemistry analysis from each lake using pre-cleaned polyethylene bottles and kept cool and in the dark following the protocol by the Environment Canada, Pacific Environmental Science Centre (PESC) in Vancouver, Canada. Water samples were collected for $\delta^{13}C_{DIC}$ analysis from each lake in crimp

sealed gas serum bottles with no headspace and fixed with mercuric chloride to prevent further microbial activity.

One sediment core was collected from each lake near the shoreline using a 10 cm diameter polycarbonate core tube. Cores were collected to depths of 21, 31, and 25 cm for Probe Lake, Deer Lake and Goodenough Lake, respectively. Cores were visually characterized and subsectioned into 1-2 cm sections on site and stored in 50 mL pre-combusted glass jars and stored frozen until analysis. Four samples from each core were selected for laboratory analysis based on visual appearance and were believed to represent the variations present downcore (Figure 3.2). Laboratory analysis was performed on Probe Lake samples at the following depths: 1-2, 5-7, 15-16 and 19-20 cm. Deer Lake samples were used at the following depths: 1-2, 8-10, 13-14, and 22-24 cm. Goodenough Lake samples were analyzed at the following depths: 1-2, 7-9, 11-12, and 17-19 cm.

3.2.3 Laboratory Analysis

Organic and inorganic carbon contents of selected core samples of each lake were determined using loss on ignition (LOI). Percent total organic carbon content (TOC) was determined by the mass difference of the original dry sample (105°C overnight) to that after combustion at 550°C for 4hrs. Percent inorganic carbon content was determined by the mass difference of the dry weight sample after combustion at 550°C to that after heating at 950°C for 2 hrs (Heiri et al., 2001). Percent error was < 2.5 %.

Core samples of each lake were freeze-dried prior to extraction of phospholipid fatty acids (PLFA). PLFA were extracted using a modified Bligh and Dyer method (Bligh and Dyer, 1959) and fractioned into non-polar, neutral and polar lipids using silica gel chromatography. The polar fraction was subjected to mild alkaline methanolysis to convert PLFA into fatty acid methyl esters (FAMEs) (Guckert et al., 1985). Methanolyzed samples were fractionated using silica gel chromatography to remove any impurities. Microbial FAMEs were separated using

gas chromatography mass spectrometry (GC/MS) on an Agilent GC/MS with DB-XLB capillary column (30 m x 0.25 mm I.D. x 0.25 μm film thickness) using a temperature program of 40°C (1 min.), 20°C/min to 130°C, 4°C/min to 160°C, 8°C/min to 300°C (5 min). PLFA were identified based on retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc., Pleasant Gap, Pennsylvania, USA, and PUFA-3 Menhaden Oil, Supelco Analytical, Oakville, ON, Canada) and characteristic ion fragments. Monoenoic double-bond positions and geometry were determined by derivitization with dimethyl disulfide and subsequent analysis by GC/MS (Nichols et al., 1986). The presence of archaeal diether lipids was determined by derivitization of polar lipids using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and subsequent analysis by GC/MS (Teixidor and Grimalt, 1992). Cell density was calculated from the total PLFA using the conversion factor $2x10^4$ cells/pmol PLFA from Balkwill et al. (1988).

Fatty acid nomenclature is designated as follows; A:BΔC, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position (Δ) of the double bond from the carboxyl end of the molecule. The geometry of the bond is indicated as 'c' for *cis* and 't' for *trans*. The prefix 'br' indicates a branched fatty acid whose branch position is unknown. Known branch positions are denoted as the branch location followed by the total number of carbon atoms. *Iso-* and *anteiso-* branching are denoted by the prefixes 'i' or 'a', respectively and cyclopropyl fatty acids are denoted by the prefix 'cy'.

3.2.4 Isotopic Analysis

The carbon isotopic composition of the PLFA ($\delta^{13}C_{PLFA}$), total organic carbon ($\delta^{13}C_{TOC}$), and dissolved inorganic carbon ($\delta^{13}C_{DIC}$), are reported using standard delta notation ($\delta^{13}C$) and referenced to the internationally accepted standard carbonate rock Vienna Pee Dee Belemnite (VPDB) (Sessions, 2006). The relative $\delta^{13}C$ of a given sample was calculated as follows:

$$\delta^{13}C_{sample} = \frac{(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{std}) \times 1000‰}{^{13}C/^{12}C_{std}} \quad (3.1)$$

Aliquots of microbial FAMEs were injected into a split/splitless injector set to splitless mode at 300°C prior to separation using a GC/MS on an Agilent GC/MS with DB-XLB capillary column (30 m x 0.32 mm I.D. x 0.25 μm film thickness) and a temperature program of 80°C (1 min.), 4°C/min to 280°C, 10°C/min to 320°C (20 min). Individual FAMEs were eluted from the column via a combustion oven set at 960°C , were combusted to CO_2 and analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS).

The methanol added to the FAMEs during mild alkaline methanolysis was isotopically characterized and $\delta^{13}C_{PLFA}$ values were corrected using the following equation:

$$\delta^{13}C_{PLFA} = [(N+1)* \delta^{13}C_{measured} - \delta^{13}C_{MeOH}]/N \quad (3.2)$$

where N is the number of carbon atoms. Individual samples were analyzed in triplicate and precision is reported as one standard deviation (s.d.).

Carbonate stable isotope analyses were performed on an Optima isotope ratio mass spectrometer with an Isocarb common acid bath at 90°C at McMaster University. Triplicate analysis of carbonate samples generally gave precisions of ± 0.4 ‰ (1 σ) for $\delta^{13}C_{carb}$ and ± 0.5 ‰ (1 σ) for $\delta^{18}O_{carb}$. $\delta^{13}C_{TOC}$ of each lake's core samples was determined by treating dry samples with 1 M HCl to remove carbonate. Isotopic values were determined on an EA-Delta XL at McMaster University and triplicate analyses gave a precision of ± 0.4 ‰ (1 σ) for the $\delta^{13}C_{TOC}$. $\delta^{13}C_{DIC}$ of the water samples was determined by acidification and conversion to CO_2 and analyzed by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa (St-Jean, 2003). Analytical precision was ± 0.2 ‰.

The equilibrium concentration of dissolved CO_2 was calculated according to Henry's law:

$$[CO_2] = K_h * pCO_2 \quad (3.3)$$

where K_h is Henry's law constant for CO_2 that varies with temperature and salinity (mol L^{-1} bar^{-1}) and pCO_2 represents the partial pressure of atmospheric CO_2 (bar) (Deines et al., 1974). The concentrations of various carbonate species were determined using the measured pH and temperatures of the waters and the expected $\delta^{13}C_{DIC}$ (without microbial influence) was determined based on known fractionation factors for carbonate species relative to gaseous CO_2 (Deines et al., 1974) (see Appendix A).

3.3 RESULTS

3.3.1 Cariboo Plateau Lakes Characterization: Organic and Carbonate Content, Cell Density, and Water Chemistry

Visual appearances of core samples and lakes are depicted in Figures 3.2 and 3.3. Probe Lake was the deepest of the three lakes with a mid-summer depth of approximately 1.75 meters (Figure 3.3a). Evaporation of the lake led to the formation of efflorescent salt crusts in surrounding saline mudflats. Microbial mats were common within the lake and are demonstrated in Figure 3.3c. The core sample taken from Probe lake was to a depth of approximately 20 cm with the upper 13 cm representing organic rich disrupted microbial mat material followed by a transition to charcoal-grey silty-clay sediment. Deer Lake was a shallow lake with water depth measuring ~25 cm in mid-summer. Extensive microbial mat development was visible at the lake (Figure 3.3b) and evaporation led to the formation of an organic-rich, salt-encrusted mudflat surrounding the lake. The core taken from Deer Lake was to a depth of approximately 30 cm. The core transitioned from a green and pink disturbed microbial mat material into green organic rich material with the bottom of the core being grey, clay-like sediment. Goodenough Lake was also a shallow lake with a depth of ~20 cm during the sampling period. The surrounding mudflats consisted of polygonally cracked microbial mats encrusted with sodium carbonate efflorescence (Renaut and Long, 1989) (Figure 3.3d). A core depth of ~25 cm was taken from the margin of the lake and consisted

of dark-green disrupted microbial mat to a depth of 10 cm which transitioned into grayish-black sediment for the rest of the core.

Organic carbon and carbonate contents of the three lakes are shown in Figure 3.4 a. and b. Cell density downcore is shown in Figure 3.5. Of the three lakes, Probe Lake sediments had the lowest organic carbon and carbonate content. Organic carbon contents of the Probe Lake core ranged from ~23 % at the top of the core decreasing to ~2 % at the bottom of the core. Carbonate contents exhibited a similar trend with ~5.5 % carbonate content at the top of the core and decreasing to ~1 % at the bottom of the core. Similarly, cell density, based on PLFA concentrations, decreased by two orders of magnitude between the top and bottom of the core. Deer Lake had the highest organic and carbonate contents as well as cell density, all of which decreased downcore. Organic carbon contents were approximately 30-40 % for the length of the core and carbonate contents ranged from 15 % at the top of the core to 8 % at the bottom of the core. Similar to Probe and Deer Lake, Goodenough Lake organic carbon content, carbonate content, and cell density decreased throughout the length of the core with cell density being the lowest of the three lakes. Organic carbon content ranged from ~ 31 % at the top of the core to ~7 % at the bottom while carbonate content ranged from 15 % at the top of the core and decreased to 1 % in the bottom sediments.

The water chemistry analysis of the three Cariboo Plateau lakes is listed in Table 3.1. All three lakes had pH values of ~10.1-10.3 during the sampling period which was consistent with measurements made during previous studies of the lakes (Slater, 1997; Brady et al., 2009). All three lakes had high salinity values of 6.3, 7.6, and 16.3 % for Deer Lake, Probe Lake, and Goodenough Lake, respectively, and may be classified as hypersaline lakes in the summer according to Renaut and Long (1989). All three lakes had high dissolved inorganic carbon (DIC) (~4000-9500 mg/L) and Na (~13600-40000 mg/L) contents and were poor in Ca and Mg, consistent with the classification as sodium carbonate lakes by Renaut and Long (1989). Goodenough Lake had a high concentration of SO_4^{2-} (3850 mg/L) compared

to Probe and Deer Lake (63 and 50 mg/L, respectively) consistent with previous identification of elevated surface and porewater concentrations of SO_4^{2-} by Slater (1997) and Brady et al. (2009).

3.3.2 Phospholipid Fatty Acid Distributions

The phospholipid fatty acids identified in the three lakes of the Cariboo Plateau are listed in Tables 3.2 to 3.4. PLFA were grouped into polyunsaturated, terminally branched, mid-chain branched, monounsaturated and cylopropyl, and saturated PLFA. Distributions of microbial PLFA groups are shown in Figure 3.6. The PLFA in all three of the Cariboo Plateau lakes exhibited some variations in PLFA distribution, however, overall the microbial community composition within the lakes were quite similar. All three lakes had saturated fatty acids, with 14:0, 16:0, and 18:0 being predominant, which are widespread in bacteria and eukaryotes (Boschker and Middelburg, 2002). All three lakes had high proportions of monounsaturated fatty acids (20-50 %), dominated by 16:1Δ9cis, 18:1Δ9 and 18:1Δ11, which decreased in concentration downcore, and were indicative of gram-negative bacteria including cyanobacteria (Nichols and Wood, 1968; Wilkinson, 1988; Findlay et al., 1990; Grimalt et al., 1992; Hedrick et al., 2005). Approximately 30-50 % of the PLFA distribution at each lake was composed of terminally branched fatty acids, dominated by i15:0 and a15:0, indicative of gram-positive bacteria (Kaneda, 1991; White et al., 1996). Proportions of mid-chain branched fatty acids were small, however, the sulfate reducer biomarker 10me16:0 (Wakeham, 1995) was detected in all three lakes with Goodenough Lake having the highest concentrations throughout the entire core (~3.5-5.5 mol %). The archaeal biomarker archaeol was detected in the Deer Lake core, indicative of the presence of methanogens, and was consistent with previous identification of methane gas in porewaters and bubbling up from sediments as well as identification of methanogens in surface mats of Deer Lake by Brady et al. (2009).

3.3.3 Organic, Inorganic, and PLFA Isotopes

The measured organic and inorganic carbon, and bulk PLFA isotopes for the three lakes of the Cariboo Plateau are shown in Table 3.6 and Figures 3.7 to 3.8. The carbon isotopic composition of individual PLFA in the three lakes is listed in Table 3.5. The $\delta^{13}C$ of the atmosphere was measured previously by a nearby lake by Brady et al. (2009) and determined to be -9.6 ± 0.2 ‰. Based on pH, temperature, and Henry's law for the solubility of gases, the dominant carbonate species at all three lakes were bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) and the lakes were saturated with respect to atmospheric CO_2. Using known fractionation factors (Deines et al., 1974) the expected $\delta^{13}C_{DIC}$ without microbial influence was calculated to be ~ -2.2 ‰ for all three lakes. However, the measured $\delta^{13}C_{DIC}$ for the three lakes was 0.0, 3.1, and -1.1 ± 0.2 for Probe Lake, Deer Lake, and Goodenough Lake, respectively.

