MICROBIAL CARBON CYCLING AND ISOTOPE BIOSIGNATURES

ISOTOPIC INVESTIGATIONS OF CARBON CYCLING AND MICROBIALLY INFLUENCED CARBONATE PRECIPITATION IN FRESHWATER MICROBIALITES AND CARBONATE-RICH MICROBIAL MATS

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ABSTRACT

Modern microbialites and microbial mats are the focus of ongoing research as they provide an opportunity to understand microbial-mineral interactions during carbonate precipitation and the generation of biosignatures that can inform our interpretation of the geological record. This study determined the natural abundance isotopic compositions (^{13}C, ^{14}C) of the primary carbon pools and microbial communities associated with modern freshwater microbialites located in Pavilion Lake and in carbonate rich microbial mats on the nearby Cariboo Plateau in British Columbia, Canada.

Natural abundance ^{14}C analysis of carbon pools associated with the Pavilion Lake microbialites demonstrated that structures were actively growing and that groundwater carbon inputs to the lake and microbialites were minimal. Rather, ambient dissolved inorganic carbon (DIC) was the primary carbon source for both microbial communities and recent carbonate.

Isotopic enrichment of calcium carbonate within microbial communities associated with the microbialites was identified as a biosignature of microbial photosynthetic influence driving precipitation. Elevated oxygen concentrations and pH within the microenvironment of small, sporadic nodular microbial surface communities was concurrent with *in situ* precipitation of carbonate with $\delta^{13}C$ values higher than predicted abiotic values and $\delta^{13}C$ of bulk organic matter and phospholipid fatty acids (PLFA) that were consistent with a photosynthetically dominated community. Elevated carbonate $\delta^{13}C$ values were also noted in the thin surface microbial mat recovered from shallow (11 m) microbialites. These samples showed increased biomass during summer

sampling periods as compared to deeper samples, consistent with expected high rates of photosynthetic activity due to higher light levels and temperature at these depths. These results contrast other recent studies of modern microbialite systems that identified biosignatures of heterotrophic influences on precipitation of carbonates. PLFA profiles demonstrated that the surface microbial mat community consisting of both photosynthetic and heterotrophic microbes was stable over seasonal and spatial changes in light and temperature. However, changes in microbial biomass with depth and season indicated that microbial activity and growth plays an important role in the development of isotopic biosignatures.

Biosignatures of high levels of photosynthetic activity were also observed in carbonate, rich microbial mats that exhibited undersaturated pCO_2 concentrations during the summer and DIC $\delta^{13}C$ values enriched above values predicted for isotopic equilibrium with atmospheric CO_2. Seasonal and annual shifts in the balance of heterotrophy and autotrophy in the lakes and microenvironment of the mat accounted for observed variations in DIC and associated carbonate $\delta^{13}C$ values. In contrast to other organic rich microbial mats, bulk organic $\delta^{13}C$ values were not enriched and the systems did not show evidence of CO_2 limitation. Rather, these results indicated that low bulk organic $\delta^{13}C$ values and large isotopic discriminations can exist under conditions of high DIC concentrations and carbonate content that provide a non limiting carbon source to replenish photosynthetic drawdown.

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This dissertation represents not only many years of reading, field work, long hours in the lab, more reading but also many years filled with developing new skills, exciting discoveries, wonderful research opportunities and good friends. I feel very fortunate to have been supported throughout this research by numerous invaluable people all of whom deserve my sincerest thanks. First and foremost, my biggest thank you goes to my supervisor, Dr. Greg Slater. I have had so many fun and unique research experiences during my time at Mac due largely to your encouragement and dedication to getting students involved that I can't even begin to list them. Not many grad students get the chance to hang with astronauts and submarines. Thanks for not only being a fantastic mentor, but a good friend.

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PREFACE

This dissertation is a compilation of individual research papers that are presently published, under review or in the process of being submitted. The objectives and relationships between the papers are described in the opening chapter. Chapter six represents the final chapter summarizing the major findings and contributions obtained during this research. The individual research papers presented in this dissertation are:

Chapter Two Brady, A.L., G.F. Slater, B. Laval and D.S.S. Lim (2009) Constraining carbon sources and growth rates of freshwater microbialites in Pavilion Lake, using ^{14}C analysis. **Geobiology** 7, 544-555.