For all three lakes, the $\delta^{13}C_{carb}$ at the top of the cores was the same as the $\delta^{13}C_{DIC}$ of surface waters. In general, the $\delta^{13}C_{carb}$ became more enriched downcore with enrichments of 1-4 ‰ at the bottom of the core compared to the top and with Probe Lake showing the least enrichment. $\delta^{18}O_{carb}$ values showed no correlation with $\delta^{13}C_{carb}$ values and no visible trends with depth. The $\delta^{13}C_{TOC}$ also showed a general trend of enrichment downcore for the three lakes. However, the bottom of Deer Lake is depleted by ~ 3 ‰ compared to the rest of the core and is associated with an enrichment of $\delta^{13}C_{carb}$ of 2-3 ‰. The average $\Delta^{13}C_{DIC\text{-}TOC}$ and $\Delta^{13}C_{carb\text{-}TOC}$ of all three lakes was approximately 23 ‰ which is the expected offset produced through C3 photosynthesis (Preuβ et al., 1989; Guy et al., 1993). Deer Lake had the most enriched $\delta^{13}C_{BULK\ PLFA}$ of -25 ‰ compared to Probe Lake and Goodenough Lake whose bulk PLFA values were ~ -29.0 ‰ and -29.5 ‰, respectively. However, in comparison to the $\delta^{13}C_{TOC}$ all three lakes had $\Delta^{13}C_{TOC\text{-}PLFA}$ in the range of 4.5-6.5 ‰. There was a variation of ~4 ‰ for the $\delta^{13}C$ of individual PLFA for each lake depth indicative of the presence of both autotrophic and heterotrophic bacteria. The $\Delta^{13}C_{TOC\text{-}16:1\Delta9\ AND\ 11}$ for each lake was approximately 7 ‰ which was consistent with

isotopic offsets between PLFA and bulk organic matter produced by cyanobacteria (Bauld, 1981; Jones et al., 1998; Dupraz and Visscher, 2005).

3.4 DISCUSSION

3.4.1 Generation of Biosignatures

Probe Lake, Deer Lake, and Goodenough Lake are only three of many saline lakes of the Cariboo Plateau, British Columbia. They were sodium carbonate lakes poor in Ca and Mg and had extensive microbial mat communities that were dominated by cyanobacteria. Cyanobacterial photosynthesis and microbial carbon cycling within all three lakes influenced the isotopic compositions of the organic and inorganic carbon pools. The primary goal of this research was to assess the preservation of microbial biosignatures within saline environments and their applications for the identification of biosignatures within the early Earth geologic record and on Mars.

3.4.1.1 Surface Water DIC Biosignature

All three Cariboo Plateau lakes were saturated with respect to atmospheric CO_2. Under saturated conditions the isotopic composition of lake DIC ($\delta^{13}C_{DIC}$) was expected to reflect equilibrium with atmospheric CO_2 $\delta^{13}C$ values. The $\delta^{13}C_{DIC}$ of all three lakes was expected to be ~-2.2 ‰ if in equilibrium with atmospheric CO_2. However, the measured $\delta^{13}C$ of surface water DIC was 0.0, 3.1, and -1.1 ± 0.2 ‰ for Probe Lake, Deer Lake, and Goodenough Lake, respectively. These values reflect a ^{13}C enrichment of DIC, relative to the expected values, by 2.1, 5.3, and 1.3 ‰. Enrichment of lake DIC could be the result of both biological and physical process. It is important to be able to distinguish biological and physical influences on $\delta^{13}C$ values in order to be able to identify biosignatures in the geologic record both on Earth and on Mars.

Microbial mats in most saline environments are dominated by cyanobacteria, which are photosynthetic primary producers that couple light energy and CO_2

fixation to produce organic matter (Dupraz and Visscher, 2005). The PLFA distribution within the microbial mats of all three Cariboo lakes consisted of high proportions of saturated (16-20 mol %), monounsaturated (35-50 mol %), and terminally branched (30-35 mol %) fatty acids, which are dominant PLFA in cyanobacteria-dominated mats (Sakata et al., 1997; Schouten et al., 2001; Jahnke, 2004). The isotopic difference between bulk organic matter and the monounsaturated PLFA 16:1Δ9 and 11 ($\Delta^{13}C_{TOC\text{-}16:1\Delta9\ AND\ 11}$) was approximately 7 ‰ which was also indicative of PLFA synthesis by cyanobacteria (Bauld, 1981; Jones et al., 1998; Dupraz and Visscher, 2005). All of this evidence, along with visual evidence of the presence of dark green microbial mats at each lake, was consistent with cyanobacteria being dominant members of the microbial community within the microbial mats at all three Cariboo Plateau lakes. Cyanobacterial metabolism produces organic carbon that is isotopically depleted relative to the inorganic carbon source (O'Leary, 1988; Preuβ et al., 1989; Guy et al., 1993). The average isotopic offset between the inorganic carbon of the surface waters and the organic carbon ($\Delta^{13}C_{DIC\text{-}TOC(avg)}$) measured at each lake was approximately 23 ‰. This was consistent with isotopic offsets produced through non-CO_2 limited photosynthesis by cyanobacteria (Preuβ et al., 1989; Guy et al., 1993). Therefore, this isotopic difference between inorganic and organic carbon ($\Delta^{13}C_{DIC\text{-}TOC(avg)}$) can be considered a signature of photosynthetic activity. Preferential utilization of ^{12}C over ^{13}C during photoautotrophy by cyanobacteria can also lead to an enrichment of the residual inorganic carbon pool and has been observed in many saline environments (O'Leary, 1988; Preuβ et al., 1989; Hollander and McKenzie, 1991; Merz, 1992; Guy et al., 1993). Therefore, photosynthetic uptake of DIC by cyanobacteria within the Cariboo Plateau is a biological mechanism by which the $\delta^{13}C_{DIC}$ of surface water can become enriched in ^{13}C.

The Cariboo lakes also experienced seasonal evaporative concentration of lake waters, which may influence $\delta^{13}C_{DIC}$ values. Evaporation has been shown to cause enrichment in $\delta^{13}C_{DIC}$ values of surface waters due to outgassing of ^{13}C-

depleted CO_2 (Stiller et al., 1985; Valero-Garcés et al., 1999). All three lakes were saturated with respect to CO_2, which suggested that outgassing of ^{13}C-depleted CO_2 could be occurring thereby resulting in the enriched $\delta^{13}C_{DIC}$ values detected in lake surface waters. However, despite CO_2 saturation within all three lakes, correlations between $\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$ values expected for evaporative influences on surface water DIC (McConnaughey, 1989; Léveillé et al., 2007; Kremer et al., 2008) was not observed within lake core sediments. This indicated that evaporation was not a controlling factor on the isotopic composition of surface water DIC. Rather, based on the evidence of cyanobacterial (autotrophic) dominance within all three lakes, the isotopic offset between $\Delta^{13}C_{DIC\text{-}TOC(avg)}$, and lack of correlation evidence for evaporative influences on lake DIC, the observed enrichment in surface water DIC was determined to be due to cyanobacterial photosynthesis resulting in a ^{13}C-enrichment of the residual DIC pool and represents a photosynthetic biosignature.

3.4.1.1.1 Other Influences on the Surface Water DIC Signature

Replenishment of lake DIC through diffusion of atmospheric CO_2 can result in a depletion of lake water $\delta^{13}C_{DIC}$ values. During periods of undersaturation, invasion of ^{13}C-depleted CO_2 from the atmosphere occurs due to faster kinetics for ^{12}C versus ^{13}C (Herczeg and Fairbanks, 1987; Lazar and Erez, 1990). This can result in depleted $\delta^{13}C_{DIC}$ values of lake water. However, the Cariboo Plateau lakes were saturated with respect to atmospheric CO_2 during the sampling period. This suggested that CO_2 was in equilibrium with the atmosphere and that invasion of ^{13}C-depleted atmospheric CO_2 was a minimal process. Therefore, the enriched $\delta^{13}C_{DIC}$ values of surface waters were likely not diluted by diffusion of ^{13}C-depleted atmospheric CO_2.

Heterotrophic activity can also result in a ^{13}C-depletion of lake DIC. Heterotrophs consume isotopically depleted organic carbon and produce isotopically depleted inorganic carbon (Blair et al., 1985). Inputs of isotopically depleted DIC to surface waters can deplete the $\delta^{13}C_{DIC}$ thereby narrowing the

$\Delta^{13}C_{DIC\text{-}TOC(avg)}$ margin (Blair et al., 1985). Heterotrophs were present within the microbial mats of the Cariboo Plateau lakes, which was evident by the presence of heterotrophic markers *iso* and *anteiso* and mid-branched PLFA (Blair et al., 1985; Dowling et al., 1986; Vestal and White, 1989; Parkes et al., 1993; Londry et al., 2004). Heterotrophic sulfate reducers of the genus *Desulfobacter* were also important components of the microbial communities at Probe Lake, Goodenough Lake, and to a lesser extent Deer Lake, which was indicated by the presence of the sulfate reducer biomarker 10me16:0 (Wakeham, 1995). Therefore, heterotrophic activity within the lakes would be contributing ^{13}C-depleted DIC into lake waters thereby influencing lake $\delta^{13}C_{DIC}$ values.

Regardless of inputs of isotopically depleted DIC, lake $\delta^{13}C_{DIC}$ values were still enriched relative to expected values. This indicated that production of isotopically depleted DIC through atmospheric influx of CO_2 or through heterotrophic activity did not overprint the enriched signal produced through cyanobacterial photosynthesis. The enriched $\delta^{13}C_{DIC}$ of lake surface water was thus indicative of photosynthetic influences on surface water DIC. Furthermore, the isotopic offset of organic carbon from the inorganic carbon pool ($\Delta^{13}C_{DIC\text{-}TOC(avg)}$) was also indicative of photosynthetic activity. Therefore, enriched $\delta^{13}C_{DIC}$ values and isotopic offsets between DIC and organic carbon ($\Delta^{13}C_{DIC\text{-}TOC(avg)}$) are both biosignatures of photosynthetic activity detected within the Cariboo Plateau lakes.

3.4.1.2 Record of Microbial Biosignatures in Precipitated Carbonates

Biological influences on lake DIC may also be reflected in the isotopic composition of precipitated carbonates, thereby preserving the biosignature within lake sediments. The surface water $\delta^{13}C_{DIC}$ of the Cariboo Plateau lakes were 0.0, 3.1, and -1.1 ± 0.2 ‰ for Probe Lake, Deer Lake and Goodenough Lake, respectively. Corresponding isotopic values of carbonates ($\delta^{13}C_{carb}$) at the top of each lake core were -0.5, 3.1, and -1.1 ± 0.2 ‰. The difference between the isotopic composition of surface water DIC and surface sediment carbonates ($\Delta^{13}C_{DIC\text{-}carb}$) was = 0.5, 0.02,

and 0.07 ‰ for Probe Lake, Deer Lake and Goodenough Lake, respectively. The similarity in the $\delta^{13}C_{DIC}$ and $\delta^{13}C_{carb}$ values indicated that carbonates precipitating out of solution were reflecting the isotopic composition of surface water DIC. As precipitation rates of carbonate minerals such as $CaCO_3$ increase, there is a decreased fractionation between the isotopic value of bicarbonate (HCO_3^-) and $CaCO_3$ ($\varepsilon_{HCO3\text{-}\ CaCO3}$) (Turner, 1982). Therefore, the similarity in $\delta^{13}C_{DIC}$ and $\delta^{13}C_{carb}$ reflected rapid precipitation of carbonates and a preservation of the isotopic composition of lake DIC values in the $\delta^{13}C_{carb}$. As discussed above, the enriched DIC values were biosignatures of photosynthetic activity. Therefore, the preservation of enriched surface water $\delta^{13}C_{DIC}$ values in precipitated carbonates ($\delta^{13}C_{carb}$) reflects a preservation of this photosynthetic biosignature within the sediments of the three lakes.

In addition to enriched $\delta^{13}C_{DIC}$ values, the $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ was also a signature of photosynthetic activity within the Cariboo Plateau lakes. If precipitating carbonates are reflecting the isotopic composition of lake DIC, then the photosynthetic signature of $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ should also be preserved as offsets between the isotopic compositions of carbonate and organic carbon ($\Delta^{13}C_{carb\text{-}TOC}$). In fact, the $\Delta^{13}C_{carb\text{-}TOC}$ at the top of each lake core was 23.2, 22.2, and 23.6 ‰ for Probe Lake, Deer Lake, and Goodenough Lake, respectively. These values reflect isotopic offsets produced through non-CO_2 limited photosynthesis by cyanobacteria. Therefore, the isotopic offsets between carbonates and organic matter within lake sediments can be used as a biosignature of photosynthetic activity. The influences of photosynthetic activity within these saline lakes are therefore not only preserved within the enriched isotopic composition of precipitated carbonates ($\delta^{13}C_{carb}$) as discussed above, but also in the offsets between preserved carbonates and organic matter ($\Delta^{13}C_{carb\text{-}TOC}$).

3.4.2 Preservation of Biosignatures

3.4.2.1 Preservation of Biosignatures Downcore

As discussed above, the photosynthetic influences by cyanobacteria on lake $\delta^{13}C_{DIC}$ values were preserved as a biosignature in precipitated carbonates ($\delta^{13}C_{carb}$). The biosignature of isotopically depleted organic matter relative to lake DIC ($\Delta^{13}C_{DIC\text{-}TOC(avg)}$) was also preserved as the difference in isotopic composition between carbonates and organic matter ($\Delta^{13}C_{carb\text{-}TOC(avg)}$). In order to assess the applicability of such biosignatures to the identification and interpretation of life on early Earth and on Mars the biosignatures must endure burial into lake sediments.

The percent organic and carbonate content decreased downcore within all three lakes, however, both organic matter and carbonate are present at the bottom of each lake core. The $\delta^{13}C$ of corresponding carbonates were all enriched (+ 1.2 – 7.5 ‰) relative to the expected $\delta^{13}C$ of carbonates precipitating from DIC in equilibrium with atmospheric CO_2 (~-2.2 ‰). Therefore, the photosynthetic biosignature of enriched carbonates was preserved in the downcore record at all three lakes. Furthermore, the $\Delta^{13}C_{carb\text{-}TOC}$ downcore for all three lakes was in the range of +20.5 to 27.1 ‰. This is consistent with isotopic offsets produced through non-CO_2 limited photosynthesis by cyanobacteria (Preuβ et al., 1989; Guy et al., 1993). The $\Delta^{13}C_{carb\text{-}TOC}$ biosignature was thus also preserved downcore within all three Cariboo Plateau lakes.

3.4.2.2 Influences on Downcore Biosignatures

The downcore biosignature of enriched carbonates and isotopic offset between carbonates and organic carbon within the Cariboo Plateau lakes display some variations. The bottom sediment $\delta^{13}C_{carb}$ values were enriched relative to surface sediment values by 1.2, 2.3, and 3.6 ‰ for Probe Lake, Deer Lake, and Goodenough Lake, respectively. Correspondingly, the $\Delta^{13}C_{carb\text{-}TOC}$ values downcore were 23.7 ± 0.4, 22.8 ± 3.0, and 25.0 ± 1.1 ‰ for the three lakes. Different biological metabolisms can influence the downcore biosignatures and can cause some variations in the biosignatures that are preserved.

First and foremost, variations in photosynthetic activity within the lakes could cause differences in lake DIC values over time. Hypersaline environments are often undersaturated with respect to CO_2 due to high rates of photosynthetic activity (Schidlowski, 1984; O'Leary, 1988; Schouten et al., 2001). The decrease in CO_2 concentration results in less discrimination between ^{12}C and ^{13}C resulting in relatively ^{13}C-depleted surface water DIC and a decreased fractionation between the DIC and resulting organic carbon pool ($\Delta^{13}C_{DIC\text{-}TOC}$) (Schidlowski, 1984; Des Marais et al., 1989; Hollander and McKenzie, 1991; Schouten et al., 2001). Although the lakes were saturated with respect to CO_2, periods of undersaturation and variations in lake DIC values have been identified in previous sampling periods (Slater, 1997; Brady et al., 2009). Such variations in CO_2 availability and photosynthetic rates could therefore influence lake $\delta^{13}C_{DIC}$ values and corresponding $\delta^{13}C_{carb}$ as well as the $\Delta^{13}C_{carb\text{-}TOC}$ over time. Periods of undersaturation may be detected by relatively depleted $\delta^{13}C_{carb}$ values and decreased $\Delta^{13}C_{carb\text{-}TOC}$. Such influences may be reflected, for example, in the Deer Lake 13-14 cm sample, which had the most depleted carbonate values downcore ($\delta^{13}C_{carb}$ of 1.9 ‰) and a $\Delta^{13}C_{carb\text{-}TOC}$ of 20.4 ‰. This is consistent with depleted surface water $\delta^{13}C_{DIC}$ and decreased $\Delta^{13}C_{DIC\text{-}TOC}$ values of surface microbial mats during periods of CO_2 undersaturation in previous sampling seasons of the three lakes by Brady et al. (2009). In contrast, periods of CO_2 saturation would have resulted in more enriched $\delta^{13}C_{carb}$ and greater differences in $\Delta^{13}C_{carb\text{-}TOC}$ values. This may be demonstrated at the bottom of the Goodenough Lake core (17-19 cm), which had $\delta^{13}C_{carb}$ values of 2.4 ‰ and a $\Delta^{13}C_{carb\text{-}TOC}$ of 26.2 ‰. This is also consistent with measurements by Brady et al. (2009), which detected enriched surface water $\delta^{13}C_{DIC}$ and larger $\Delta^{13}C_{DIC\text{-}TOC}$ values during periods of CO_2 saturation within the three Cariboo Plateau lakes in previous sampling periods. Therefore, variations in lake DIC values due to differences in photosynthetic activity can influence preserved photosynthetic biosignatures downcore. However, despite potential variations in photosynthetic activity,

enriched $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ downcore values were consistent with non-CO_2 limited photosynthesis and preserved the photosynthetic biosignatures downcore.

Heterotrophic activity downcore can result in the production of isotopically depleted carbonates. Uptake of isotopically depleted organic carbon produced through autotrophy leads to the production of ^{13}C-depleted DIC, which can be preserved in precipitated carbonates (Blair et al., 1985; Andres et al., 2006; Breitbart et al., 2009). In particular, heterotrophic sulfate reduction is thought to have a large role in carbonate precipitation in microbial mats by influencing local pH thereby increasing carbonate saturation and the precipitation of isotopically depleted carbonates from solution (Visscher et al., 2000; Dupraz and Visscher, 2005). PLFA distributions of *iso*, *anteiso* and mid-branched PLFA indicated that heterotrophs were present within all three Cariboo lakes and generally increased in concentration downcore. This included the presence of heterotrophic sulfate reducers whose concentrations also increased downcore, evident by the presence of the sulfate reducer biomarker 10me16:0 (~3.5-5.5 mol %). Heterotrophic activity within all three lakes would be producing isotopically depleted inorganic carbon thereby diluting the isotopic composition of precipitated carbonates. Brady et al. (2009) demonstrated that there are seasonal variations in lake DIC and carbonate values depending on the dominance of heterotrophy vs. autotrophy. However, the enriched $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ signature preserved downcore demonstrated that heterotrophic production of isotopically depleted DIC does not overprint the enriched autotrophic signature. Therefore, despite heterotrophic activity, the photosynthetic biosignatures of enriched $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ in the range of non-CO_2 limited photosynthesis were still preserved downcore.

3.4.2.2.1 Potential Impacts of Methanogenesis

Methanogensis can also influence the isotopic composition of precipitated carbonates downcore. Methanogens operating through the CO_2 reduction pathway convert CO_2 to CH_4 (Konhauser, 2007) and their strong preference for ^{12}C over ^{13}C

results in the enrichment of the residual DIC pool and the production of isotopically depleted organic matter (Botz et al., 1996; Whiticar, 1999; Gu et al., 2004). Brady et al. (2009) previously identified that isotopic values of methane gas ($\delta^{13}C$ = -70.6 ± 2.6 ‰) emitted from Deer Lake indicated that methanogenesis was occurring through the CO_2 reduction pathway. The identification of the archaeal biomarker, archaeol, in this study and the presence of methane gas in porewater and surface water measured by Brady et al. (2009) indicated that methanogens were active members of the microbial community at depth within Deer Lake. Therefore, the enriched $\delta^{13}C_{carb}$ (5.3 ‰) and depleted $\delta^{13}C_{TOC}$ (-21.8 ‰) measured at the bottom of the Deer Lake core may reflect the influence of methanogens on these carbon isotopic signatures. Previous detection of methane gas in surface and sediment porewaters of Probe Lake by Slater (1997) and Brady et al. (2009) also provide evidence for the occurrence of methanogenesis operating through the CO_2 reduction pathway ($\delta^{13}C$ = -70.0 ± 2.5 ‰). However, the lack of detection of the archaeal biomarker archaeol and lack of significantly enriched $\delta^{13}C_{carb}$ in this study indicated that methanogenesis was not a major process influencing the isotopic composition of precipitated carbonates downcore. Methanogenesis may not be the primary driver of $\delta^{13}C_{carb}$ and $\delta^{13}C_{TOC}$ values in any of the three Cariboo lakes studied as Brady et al. (2009) determined that porewater methane concentrations were much less than DIC concentrations and there was a lack of significantly enriched porewater $\delta^{13}C_{DIC}$ values. However, methanogenesis may still play a role in maintaining enriched $\delta^{13}C_{DIC}$ and corresponding $\delta^{13}C_{carb}$ values at depth within the Cariboo lakes.

Methanogenesis was unlikely to be a major process in Goodenough Lake due to high concentrations of sulfate detected within the lake. Sulfate reduction is a more thermodynamically favourable redox reaction than methanogenesis allowing sulfate reducers to outcompete methanogens in environments where sulfur concentrations are high (Oremland, 1982; Botz et al., 1996; Summons et al., 1998; Thaur et al., 2008). Goodenough Lake was characterized by high concentrations of

sulfate in surface waters (3850 mg/L) compared to Probe Lake (63 mg/L) and Deer Lake (50 mg/L). Furthermore, sulfate reducers were present within the lake, which was indicated by the presence of the biomarker 10me16:0 (3.4-5.5 mol % of the total PLFA). Therefore, sulfate reduction was likely a more dominant process than methanogenesis in Goodenough Lake, which was consistent with the high sulfate concentrations in surface waters compared to Probe Lake and Deer Lake.

Overall, despite potential impacts of variations in photosynthetic activity, heterotrophic activity, and methanogenesis the downcore record in all three lakes of the Cariboo Plateau demonstrated enriched $\delta^{13}C_{carb}$ values and $\Delta^{13}C_{carb\text{-}TOC}$ values consistent with non-CO_2 limited photosynthesis over time. This demonstrated that the isotopic composition of precipitated carbonates and their offsets from produced organic matter are biosignatures of photosynthetic activity that are preserved in the downcore record. Therefore, such biosignatures can help us interpret the geologic record on Earth as well as identify biosignatures of life that may be preserved on Mars.

3.4.3 Implications for the Identification of Biosignatures on Early Earth and Mars

The Cariboo Plateau lakes provide a unique opportunity for investigating environmental conditions that may have been present during the formation of microbial mat communities on early Earth. The isotopic composition of carbonates and organic carbon preserved within the Cariboo Plateau lakes suggested that concentrations of atmospheric CO_2 on early Earth did not necessarily exceed current levels. High rates of photosynthetic activity within many saline environments, such as the salt ponds of Guerrero Negro and Solar Lake, has led to CO_2 limitation and the subsequent production of ^{13}C-enriched organic matter. Enriched $\delta^{13}C_{org}$ values in these saline environments were inconsistent with $\delta^{13}C$ values in the early Earth geologic record, which led to the suggestion that atmospheric levels of CO_2 must

have exceeded current levels in order to maintain non-CO_2 limited photosynthesis (Schidlowski et al., 1984; Des Marais et al., 1989; Schouten et al., 2001). However, the isotopic values of organic and inorganic carbon detected within the Cariboo Plateau lakes were comparable to $\delta^{13}C$ values in the early Earth rock record (Schidlowski, 2001). The $\delta^{13}C_{carb}$ values and $\Delta^{13}C_{carb\text{-}TOC(avg)}$ of ~ 23 ‰ downcore demonstrated that the Cariboo lakes were able to maintain non-CO_2 limited photosynthesis under modern atmospheric CO_2 levels. Periods of CO_2 undersaturation had been identified in previous sampling periods within the Cariboo lakes, however, evidence of non-CO_2 limited photosynthesis indicated that cyanobacteria were utilizing an alternative carbon source during periods of CO_2 undersaturation (Brady et al., 2009). At low concentrations of CO_2, cyanobacteria can still photosynthesize rapidly by increasing intracellular concentrations of DIC and by utilizing bicarbonate as an inorganic carbon source (Merz, 1992). Based on the high pH of the Cariboo Plateau lakes (~10.1-10.3) bicarbonate would have been the dominant inorganic carbon species and concentrations of lake DIC (4080-9530 mg/L) indicated that bicarbonate would have been present in high concentrations. Furthermore, PLFA profiles confirm the presence of heterotrophs in all three lakes, which could have supplied inorganic carbon for cyanobacterial photosynthesis. Therefore, the high concentrations of DIC (predominantly as bicarbonate) and heterotrophic activity could have maintained non-CO_2 limited photosynthesis during periods of CO_2 undersaturation. The preservation of enriched $\delta^{13}C_{carb}$ values and $\Delta^{13}C_{carb\text{-}TOC(avg)}$ of ~ 23 ‰ downcore demonstrated the stability of the Cariboo Plateau lakes and their ability to maintain non-CO_2 limited photosynthesis over time. The production of such isotopic values under modern atmospheric conditions suggested that concentrations of CO_2 did not need to exceed modern levels in order to produce the isotopic signatures detected within the geologic record.