Chapter Three Brady A.L., G. Slater, C.R. Omelon, G. Southam, G. Druschel, D.T. Andersen, I. Hawes, B. Laval and D.S.S. Lim. Photosynthetic isotope biosignatures in laminated micro-stromatolitic and non-laminated nodules associated with modern, freshwater microbialites in Pavilion Lake, B.C. *Chemical Geology (submitted October 2009, in review)*

Chapter Four Brady, A.L., G.F. Slater, B. Laval and D.S.S. Lim. Investigation of autotrophic and heterotrophic dominance in modern, freshwater microbialites in Pavilion Lake, B.C. *Prepared for Limnology and Oceanography*

Chapter Five Brady A.L., G. Slater, G. Druschel, and D.S.S. Lim. Carbon cycling in carbonate-rich, cyanobacteria dominated microbial mats of the Cariboo Plateau, B.C. *Prepared for Environmental Microbiology*

Although all of the papers were co-authored, the first author on all papers (dissertation author) conducted all of the research surrounding the objective of this investigation, literature review, selection of samples, sample collection, lipid and isotope analysis and manuscript writing. Dr. Slater provided valuable guidance on the direction of the research, discussion of the results at various stages of research and editorial comments during the writing of the manuscripts.

Different levels of collaboration exist on multi-authored papers. As principal investigators on the Pavilion Lake Research Project, Dr. Bernard Laval and Dr. Darlene Lim are included as authors on various papers for assistance with site-access and logistical support required for my dissertation. In Chapter 3, microelectrode profiling was performed in the field by collaborators from the University of Vermont, SETI and Aquatic Research Solutions Ltd. with sample location and collection input from the author of this thesis. SEM analysis was conducted in collaboration with C. Omelon and G. Southam at the University of Western Ontario. Sample field collection, sub-sampling and selection for SEM were done by the author. In Chapter 5, microelectrode profiling was conducted in the field in collaboration with G. Druschel from the University of Vermont. The author contributed to site selection for profiling and assisted in obtaining the profiles. All co-authors have reviewed and approved final manuscripts before submission to journals.

The chapters in this dissertation show different formatting styles are each was prepared following the style requirements for each journal to which they were submitted or are to be submitted. Due to the 'sandwich' style of the dissertation, the reader may find some unavoidable overlap of information throughout the chapters with respect to study area, general theoretical background and methods. In compliance with McMaster University copyright regulations, appropriate permissions were requested for research papers that were already published.

CHAPTER 1

To place the findings outlined in the subsequent thesis chapters in perspective, the following sections of Chapter 1 provide an introduction to the fundamental concepts related to proposed mechanisms for microbialite formation and the role of biology in carbonate precipitation. Specifically these introductory sections focus on the challenges associated with identification of biosignatures and the use of modern analogues to address questions relevant to understanding biosignatures in modern systems and the geologic record.

1.1. Applications of microbialite and microbial mat research

Questions abound regarding the timing of the rise of early life on Earth, what types of geochemical environments existed, how early microbial communities interacted with these environments and how these outcomes are reflected in the way modern microbial systems function today. Critical to the ability to answer these questions is the recognition of 'biosignatures' that are unambiguous signals of past biological activity. Putative microfossils have been purported to represent some of the earliest evidence for photosynthetic life in the geologic record (Schopf, 1993), however debate exists over the biogenicity of such fossil evidence (Brasier et al., 2002). Stromatolites are lithified layered organo-sedimentary structures that are found throughout the geologic record as far as ~ 3.4 billion years ago that are purported to be of biotic origin (Awramik, 1992; Riding, 2000; Walter et al., 1980). 'Microbialite' is a general term commonly used for

organo-sedimentary structures hypothesized to have been constructed under biological influences (Burne and Moore, 1987). The discovery of early stromatolites in association with bacterial microfossils (see Grotzinger and Knoll, 1999; Tyler and Barghoorn, 1954), and early isotopic studies of organic material associated with ancient stromatolites consistent with values expected for photosynthesis led to the early interpretation of stromatolites as biogenic structures (Schopf et al., 1971). Despite the use of microbialites as evidence for early life on earth, the recognition of biosignatures remains challenging and the exact role, if any, of biology in the formation of such structures is debated e.g. (Awramik, 1992; Grotzinger and Rothman, 1996; Kempe et al., 1991; Lowe, 1994; Merz-Preiß and Riding, 1999; Noffke, 2009; Walter, 1996). Microbes are believed to play a significant role in the formation of microbialites through trapping and binding of sediment and/or influences on carbonate precipitation (e.g. Altermann et al., 2006; Burne and Moore, 1987; Merz-Preiß, 2000; Thompson et al., 1997). However, abiotic processes resulting from changes in geochemical conditions and/or sedimentation patterns have also been proposed as a mechanism for the formation of large carbonate structures (Council and Bennett, 1993; Grotzinger and Rothman, 1996; Walter, 1996). A clear understanding of the mechanisms of microbialite formation, microbial influences on carbonate precipitation and the potential for biosignatures is required for interpreting the geologic record and evidence for life on early earth.