The preservation of microbial isotopic signatures within the sediments of the Cariboo Plateau lakes also made them an analogue environment for studying the preservation of biosignatures that may exist within salt and carbonate deposits on

Mars. Measurements of the isotopic composition of carbonates, organic matter, and their offsets on Mars could indicate the presence of past microbial activity on the planet. The influence of the microbial communities on the organic and inorganic carbon isotopes was preserved in buried organic matter and precipitated carbonates within the sediments of the Cariboo lakes. Photosynthetic enrichments of the $\delta^{13}C_{DIC}$ resulted in the precipitation of carbonates that reflected the photosynthetic influences on the DIC pool. Furthermore, the $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ and $\Delta^{13}C_{carb\text{-}TOC}$ values reflected the C3 photosynthetic pathway used by cyanobacteria. Despite heterotrophic activity within all three lakes, the $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ values were preserved at depth within the sediments of the Cariboo Plateau lakes and preserved the photosynthetic influences on the local carbon pools and were biosignatures of photosynthetic activity. Therefore, salt and carbonate deposits are favourable environments for the search for biosignatures on Mars and the isotopic compositions of organic and inorganic carbon pools could preserve biosignatures of past microbial activity.

Based on current knowledge of the isotopic composition of atmospheric CO_2 on Mars and using the data from the Cariboo Plateau lakes, it is possible to estimate the isotopic value of biosignatures that may potentially be preserved on Mars. The current estimate of atmospheric $\delta^{13}C_{CO2}$ on Mars is enriched, relative to the $\delta^{13}C_{CO2}$ of atmospheric CO_2 on Earth ($\delta^{13}C_{CO2} \sim -7$ ‰), and is approximately +40 ‰ (Carr et al., 1985; Grady and Wright, 2006). Assuming the aqueous environment on early Mars is comparable to conditions within the saline Cariboo Plateau lakes of this study, the expected $\delta^{13}C_{DIC}$ in solution would have been ~+47.9 ‰. However, as demonstrated in the Cariboo Plateau lakes, photosynthetic influences on surface water DIC could enrich the $\delta^{13}C_{DIC}$ by 1.3 to 5.3 ‰, which could result in a $\delta^{13}C_{DIC}$ of +49.1 to +53.1 ‰. As seen in the Cariboo lakes, rapid precipitation of carbonates could result in little fractionation relative to surface water DIC values resulting in preserved $\delta^{13}C_{carb}$ values ranging from +47.9 to +53.1 ‰. The degree of photosynthetic, heterotrophic and methanogenic activity would cause some

variations in the $\delta^{13}C$ values; however, as was demonstrated in the Cariboo Plateau lakes, despite these potential variations, the photosynthetic biosignature was still preserved within sediments. Thus, despite variations in microbial activity and metabolisms that may have developed on Mars, a photosynthetic biosignature of enriched carbonates may still be preserved on the planet. Photosynthetic production of organic matter in the Cariboo lakes resulted in a $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ of ~23 ‰. Similar photosynthetic processes operating on Mars could therefore lead to organic matter with $\delta^{13}C_{TOC}$ values of ~ 24.7 to 30.0 ‰ depending on the degree of photosynthetic enrichment that occurred to the residual inorganic carbon pool. Current measurements of the $\delta^{13}C$ of organic matter in Martian meteorites predicts a $\delta^{13}C_{TOC}$ value of ~ -15 ‰ on Mars (Jull et al., 2000; Sephton et al., 2002). The similarity of organic matter $\delta^{13}C$ to lithospheric carbon on Mars ($\delta^{13}C$ ~ -21.2) is thought to indicate an abiotic source for the organic matter (Grady and Wright, 2006). Thus, the identification of significantly ^{13}C-enriched organic matter on Mars could point to a biotic origin. Such measurements of enriched organic matter, in conjunction with measurements of ^{13}C-enriched preserved carbonates and a $\Delta^{13}C_{carb\text{-}TOC(avg)}$ of ~23 ‰, within salt and carbonate deposits on Mars could therefore preserve a biosignature of photosynthetic activity.

Isotopic characterization of carbon pools on Mars will nevertheless require careful interpretation in order to identify biosignatures of life. Solar wind stripping has changed the isotopic composition of atmospheric CO_2 over time (Krasnopolsky et al. 1996; van Zuilen, 2008), which could cause variability in the $\delta^{13}C$ values of the organic and inorganic carbon pools. Volcanic and geologic activity is believed to have ended on Mars approximately 3.5 billion years ago, leaving any preserved organic matter isotopically unaltered. However, the strong oxidizing conditions on Mars today would have stripped away any organic matter present on the surface (Klein et al., 1976; van Zuilen, 2008). Organic matter may still be present in the subsurface; however, such sample retrieval poses challenges for the unmanned Mars rovers. The acidic conditions that were believed to have been widespread on

Mars may have destroyed much of the carbonate biosignatures that may have been preserved on the planet (Squyres et al., 2004a; Squyres et al., 2004b; Bibring et al., 2006). However, the identification of carbonates in the Nili Fossae region of Mars could indicate that such acidic conditions did not impact the entire planet (Fairen et al., 2004; Ehlmann et al., 2008), and biosignatures of life may still be preserved on the planet today. Many more salt and carbonate deposits may also be preserved on Mars as more than 50 % of the surface dates back to the end of the heavy bombardment ~3.5-4.0 Ga (McKay, 1997). Characterization of the isotopic composition and relative offsets of the organic and inorganic carbon pools in non-impacted deposits could lead to the identification of the presence of past life on the planet.

3.5 CONCLUSIONS

The Cariboo Plateau lakes of this study were sodium carbonate lakes that contained benthic microbial mat communities that influenced local water chemistry and isotopic signatures. Despite geochemical variability in surface waters, the microbial mat communities of each lake were dominated by cyanobacteria. This was evident by the PLFA composition in lake sediments and the $\Delta^{13}C_{TOC\text{-}16:1\Delta9 \text{ AND } 11}$ of ~7 ‰ which was indicative of PLFA synthesis by cyanobacteria. Photosynthesis by cyanobacteria was the dominant process controlling the isotopic composition of the organic and inorganic carbon pools within each lake. The $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ of ~23 ‰ for each lake reflected organic matter production by cyanobacteria through the C3 photosynthetic pathway. Furthermore, photosynthetic activity was leading to an enrichment of $\delta^{13}C_{DIC}$ due to preferential utilization of ^{12}C over ^{13}C during uptake of inorganic carbon. The enriched $\delta^{13}C_{DIC}$ values were being preserved through rapid precipitation of isotopically enriched carbonates ($\delta^{13}C_{carb}$). Both enriched carbonates and $\Delta^{13}C_{carb\text{-}TOC}$ were biosignatures of photosynthetic activity within the lakes and were not overprinted by other physical and biological factors. The preservation of these biosignatures suggested that concentrations of CO_2 on early

Earth did not necessarily exceed modern levels and that measuring the carbon isotopic compositions of carbon pools within saline, carbonate-rich deposits on Mars could lead to the identification of past life on the planet.

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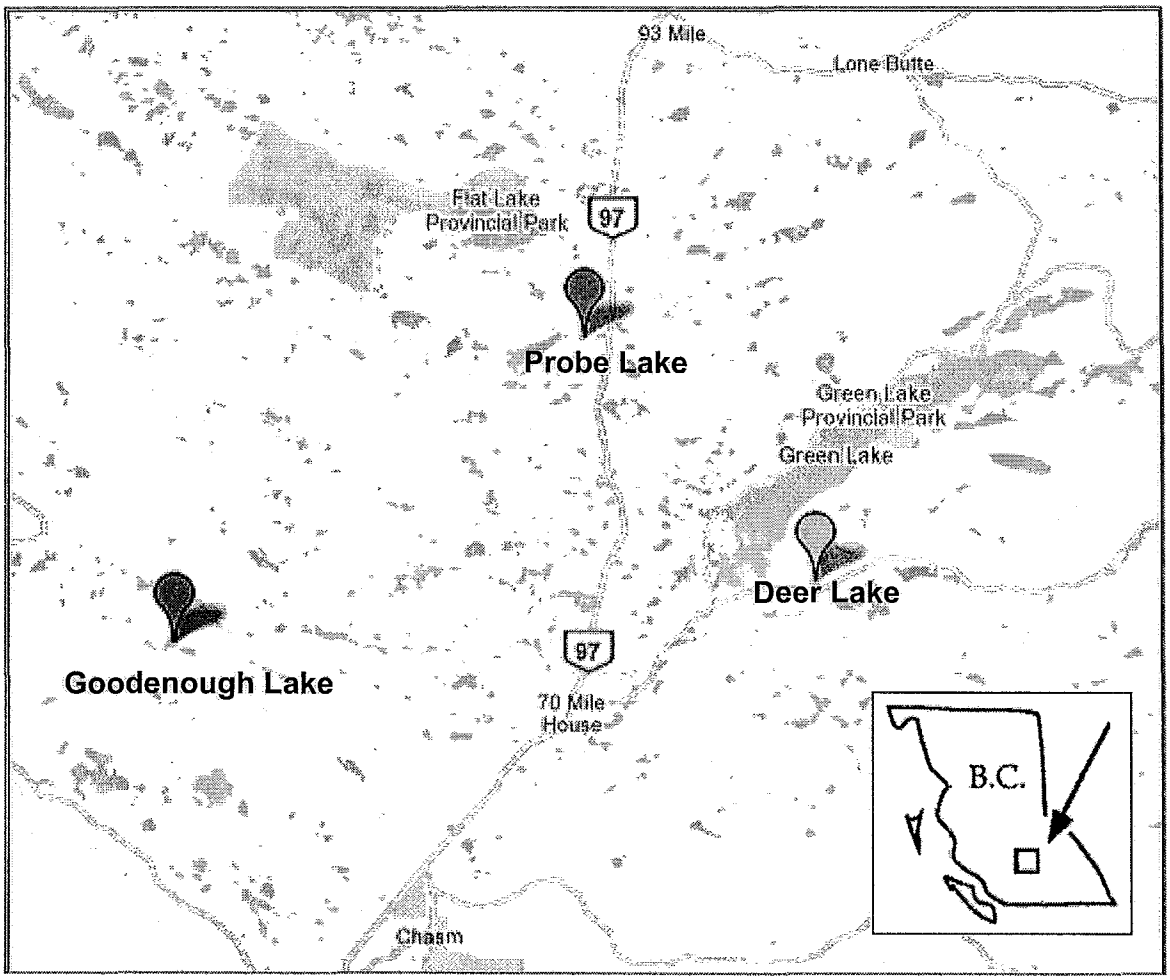


Figure 3.1: Location of the three lakes of this study located within the Cariboo Plateau in the southern interior of British Columbia.

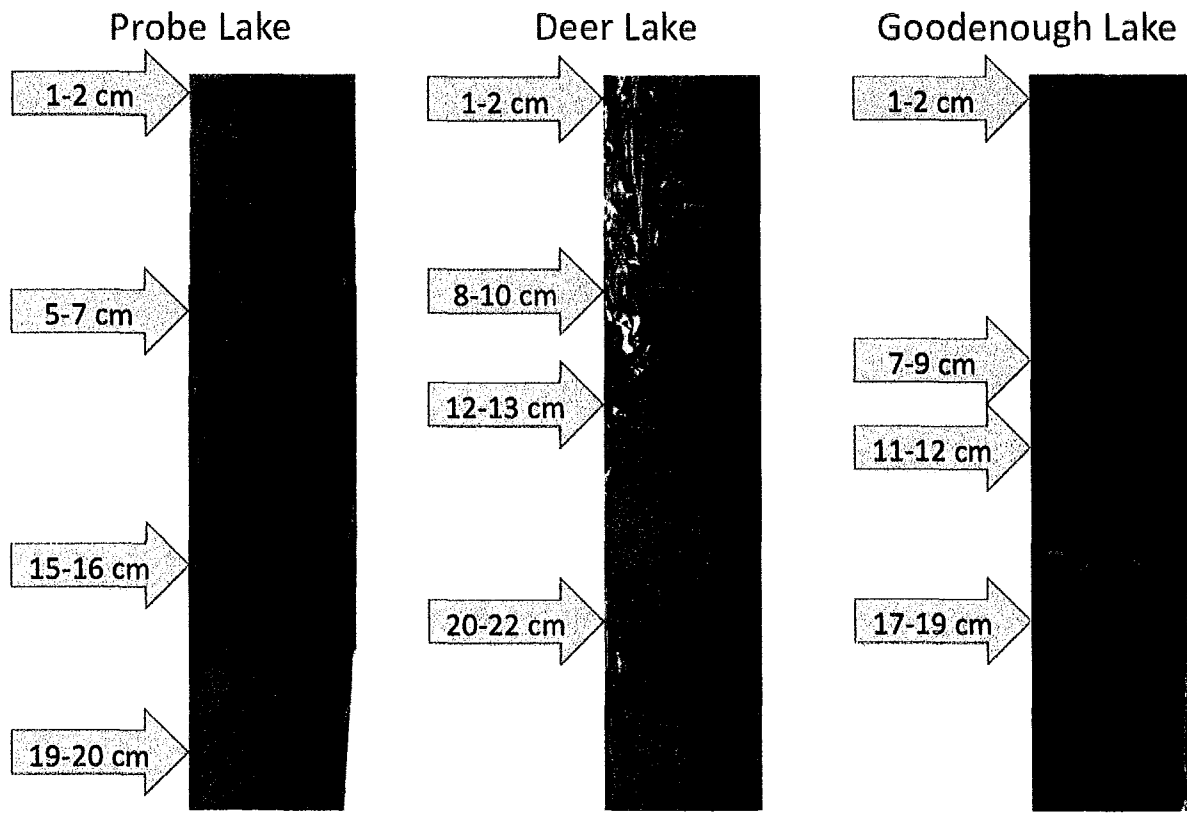


Figure 3.2: Cores of each lake showing sampling depths used for laboratory analysis