Modern microbial systems have been identified in a wide variety of environments on Earth including polar regions (e.g. Fritsen and Priscu, 1998; Niederberger et al., 2009), hot springs (e.g. van der Meer et al., 2000; Ward et al., 1998), and hypersaline lakes (e.g.

Des Marais, 2003; Schidlowski et al., 1992). The ubiquitous nature and metabolic diversity of microbes makes microbial communities an integral component of modern cycling of biogeochemically important elements, such as carbon and sulfur, on a local and global scale. Studies of modern microbial mat and microbialite systems not only offer insight into past environmental conditions and the role of microbes in mineral precipitation and dissolution, but these mats are ideal models for investigating the current role of microbes in the carbon cycle and microbe-mineral interactions.

1.2. Proposed mechanisms of microbialite formation

Despite nearly 100 years of research on ancient and modern microbialites the exact mechanisms of formation and significance of these structures with respect to early ecosystems remains unresolved. The four major processes proposed for the formation of microbialites are shown in Figure 1.1.

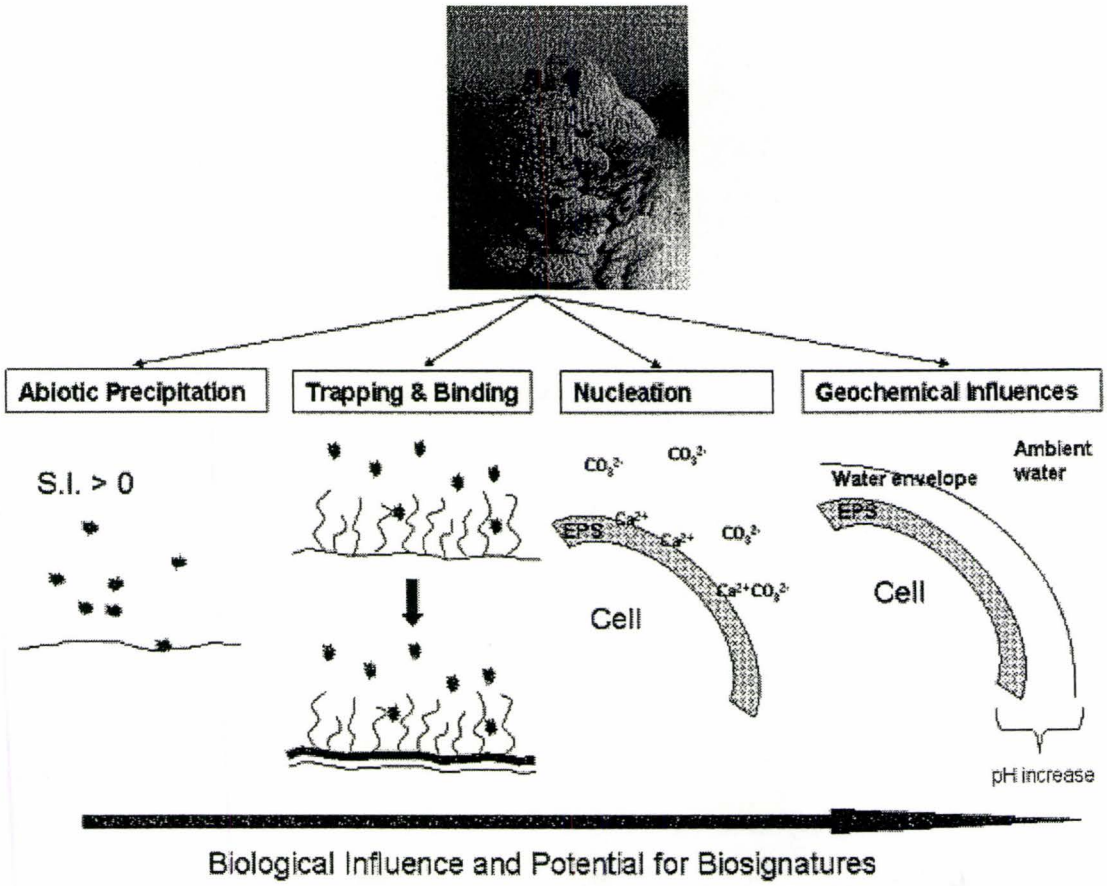

Figure 1.1: Schematic illustrating proposed abiotic and biotic mechanisms of microbialite formation listed in order of increasing potential for creation and preservation of biosignatures. The mechanism at the far left illustrates abiotic precipitation while the image at the far right represents the highest potential for biosignature creation through microbial influences on the geochemical environment.

1.2.1. Abiotic influences

Saturation of the water with respect to calcium carbonate (calcite) is the major factor controlling calcification. The saturation of a system may be modeled using a saturation index (S.I.), which is defined as the log (IAP/K_{sp}) where IAP is the ion activity product of the ions of interest (e.g. calcium and carbonate), and K_{sp} is the solubility product of the mineral of interest. Temperature and pH influence the solubility of

carbonate and are used in conjunction with calcium and carbonate concentrations to determine saturation indices (Langmuir, 1971). A S.I. value of 0 or above indicates that the solution is saturated or supersaturated with respect to calcium carbonate and precipitation is predicted to occur.