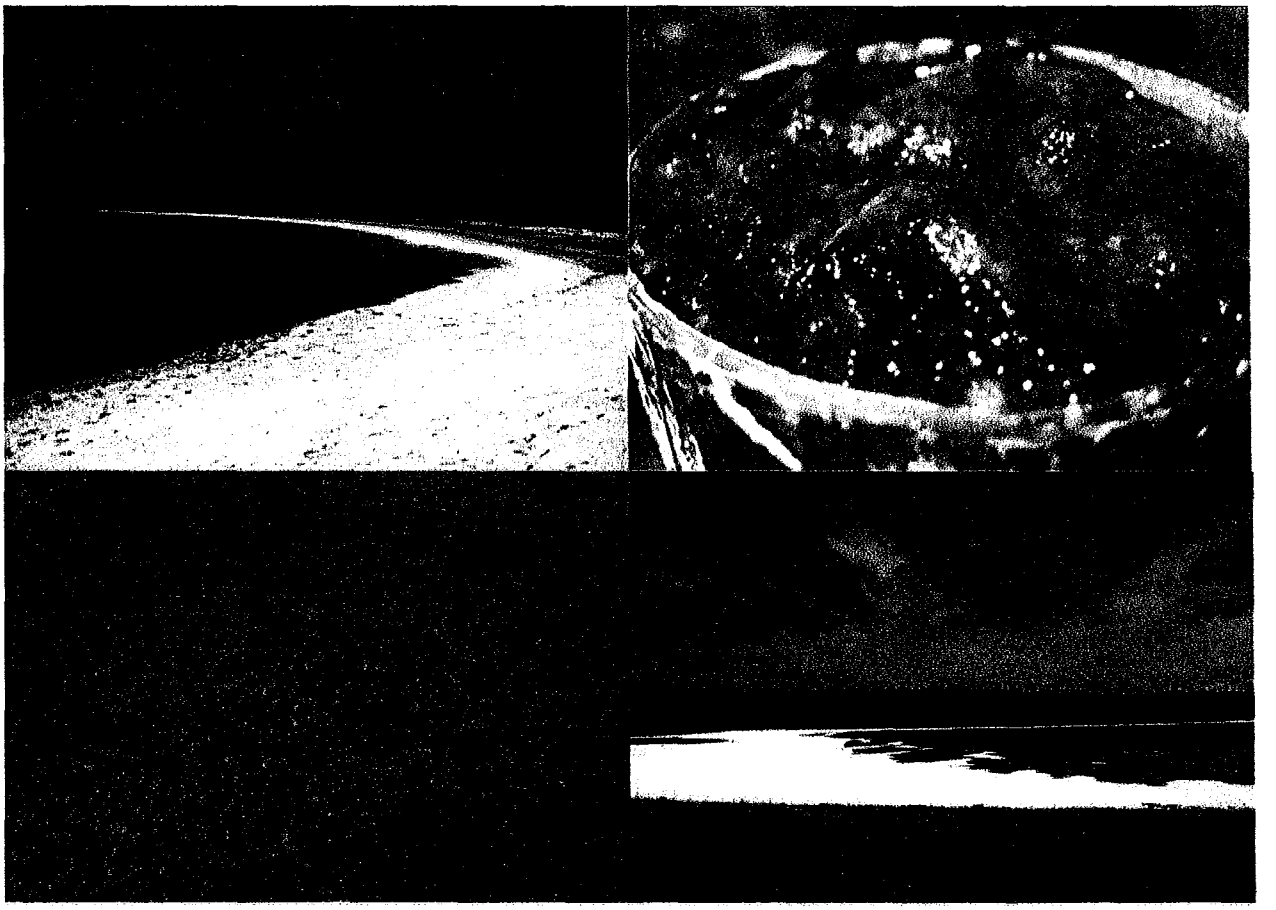


Figure 3.3: The saline lakes of the Cariboo Plateau. a. Probe Lake b. Disturbed microbial mat of Deer Lake showing some laminations c. Benthic microbial mats in Probe Lake d. Goodenough Lake

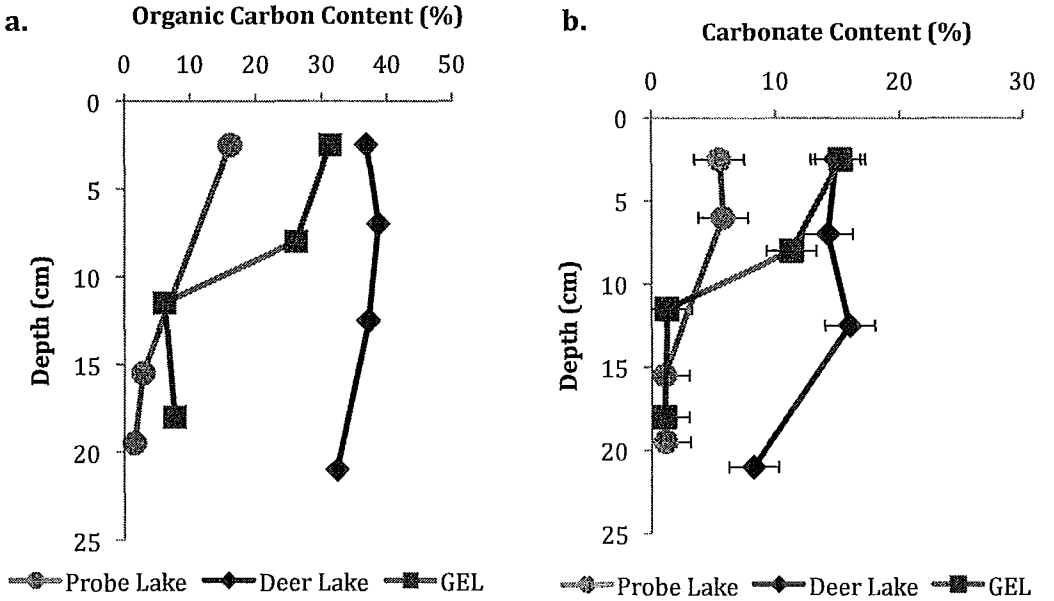


Figure 3.4: a. Organic carbon content of the three Cariboo Lakes. Deer Lake had the highest organic carbon content with all three lakes demonstrating decreases in organic carbon content downcore b. Carbonate content of the three Cariboo Lakes. Deer Lake had the highest carbonate content throughout the entire core. All three lakes showed decreases in carbonate content downcore.

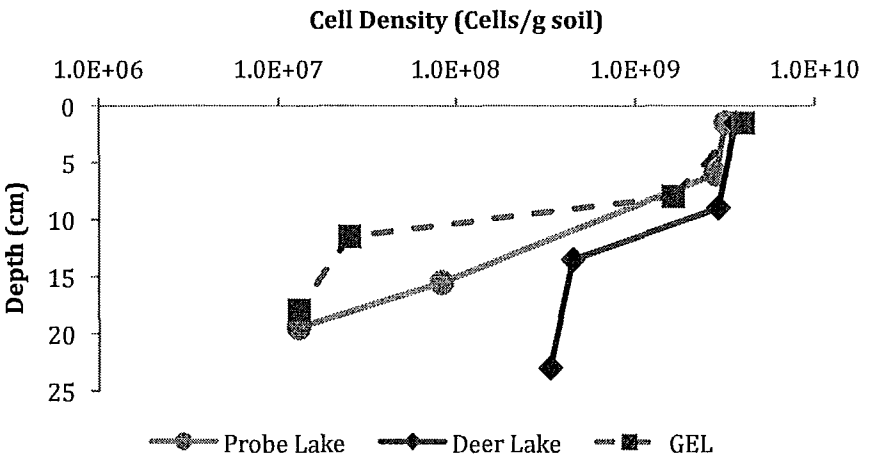


Figure 3.5: Cell density, as determined by PLFA, of the three Cariboo lakes. All three lakes showed decreasing cell densities downcore with Deer Lake maintaining the highest cell density downcore.

Table 3.1: Water chemistry of the three Cariboo Lakes

Sample Site	Depth (m)	Temp (°C)	pH	sp. Conductivity (µS/cm)	Salinity (ppt)	% DO	DIC (mg/L)	DOC (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	SO4 (mg/L)
Probe Lake	surface	19.2	10.1	83152	67.1	127.6						
	0.5	19.2	10.1	83108	67.1	129.3						
	1	23.6	10.1	107843	82.1	235.5						
	1.5	23.6	10.1	108023	82.2	238.8						
	1.75	23.7	10.1	108029	82.1	254.9						
	Average	**21.9**	**10.1**	**98031**	**76.1**	**197.2**	**4430**	**158**	**5**	**10**	**15900**	**63**
Deer Lake	surface	24.2	10.0	87443	63.2	160.3						
	25cm	24.2	10.1	87469	63.2	165.5						
	Average	**24.2**	**10.1**	**87456**	**63.2**	**162.9**	**4080**	**510**	**3**	**72**	**13600**	**50**
GEL	surface	22.7	10.3	181580	163.2	127.2						
	~20cm	22.6	10.3	181044	162.9	128.3						
	Average	**22.6**	**10.3**	**181312**	**163.1**	**127.8**	**9530**	**510**	**4**	**93**	**39200**	**3850**

Table 3.2: PLFA profiles of Probe Lake samples expressed in ug/g and mol % for each depth

PLFA I.D.	Probe 1-2 cm		Probe 5-7cm		Probe 15-16 cm		Probe 19-20 cm	
	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)
12:0	0.00	0.00	0.59	1.99	0.00	0.00	0.01	7.14
i-13:0	0.00	0.00	0.56	1.76	0.00	0.00	0.00	0.00
a-13:0	0.00	0.00	0.57	1.80	0.00	0.00	0.00	0.00
I or a 14:0	1.34	3.47	0.90	2.68	0.07	6.81	0.01	6.16
14:0	1.38	3.59	0.90	2.68	0.06	6.46	0.01	6.13
i-15:0	4.10	10.06	4.83	13.55	0.09	8.70	0.02	9.74
a-15:0	4.69	11.49	5.35	15.00	0.14	13.23	0.02	12.05
15:0	1.18	2.90	0.63	1.77	0.00	0.00	0.00	0.00
br15:0	1.30	3.02	0.69	1.83	0.00	0.00	0.00	0.00
i-16:0	1.89	4.39	1.58	4.19	0.08	7.32	0.01	5.89
16:1Δ9 cis	3.63	8.50	2.68	7.17	0.07	6.56	0.01	6.04
16:1	1.36	3.19	0.76	2.05	0.09	8.33	0.02	9.02
16:0	3.38	7.85	2.56	6.81	0.00	0.00	0.00	0.00
17:1	0.00	0.00	0.76	1.92	0.00	0.00	0.00	0.00
10me16:0	1.38	3.04	0.72	1.81	0.07	5.92	0.01	5.49
17:1	0.00	0.00	0.66	1.68	0.00	0.00	0.00	0.00
i-17:0	1.36	3.00	0.77	1.94	0.06	5.49	0.01	5.09
a-17:0	1.55	3.42	1.02	2.57	0.07	6.09	0.01	5.33
17:0	1.31	2.89	0.67	1.70	0.00	0.00	0.00	0.00
18:2Δ9,12	1.47	3.14	0.76	1.84	0.00	0.00	0.00	0.00
18:1Δ9	3.19	6.76	2.09	5.07	0.09	7.48	0.01	6.28
18:1Δ11	7.46	15.81	5.79	14.04	0.12	10.13	0.02	8.37
18:0	1.66	3.49	1.02	2.46	0.09	7.48	0.01	7.25
20:0	0.00	0.00	0.76	1.66	0.00	0.00	0.00	0.00

Table 3.3: PLFA profiles of Deer Lake samples expressed in ug/g and mol % for each depth

PLFA I.D.	Deer 1-2 cm		Deer 8-10 cm		Deer 13-14 cm		Deer 22-24 cm	
	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)
12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.09	2.40
i13:0	0.00	0.00	0.00	0.00	0.16	3.10	0.07	1.74
a13:0	1.08	2.60	0.62	1.90	0.17	3.28	0.08	2.11
I or a 14:0	1.19	2.71	0.86	2.46	0.27	5.03	0.15	3.71
14:0	1.22	2.79	0.72	2.06	0.21	3.92	0.15	3.64
br14:0	0.00	0.00	0.00	0.00	0.00	0.00	0.07	1.59
i15:0	4.46	9.61	3.03	8.24	0.52	9.09	0.37	8.72
a15:0	4.30	9.27	4.85	13.19	1.22	21.21	0.88	20.66
15:0	1.07	2.31	0.59	1.59	0.16	2.82	0.07	1.64
br15:0	0.00	0.00	0.00	0.00	0.23	3.74	0.09	2.05
i16:0	1.54	3.15	1.95	5.03	0.45	7.37	0.32	7.07
br15:0	0.00	0.00	0.00	0.00	0.00	0.00	0.07	1.48
16:1Δ9cis	3.19	6.57	2.81	7.25	0.26	4.41	0.11	2.39
16:1Δ9trans	0.00	0.00	0.47	1.22	0.00	0.00	0.06	1.39
16:1Δ11	1.02	2.11	0.57	1.48	0.00	0.00	0.00	0.00
16:0	3.73	7.63	3.09	7.95	0.44	7.23	0.41	9.14
17:1Δ	0.00	0.00	0.00	0.00	0.00	0.00	0.08	1.78
10me16:0	0.00	0.00	0.50	1.23	0.21	3.34	0.11	2.31
br16:0	0.00	0.00	0.00	0.00	0.00	0.00	0.07	1.46
i17:0	0.93	1.81	0.53	1.30	0.17	2.71	0.08	1.79
a17:0	1.26	2.45	1.30	3.19	0.30	4.68	0.24	5.02
17:1Δ11	0.96	1.88	0.59	1.46	0.00	0.00	0.00	0.00
17:1Δ	0.00	0.00	0.53	1.30	0.00	0.00	0.00	0.00
17:0	0.90	1.76	0.50	1.24	0.00	0.00	0.00	0.00
18:2Δ9,12	1.19	2.24	0.68	1.61	0.00	0.00	0.00	0.00
18:1Δ9	2.85	5.31	3.06	7.19	0.27	4.04	0.14	2.77
18:1Δ11	16.77	31.28	10.79	25.35	0.69	10.38	0.21	4.25
18:0	1.33	2.47	0.85	1.99	0.24	3.63	0.23	4.57
br18:0	0.00	0.00	0.00	0.00	0.00	0.00	0.09	1.67
cy19:0	1.17	2.08	0.79	1.77	0.00	0.00	0.00	0.00
20:0	0.00	0.00	0.00	0.00	0.00	0.00	0.15	2.77
22:0	0.00	0.00	0.00	0.00	0.00	0.00	0.11	1.87

Table 3.4: PLFA profile of Goodenough Lake samples expressed in ug/g and mol % for each depth

PLFA I.D.	GEL 1-2 cm		GEL 7-9 cm		GEL 11-12 cm		GEL 17-19 cm	
	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)	(ug/g)	(mol %)
12:0	0.00	0.00	0.73	4.26	0.02	6.89	0.01	7.14
i13:0	1.02	2.25	0.74	4.02	0.00	0.00	0.00	0.00
a13:0	1.04	2.29	0.74	4.03	0.00	0.00	0.00	0.00
I or a 14:0	1.32	2.74	0.83	4.27	0.02	6.13	0.01	6.16
14:0	1.26	2.60	0.75	3.87	0.02	6.19	0.01	6.13
i15:0	4.97	9.71	1.86	9.05	0.03	9.76	0.02	9.74
a15:0	3.89	7.61	2.12	10.29	0.04	12.08	0.02	12.05
15:0	1.16	2.26	0.73	3.53	0.00	0.00	0.00	0.00
br15:0	0.00	0.00	0.81	3.73	0.00	0.00	0.00	0.00
i16:0	1.27	2.35	0.97	4.46	0.02	5.91	0.01	5.89
16:1Δ9cis	3.57	6.67	0.91	4.23	0.02	6.05	0.01	6.04
16:1Δ11	1.10	2.06	0.00	0.00	0.00	0.00	0.00	0.00
16:0	4.46	8.28	1.19	5.48	0.03	9.04	0.02	9.02
17:1Δ9cis	1.01	1.79	0.83	3.64	0.00	0.00	0.00	0.00
10me16:0	1.95	3.44	0.86	3.78	0.02	5.50	0.01	5.49
17:1Δ9trans	0.96	1.71	0.00	0.00	0.00	0.00	0.00	0.00
i17:0	1.05	1.85	0.83	3.63	0.02	5.15	0.01	5.09
a17:0	1.16	2.05	0.91	3.99	0.02	5.35	0.01	5.33
17:1Δ11	1.06	1.89	0.00	0.00	0.00	0.00	0.00	0.00
17:0	0.90	1.59	0.80	3.51	0.00	0.00	0.00	0.00
18:2Δ9,12	1.29	2.20	0.00	0.00	0.00	0.00	0.00	0.00
18:1Δ9	3.28	5.55	1.16	4.89	0.02	6.30	0.01	6.28
18:1Δ11	12.86	21.76	1.69	7.08	0.03	8.39	0.02	8.37
18:0	1.41	2.37	0.98	4.09	0.03	7.26	0.01	7.25
cy19:0	3.08	4.97	1.04	4.16	0.00	0.00	0.00	0.00

Table 3.5: $\delta^{13}C_{PLFA}$ for Cariboo Lake samples. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB. Highlighted sections indicate integrated $\delta^{13}C$ of individual PLFA as peaks were not completely resolved.

PLFA I.D.	$\delta^{13}C$ Probe 1-2 cm	$\delta^{13}C$ Probe 5-7 cm	$\delta^{13}C$ Deer 1-2 cm	$\delta^{13}C$ Deer 8-10 cm	$\delta^{13}C$ GEL 1-2 cm	$\delta^{13}C$ GEL 7-9 cm
I or a 14:0		-29.4 ± 0.2	-25.6 ± 0.8	-25.4 ± 0.2	-30.5 ±	-28.0 ± 0.2
14:0	-27.4 ± 0.2	-27.8 ± 0.3	-24.0 ±0.2	-23.4 ± 0.1	-28.9 ±	-27.3 ± 0.2
i15:0	-30.6 ± 0.1	-30.8 ± 0.1	-26.5 ± 0.1	-25.2 ± 0.1	-31.4 ± 0.2	-30.5 ± 0.2
a15:0						
15:0		-28.6 ± 0.4				
br15:0						
i16:0		-32.4 ± 0.7			-32.8 ±	-31.3 ± 0.4
16:1Δ9cis	-30.3 ± 0.3	-29.7 ± 0.3	-26.6 ± 0.2	-26.2 ± 0.2	-29.4 ± 0.3	-26.7 ± 0.6
16:1Δ11						
16:0	-27.2 ± 0.1	-29.6 ± 0.2	-24.9 ± 0.4	-28.0 ± 0.9	-31.5 ±	-31.2 ± 0.4
17:1Δ9cis				-24.7 ± 0.7		
10me16:0					-28.7 ±	-28.7 ± 0.1
17:1Δ9trans						
i17:0		-29.7 ± 0.2			-30.1 ±	
a17:0	-28.5 ± 0.2			-25.3 ± 0.2	-29.6 ± 0.7	-30.5 ± 0.2
17:1Δ11						
17:0						
18:2Δ9,12						
18:1Δ9	-27.2 ± 0.3	-27.8 ± 0.1	-22.2 ± 0.1	-22.9 ± 0.2	-28.6 ± 0.4	-28.6 ± 0.1
18:1Δ11						
18:0						
cy19:0				-24.0 ± 0.2	-29.3 ±	-27.4 ± 0.6
AVG $\delta^{13}C_{PLFA}$	**-28.5**	**-29.5**	**-25.0**	**-25.0**	**-30.1**	**-29.0**

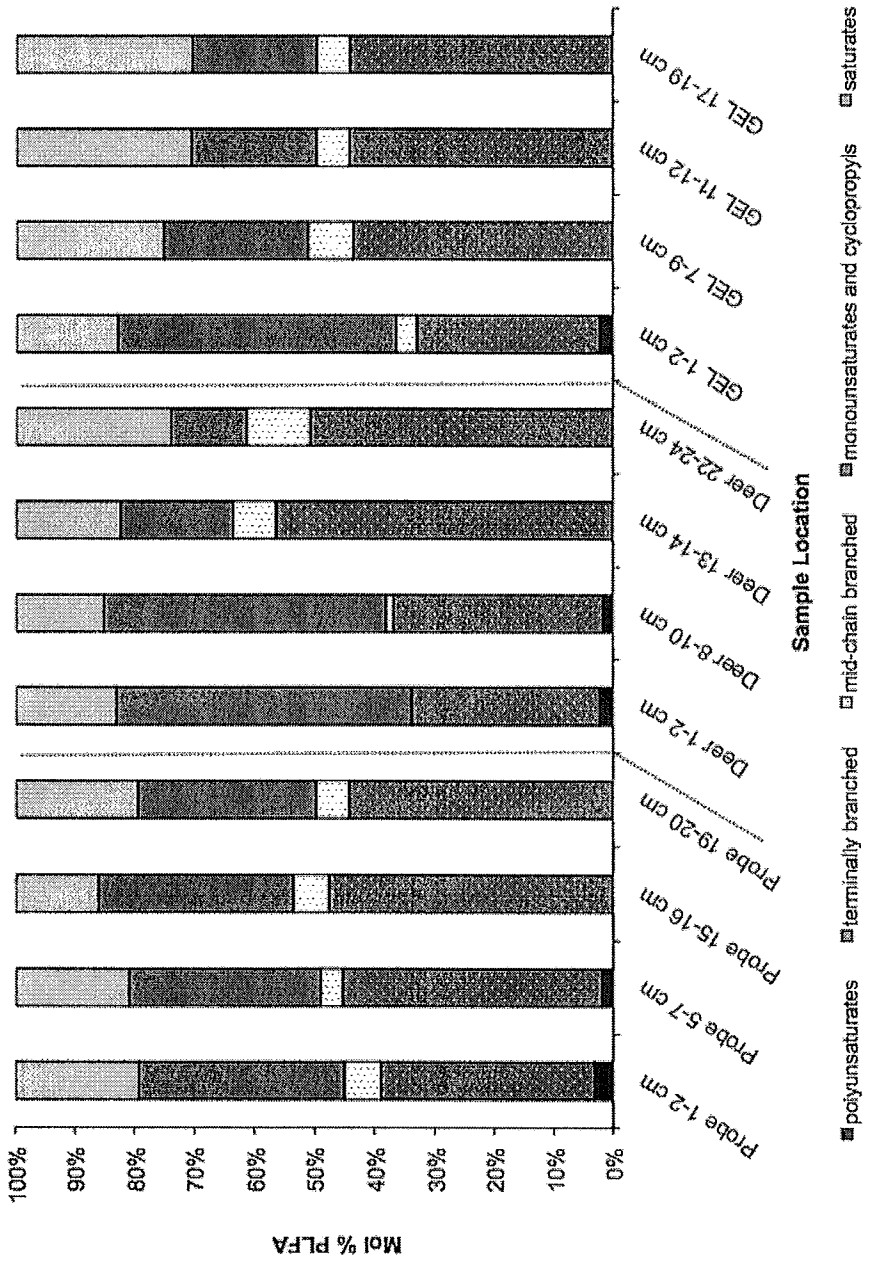


Figure 3.6: PLFA distributions of the Cariboo Lakes in mol %. Each lake had high concentrations of saturated, monounsaturated and terminally branched PLFA. PLFA distributions are indicative of cyanobacteria-dominated microbial mat communities.

Table 3.6: $\delta^{13}C$ values of DIC, TOC, carbonates and bulk avg PLFA and $\delta^{18}O$ values of carbonates. Standard deviations are reported for triplicate analyses and all values are reported relative to VPDB.