Abiotic precipitation processes have been used to primarily explain the formation of microbialites at sites such as Lake Van, Turkey (Kempe et al., 1991) and carbonate tufa at Mono Lake, California (Council and Bennett, 1993). At these sites, influxes of Ca^{2+} rich groundwater mixed with Ca^{2+} poor, HCO_3^- rich lake water creates a state of local supersaturation with respect to calcium carbonate and precipitation in the form of carbonate mounds at groundwater input locations.

1.2.2. Biological influences

Microbial communities are thought to play a significant role in the formation of microbialite structures, however the mechanisms for bacterial calcification has remained somewhat controversial (Altermann et al., 2006; Arp et al., 2001; Golubic and Seong-Joo, 1999; Merz-Preiß, 2000; Merz, 1992; Riding, 2000). The three main hypotheses regarding biogenic mechanisms of microbialite formation are; 1) trapping and binding of sediment by microbial communities; 2) acting as nucleation sites for crystal growth or 3) promotion of calcification through microbial metabolic activity (Bosak and Newman, 2003; Merz-Preiß, 2000; Merz-Preiß and Riding, 1999; Reid et al., 2000).

Trapping and binding of water column precipitated or detrital sediment particles by filamentous cyanobacteria may cause laminations observed in microbialites as

particles become trapped in the extracellular polymeric substance (EPS) produced by the microbial cells for protection and adhesion (Reid et al., 2000; Schneider and Campion-Alsumard, 1999). Continued growth of microbial filaments leads to binding of the particles within the organic substrate and preservation of these layers as laminations as the mat grows upwards. However, since the carbonate is typically precipitated in the environment external to the microbial mat and merely becomes trapped within the mats, it is difficult to distinguish biological effects associated with this process from abiotic precipitation.

Microbial cells have been cited as promoters of carbonate precipitation by acting as nucleation sites for mineral growth (Bosak and Newman, 2003; Thompson and Ferris, 1990). Homogeneous nucleation occurs within a single phase of solution at extremely high saturations, but will not occur spontaneously at lower saturations due to kinetic limitations. Heterogeneous nucleation however, can occur at much lower supersaturations as it is more energetically favorable due to decreases in the activation energy required for nucleation on a surface (De Yoreo and Vekilov, 2003; Stabel, 1986). Net negative surface charges on microbial cells act to bind Ca^{2+}, and once this nucleation site has been formed, precipitates rapidly form incorporating anions from solution (Arp et al., 2001; Phoenix et al., 2002; Schultze-Lam et al., 1996; Schultze-Lam et al., 1992).