Location/ Depth (cm)	$\delta^{13}C_{DIC}$ (‰)	Exp. $\delta^{13}C_{DIC}$ (‰)	$\delta^{13}C_{TOC}$ (‰)	$\delta^{13}C_{carb}$ (‰)	$\delta^{18}O_{carb}$ (‰)	$\delta^{13}C$ BULK AVG PLFA (‰)	$\Delta^{13}C_{DIC-TOC(avg)}$ (‰)	$\Delta^{13}C_{carb-TOC}$ (‰)
Probe 1-2	0.0 ± 0.2	-2.1	-23.7 ± 0.2	-0.5 ± 0.2	-5.9 ±0.1	-28.5	23.5	23.2
Probe 5-7			-23.8	0.2 ± 0.2	-2.4 ±0.2	-29.5		24.0
Probe 15-16			-23.4	0.7 ± 1.2	-8.1 ±2.3			24.1
Probe 19-20			-22.9 ± 0.1	0.7 ± 0.1	-8.4 ±0.0			23.6
Deer 1-2	3.1 ± 0.2	-2.2	-19.1 ± 0.1	3.1 ± 0.2	-2.6 ±0.2	-25.0	22.6	22.2
Deer 8-10			-18.5 ±0.2	3.0 ± 0.2	-4.3 ±0.2	-25.0		21.5
Deer 13-14			-18.5	1.9 ± 0.3	-8.3 ±0.3			20.4
Deer 22-24			-21.8	5.3 ± 0.2	-6.7 ±0.4			27.1
GEL 1-2	-1.1 ± 0.2	-2.3	-24.7	-1.1 ± 0.1	-4.6 ±0.1	-30.1	23.1	23.6
GEL 7-9			-24.7	0.4 ± 0.4	-5.3 ±0.8	-29.0		25.1
GEL 11-12			-23.6 ± 0.1	1.5 ± 0.6	-4.2 ±0.5			25.1
GEL 17-19			-23.8	2.4 ± 0.4	-4.2 ±0.5			26.2

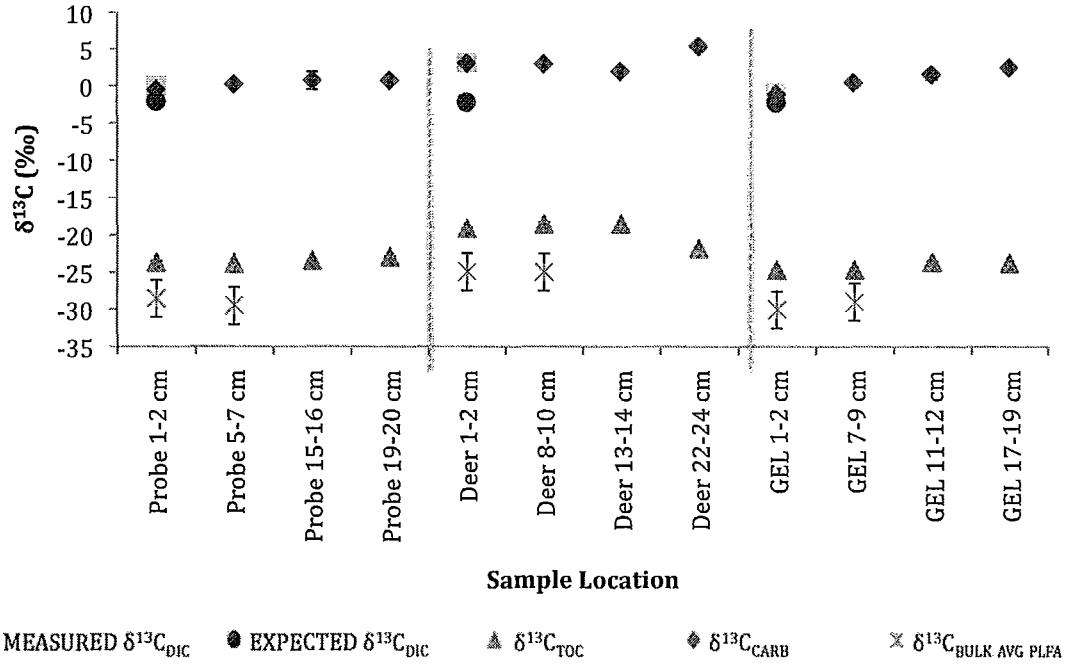


Figure 3.7: Carbon isotopic values measured within the three Cariboo Lakes. $\delta^{13}C_{DIC}$ and $\delta^{13}C_{carb}$ were enriched relative to expected $\delta^{13}C_{DIC}$ values. $\Delta^{13}C_{DIC\text{-}TOC}$ and $\delta^{13}C_{PLFA}$ was indicative of photosynthetic activity by cyanobacteria. Error for $\delta^{13}C_{DIC}$, $\delta^{13}C_{TOC}$, and $\delta^{13}C_{carb}$ was on average 0.2 ‰.

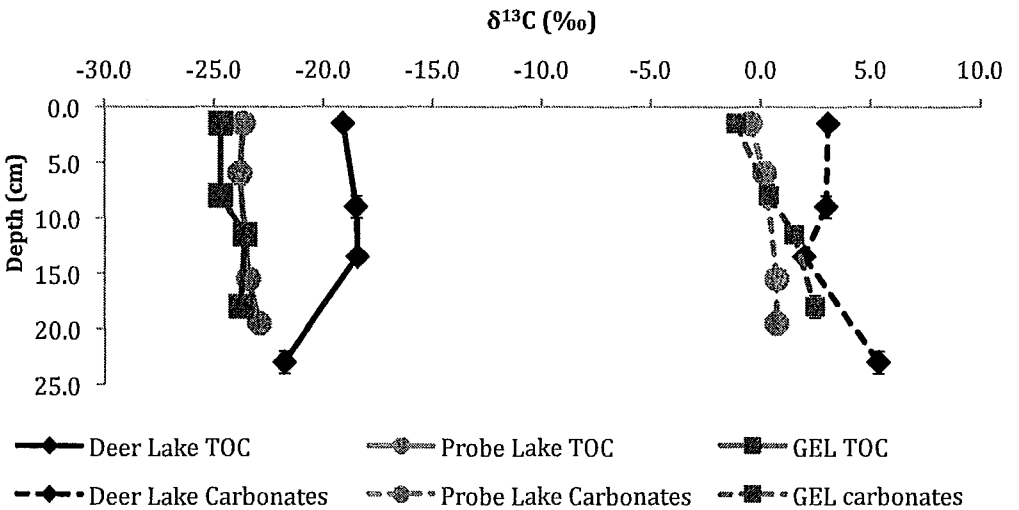
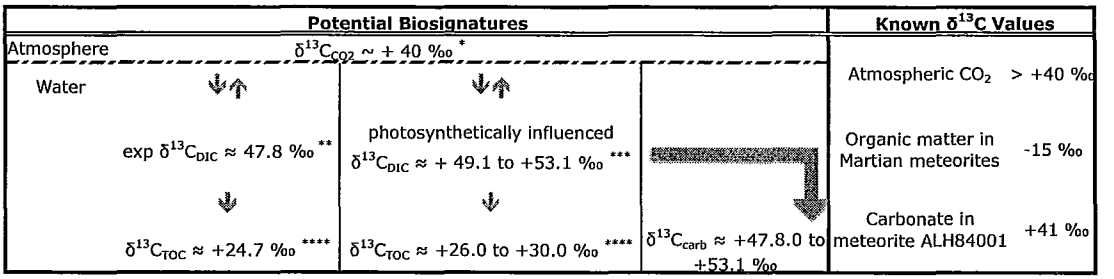


Figure 3.8: $\delta^{13}C_{TOC}$ and $\delta^{13}C_{carb}$ downcore for each lake. Each lake showed enrichments in $\delta^{13}C_{carb}$ and depletions in $\delta^{13}C_{TOC}$ downcore. Error for $\delta^{13}C_{DIC}$ and $\delta^{13}C_{TOC}$ was on average 0.2 ‰.

Figure 3.9: Potential isotopic biosignatures on Mars based on data from the Cariboo Plateau lakes as well as known $\delta^{13}C$ values of atmospheric CO_2, organic matter, and carbonates on Mars.



* based on measurements of trapped CO_2 in martian meteorites NOTE: atmospheric CO2 values have changed on Mars over time

** based on pH and temperature of Cariboo Plateau lakes

*** based on enrichments relative to expected values observed for the Cariboo Plateau lakes

**** based on a the average $\Delta^{13}C_{DIC\text{-}TOC}$ ~23.1‰ for the three lakes

CHAPTER 4
CONCLUSIONS: THESIS SUMMARY AND FUTURE AVENUES OF RESEARCH

By studying life within extreme environment on Earth we learn the physical and chemical limits of habitability allowing us to better understand the signatures of life that may be found within the geologic record on Earth as well as elsewhere in the universe. This thesis research focused on studying microbial carbon cycling and biosignatures within two Mars analogue environments that were representative of the dominant environmental conditions thought to have been present during the aqueous period on early Mars. The Golden Deposit was an acid-sulfate environment that was an analogue to the acid-sulfate conditions present on early Mars, evidence of which is preserved as iron and sulfur mineral deposits found on the surface today. The Cariboo Plateau lakes were saline, alkaline lakes dominated by benthic microbial mat communities. They were an analogue to the alkaline, evaporitic conditions present on the surface of Mars early in its aqueous history, evidence of which is preserved in the form of carbonate and chloride deposits detected on the surface today.

This thesis research utilized phospholipid fatty acid analysis (PLFA) in combination with carbon isotopic analysis ($\delta^{13}C$) of PLFA, organic and inorganic carbon to understand microbial carbon cycling and the preservation of biosignatures within these two Mars analogue environments. Chapter 1 provided an introduction to the analogue environments of interest and an overview of fundamental concepts and microbial processes that occur within acid-sulfate and saline lake environments. Chapter 2 focused on investigating the influence of geochemistry on microbial community composition and isotopic signatures within the Golden Deposit. Chapter 3 focused on investigating the influence cyanobacteria-dominated microbial mats on carbon isotopic signatures and the preservation of biosignatures within the sediments of three saline lakes of the Cariboo Plateau. The remainder of this chapter, Chapter 4, will provide an overview of the research

completed in Chapters 2 and 3, the application of this work to identifying biosignatures on Mars, and identify future avenues of research within these two analogue environments.

4.1 Microbial Carbon Cycling in the Golden Deposit, N.W.T., Canada

The Golden Deposit is located in the Northwest Territories, Canada and is a relatively unexplored naturally occurring acid-rock drainage environment. Acidic groundwater seeps (pH ~2.4) are precipitating iron and sulfur bearing minerals, such as jarosite and goethite, to produce the ~140 x 50 m deposit (Michel and van Everdingen, 1987; Battler et al., unpublished). The water chemistry at the surface of the Golden Deposit exhibited a large degree of geochemical variability, with variations in pH, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and metal and non-metal concentrations. The variation in water chemistry was caused by a mixing of inflowing surface water from the surrounding environment with the upwelling acidic groundwater. As a result, many locations exhibited variable water chemistries, which influenced the microbial community composition and isotopic signatures found on the deposit.

The organic rich peat surrounding the deposit supported a high number and diversity of organisms and appeared to influence the microbial community on the deposit at locations that had mixed water chemistries. This was demonstrated by the similarity in PLFA distributions, the $\delta^{13}C_{BULK\ AVG\ PLFA}$ (~ -27 ‰), and the largest $\Delta^{13}C$ between measured and expected $\delta^{13}C_{DIC}$ (~10-14 ‰) compared to other locations on the deposit. The absence of methane gas and lack of detection of methanogen biosignatures in surrounding peat was due to the high concentrations of S and SO_4^{2-} in the groundwater surrounding the Golden Deposit, which supported the more thermodynamically favourable redox reactions of sulfur reducers and thereby outcompeted methanogenic processes in this environment. The most acidic site on the deposit (SITE 2) was located directly beside a groundwater seep and was most representative of the acidic groundwater chemistry. The acidic pH

(~2.4) and high concentrations of iron and sulfur selected for a unique microbial community, likely dominated by organisms such as *At. ferrooxidans* and *Desulfobacter*, which was evident by the most depleted $\Delta^{13}C_{TOC\text{-}BULK\ AVG\ PLFA}$ of ~ 7.0 ‰ and a distinct PLFA distribution dominated by high proportions (51.1 mol %) of 16:1Δ9*cis*.

4.2 Microbial Carbon Cycling and Biosignatures within Three Saline Lakes of the Cariboo Plateau, British Columbia

Three lakes located in the Cariboo Plateau, British Columbia were used in this study. The lakes were saline, alkaline (pH ~10.1), and carbonate-rich lakes that contained benthic microbial mat communities. The microbial mats in all three lakes were dominated by autotrophic, photosynthetic cyanobacteria, evident by the high proportions of saturated (16-20 mol %), monounsaturated (35-50 mol %), and terminally branched (30-35 mol %) fatty acids and $\Delta^{13}C_{TOC\text{-}16:1\Delta9\ AND\ 11}$ of ~7 ‰ indicative of PLFA synthesis by cyanobacteria. Despite evaporative influences on lake $\delta^{13}C_{DIC}$, atmospheric influx of CO_2 and heterotrophic production of isotopically depleted inorganic carbon, photosynthetic activity by cyanobacteria was the dominant process controlling carbon isotopic distributions within the three lakes. Organic matter production by cyanobacteria was resulting in a $\Delta^{13}C_{DIC\text{-}TOC(avg)}$ of ~23 ‰ reflective of non-CO_2 limited photosynthesis. The isotopic composition of surface water DIC ($\delta^{13}C_{DIC}$) was enriched (+ 1.3- 5.3 ‰) relative to the expected value (-2.2 ‰) of lake DIC in equilibrium with atmospheric CO_2. Cyanobacterial photosynthesis was resulting in enriched surface water DIC values due to preferential utilization of ^{12}C over ^{13}C during uptake of inorganic carbon. Therefore, two photosynthetic biosignatures, enriched $\delta^{13}C_{DIC}$ and $\Delta^{13}C_{DIC\text{-}TOC(avg)}$, were present within all three Cariboo Plateau lakes despite influences of other physical and biological factors. The enriched $\delta^{13}C_{DIC}$ biosignature was preserved within the $\delta^{13}C_{carb}$ through rapid precipitation of carbonates from solution, evident by the $\Delta^{13}C_{DIC\text{-}carb}$ of 0.5, 0.02, and 0.07 ‰ for Probe Lake, Deer Lake and GEL, respectively. The $\Delta^{13}C_{DIC\text{-}TOC(avg)}$

biosignature was similarly preserved in $\Delta^{13}C_{carb\text{-}TOC}$ values. Both of the photosynthetic biosignatures were preserved downcore within the three lakes and were not overprinted by heterotrophic activity. Methanogenesis may also have played a role in enrichments of carbonates downcore, particularly in Deer Lake where significant isotope shifts were detected and the archaeal biomarker archaeol was identified.

4.3 Applications to the Identification of Biosignatures on Early Earth and Mars

The Golden Deposit and Cariboo Plateau lakes are terrestrial analogues to environmental conditions that are thought to have dominated the surface of Mars early in its aqueous history. The mineralogy of the Golden Deposit and its location in a cold, permafrost environment makes it an analogue to the conditions present during the formation of sulfate deposits on Mars. The preservation of photosynthetic biosignatures within the Cariboo Plateau lakes and their ability to maintain non-CO_2 limited photosynthesis makes them an analogue to environmental conditions present on early Earth as well as an analogue to the alkaline, evaporitic environment present on early Mars.

The variability in geochemistry within the Golden Deposit had a strong influence on the microbial community and carbon isotopic composition. The less acidic locations had a greater diversity of organisms and were influenced to a large degree by inflowing surface water from the surrounding environment. In contrast, the most acidic seep location displayed minimal influences from the surrounding environment and therefore served as a better analogue to environmental conditions on Mars. SITE 2 had a distinct geochemistry with an acidic pH and high concentrations of iron and sulfur that selected for a distinct microbial community. SITE 2 also had a distinct PLFA distribution likely indicative of the presence of organisms such as *At. ferrooxidans* and *Desulfobacter* and had a distinct $\Delta^{13}C_{TOC\text{-}BULK\ AVG\ PLFA}$ of ~ 7.0 ‰ compared to other locations on the deposit. Under comparable environmental conditions on Mars similar organisms may also have dominated acid-

sulfate environments as the cold, acidic, and high iron and sulfur contents would select for such organisms. However, if the same degree of variability existed on Mars as was seen at the Golden Deposit, there could have been large differences in the microbial communities and isotopic compositions that existed on the planet. Such variability would make interpretations of biosignatures and the identification of past life on the planet more difficult.

Sulfuric and carbonic acid dominate Golden Deposit waters due to the acidic pH. Consequently, the dominance of acidic conditions limits the precipitation and preservation of carbonates in acid-sulfate environments. The mineralogy and lack of carbonates detected at Meridiani Planum, Mars is indicative of past acidic conditions on the planet and thus it is unlikely that carbonate biosignatures will be preserved within iron and sulfur mineral deposits on Mars. In contrast, the saline, alkaline, waters of the Cariboo Plateau lakes were rich in inorganic carbon, predominantly bicarbonate and carbonate, and evidence of carbonate precipitation and preservation was present within lake sediments. Photosynthetic enrichments of surface water DIC were preserved in the isotopic composition of precipitated carbonates. Furthermore, the offset between surface water DIC and organic carbon indicative of photosynthesis by cyanobacteria was preserved in the $\Delta^{13}C_{carb\text{-}TOC}$. The $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ values were also preserved within downcore profiles of the lakes and were biosignatures of photosynthetic activity. Therefore, photosynthetic biosignatures of enriched $\delta^{13}C_{carb}$ and $\Delta^{13}C_{carb\text{-}TOC}$ could be preserved within salt and carbonate deposits that have been detected on the surface of Mars.

Isotopic characterization of organic and inorganic carbon pools could lead to the identification of past life on Mars, however, careful interpretation of these signatures will be required. The isotopic composition of atmospheric CO_2 on Mars has changed over time, which could result in further variability in preserved $\delta^{13}C$ values of organic and inorganic carbon pools. Although geologic activity on Mars ceased approximately 3.5 Ga, leaving any preserved organic matter isotopically unaltered, the strong oxidizing conditions would have stripped away any organic

matter present on the surface of the planet. Detection of organic matter in the subsurface may be possible, but does pose challenges for future Mars missions. Nevertheless, measurements of the isotopic composition of organic matter ($\delta^{13}C_{TOC}$) on Mars and its offset from other known carbon pools ($\delta^{13}C_{CO2\ ATM}$, $\delta^{13}C_{DIC}$, and $\delta^{13}C_{carb}$) could lead to the identification of past life on the planet.

The Cariboo Plateau lakes can also be considered an analogue to conditions that may have been present during the formation of microbial mats on early Earth. The downcore record within all three Cariboo lakes demonstrated the ability of the lakes to maintain non-CO_2 limited photosynthesis over time. The lakes did experience periods of undersaturation with respect to atmospheric CO_2, however, were able to maintain non-CO_2 limited photosynthesis through microbial utilization of bicarbonate. The ability of the lakes to maintain non-CO_2 limited photosynthesis and their production of isotopic values consistent with $\delta^{13}C$ values detected within the geologic record on Earth indicated that atmospheric levels in the past were not required exceed modern levels in order maintain non-CO_2 limited photosynthesis and produce the isotopic values detected within the geologic record.

4.4 Future Avenues of Research within the Golden Deposit and Cariboo Plateau Lakes

The detection of acid-sulfate deposits as well as salt and carbonate deposits on Mars warrants further investigation into potential biosignatures that could be detected within such environments. The Golden Deposit and Cariboo Plateau lakes provide insight into biosignatures that we may find in such environments. Both the Golden Deposit and Cariboo Plateau lakes are relatively unexplored analogue environments and therefore offer many more opportunities for future research.

The Golden Deposit displayed an interesting variability in geochemical conditions, which influenced both the microbial community composition and isotopic signatures. Further research at the Golden Deposit should be conducted to understand the conditions of formation of the deposit and controls on the variability

detected within the Golden Deposit. Battler et al. (unpublished) has characterized the mineralogy of the Golden Deposit, however, isotopic analysis of minerals could clarify conditions of mineral formation. Such research will help identify the possible conditions of formation and potential variability of similar systems on Mars.

The sample location closest to the groundwater seep (SITE 2) had a distinct microbial community composition and isotopic signatures likely driven by the acidic pH and high concentrations of metals and non-metals in the upwelling groundwater. Subsequent sampling of the seep site as well as sample collection from other seep locations was unable to be completed during the course of this research. Therefore, the widespread occurrence of the distinct isotopic signal at other seep locations was unable to be confirmed. Further research into the influences of groundwater chemistry and proximity of locations to groundwater seeps on the microbial community composition and isotopic signatures is warranted. This will not only increase our understanding of the spatial variability within the Golden Deposit but also clarify the degree of variation required in groundwater chemistry in order to influence biosignatures within this environment.

The influence of the microbial community on the organic carbon isotopic distributions was detected in surface sediments at the Golden Deposit during the sampling period. However, strong oxidizing conditions on Mars would have stripped organic matter from the surface of the planet. It is therefore important that further research on the downcore preservation of organic biosignatures within the Golden Deposit be conducted. This will help us identify the potential for the preservation of organic biosignatures within the subsurface in similar deposits on Mars.

The Cariboo Plateau lakes are also relatively unexplored and provide ample opportunity for future research. Within this thesis research, carbonate precipitation within the three lakes reflected surface water $\delta^{13}C_{DIC}$ values and preserved the photosynthetic biosignature downcore. Despite the presence of heterotrophs within the lakes, heterotrophic production of ^{13}C-depleted DIC did not overprint the

photosynthetic biosignature. Further investigation into the magnitude of DIC production by heterotrophs would clarify the relative influence of heterotrophy vs autotrophy on carbon isotopic signatures both on surface water DIC and precipitated carbonates. In addition, investigation into the variations in the relative balance of heterotrophy vs autotrophy both diurnally and seasonally could shed light on variations in biosignatures detected downcore.

The Cariboo Plateau lakes were also distinct from other microbial mat dominated saline environments as they displayed non-CO_2 limited photosynthesis over time. This has important implications for the interpretation of biosignatures within the geologic record on Earth. Comparative studies between the non-CO_2 limited Cariboo Plateau lakes and other CO_2-limited saline environments, such as Guerrero Negro and Solar Lake, could shed light on carbon dynamics present within the early Earth geologic record. Such investigations could include genetic analysis to identify differences in the microbial communities within these environments. Such research could further our understanding of life on early Earth and the environmental conditions necessary for the preservation of non-CO_2 limited photosynthetic biosignatures in the geologic record.

This thesis research used PLFA analysis to identify the microbial community composition within the Golden Deposit and Cariboo Plateau Lakes, which assisted in the interpretation of carbon isotopic signatures. However, PLFA only represent the active bacterial and eukaryotic community as they degrade rapidly upon cell death. Therefore, it is unlikely that PLFA biosignatures will be preserved on Mars, as the harsh conditions are unlikely to support life on the planet today. An investigation of other, more recalcitrant, lipids within these analogue environments can shed light on other lipid biosignatures that may be preserved on Mars. Such lipid classes include hopanoids, sterols, and carotenoids and their diagenetic products, all of which have been identified in the early Earth geologic record (Eigenbrode, 2008).

The Golden Deposit and Cariboo Plateau lakes can both be considered analogues to environmental conditions that existed on Mars. By continuing to investigate microbial carbon cycling, biosignatures, and their preservation downcore we increase our understanding of microbial processes within these environments. Through continual scientific exploration within analogue environments we come one step closer to identifying whether signatures of life exist on other planets and answering one of the most basic human questions: are we alone in the universe?

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APPENDIX A

The concentration of carbonate species in solution were calculated for each location using field-measured temperature and pH data as well as total DIC concentrations determined during water chemistry analysis. The following equations were used to determine the concentration of each carbonate species in solution:

Total Carbonate Concentration in Solution:

$$C_T = H_2CO_{3\,(aq)} + HCO_3^- + CO_3^{2-}$$

$$C_T = [H_2CO_{3\,(aq)}]\,(1 + K_{a1}/[H^+] + K_{a1}K_{a2}/[H^+]^2)$$

$$C_T = [H_2CO_{3\,(aq)}](\alpha_H)$$

Subsequent Equations:

$$[H_2CO_{3\,(aq)}] = C_T/\alpha_H \qquad \% H_2CO_{3\,(aq)} = [H_2CO_{3\,(aq)}]/C_T$$

$$[HCO_3^-] = C_T K_{a1}/[H^+]\alpha_H \qquad \% HCO_3^- = [HCO_3^-]/C_T$$

$$[CO_3^{2-}] = C_T K_{a1}K_{a2}/[H^+]^2\alpha_H \qquad \% CO_3^{2-} = [CO_3^{2-}]/C_T$$

WHERE:

C_T = Total concentration of carbonate species in solution (mg/L)

$[H_2CO_{3\,(aq)}]$ = Concentration of $H_2CO_{3\,(aq)}$ in solution (mg/L)

$[HCO_3^-]$ = Concentration of HCO_3^- in solution (mg/L)

$[CO_3^{2-}]$ = Concentration of CO_3^{2-} in solution (mg/L)

$[H^+]$ = Concentration of hydrogen ions in solution

K_{a1}= First dissociation constant for the carbonate system

K_{a2} = Second dissociation constant for the carbonate system

$\alpha_H = (1 + K_{a1}/[H^+] + K_{a1}K_{a2}/[H^+]^2)$

The isotopic composition of each carbonate species in solution relative to gaseous CO_2 was determined using fractionation factors from Deines et al. (1974) and the isotopic composition of atmospheric CO_2 ($\delta^{13}C_{CO2(g)}$).

Fractionation Factors for Each Carbonate Species:

$H_2CO_{3\,(aq)}$-$CO_{2(g)}$ $\quad 1000\ln\alpha = -0.91 + 0.0063 \times 10^6/T^2$

HCO_3^--$CO_{2(g)}$ $\quad 1000\ln\alpha = -4.54 + 1.099 \times 10^6/T^2$

CO_3^{2-}-$CO_{2(g)}$ $\quad 1000\ln\alpha = -3.4 + 0.87 \times 10^6/T^2$

Isotopic Composition of Carbonate Species:

$$\delta^{13}C_{(species)} = \alpha(\delta^{13}C_{CO2(g)} + 1000) - 1000$$

WHERE:

T = Water temperature of each location (°C)

α = isotopic fractionation factor

$\delta^{13}C$ = isotopic composition

$\delta^{13}C_{CO2(g)}$ = isotopic composition of atmospheric $CO_{2(g)}$

The expected $\delta^{13}C_{DIC}$ in equilibrium with atmospheric CO_2 was determined using the distribution and isotopic composition of each species in solution:

$$\text{Exp. } \delta^{13}C_{DIC} = \delta^{13}C_{(H2CO3)}(\%\ H_2CO_{3\,(aq)}) + \delta^{13}C_{(HCO3-)}(\%\ HCO_3^-) + \delta^{13}C_{(CO32-)}(\%\ CO_3^{2-})$$

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