Influence on the Geochemical Environment

Bacterial metabolic activity, including both autotrophic and heterotrophic metabolisms can induce changes in the local geochemical environment that can lead to

carbonate precipitation (Ludwig et al., 2005; Merz-Preiß, 2000; Merz-Preiß and Riding, 1999; Merz, 1992; Schneider and Campion-Alsumard, 1999). During photosynthesis, cyanobacteria take up CO_2 and release O_2 in the overall reaction:

$$H_2O + CO_2 \rightarrow CH_2O + O_2 \quad (1)$$

Many cyanobacteria have been shown to possess a carbon concentrating mechanism that enables the uptake of HCO_3^- under low CO_2 concentrations that can result in a 1000-fold increase in intracellular CO_2 concentrations within the cell as compared to the ambient water. When HCO_3^- is taken into the cell, it is converted into CO_2 and OH^- by the enzyme carbonic anhydrase for use in the ribulose-1,5-bisphosphate carboxylase enzyme (Badger and Price, 2003; Miller and Colman, 1980). The CO_2 is incorporated into the cell biomass while the OH^- is expelled, contributing to increased pH in the microenvironment around the cell and a shift towards a higher CO_3^{2-} concentration leading to precipitation (Merz, 1992; Revsbech et al., 1983; Schultze-Lam et al., 1992; Thompson and Ferris, 1990).

Heterotrophic metabolic activity may also have significant influence on carbonate precipitation. Sulfate reducing bacteria (SRB) oxidize organic matter to hydrogen sulfide and CO_2 through the following general reaction:

$$SO_4^{2-} + 2CH_2O + OH^- \rightarrow HS^- + 2HCO_3^- + H_2O \quad (2)$$

This inorganic carbon input into the surrounding aqueous medium leads to an increase in alkalinity and an increase in the S.I. (Altermann et al., 2006; Baumgartner et al., 2006; Visscher et al., 2000). Other heterotrophic activity may have similar effects on ion concentration during oxidation of organic matter. Furthermore, Ca^{2+} and Mg^{2+} ions

stored in cyanobacteria EPS are released during decomposition, also increasing the S.I. and promoting precipitation of carbonate (Altermann et al., 2006; Paerl et al., 2001; Reid et al., 2000). Microbially induced changes in geochemistry, and any associated biosignatures, may be observed throughout the lake in lacustrine systems (Gu et al., 2004; McConnaughey et al., 1994; Thompson et al., 1997). Alternatively, microbial effects on geochemical conditions may be restricted to microenvironments associated with the cells, such as within a sheath or EPS or within a microbial mat (Andres et al., 2006; Bissett et al., 2008; de Beer et al., 1997; Jørgensen et al., 1983; Revsbech et al., 1983).

1.3. Biosignatures of microbial influences on precipitation

The ability to recognize any of these biological influences on carbonate formation remains challenging and the mechanisms involved in most modern systems are still not completely understood. With this in mind, it is evident that any study contributing to understanding biosignature development and interpretation makes a valuable contribution to understanding microbial interactions with the environment.

Influences on mineral morphology have been proposed as biosignatures of microbial cell nucleation (Bosak and Newman, 2003; Chafetz and Buczynski, 1992). However, mineral morphology has not proven to be a reliable indication of biological influences on carbonate precipitation as many studies have been unable to conclusively link morphology to specific abiotic or biotic processes. Similar morphologies of carbonate precipitates have been observed in both biological systems and under abiotic conditions (Chafetz and Guidry, 1999; van Knorre and Krumbein, 2000).

The greatest potential for generation of a biosignature that may be preserved in the geologic record is the ability of microbial metabolic activity to change local water geochemistry and promote the precipitation of calcium carbonate (Merz, 1992; Shiraishi et al., 2008; Shiraiwa et al., 1993). In addition to influencing the saturation state of the local environment, microbial metabolic processes may also influence the isotopic composition of the dissolved inorganic carbon and the $\delta^{13}C$ value of carbonate precipitated from this carbon pool will reflect microbial effects on the isotopic composition (Merz, 1992; Sumner, 2001).

Autotrophic and heterotrophic metabolic activity may induce deviations in measured $\delta^{13}C$ carbonate values from the predicted equilibrium values. The direction of these deviations indicate autotrophic or heterotrophic dominant processes (Ferris et al., 1997; Hodell et al., 1998; McConnaughey, 1989; McConnaughey et al., 1997; Merz, 1992; Thompson et al., 1997). Biological preference for ^{12}C during photosynthesis leads to incorporation of the lighter isotope into cell biomass and a corresponding enrichment in ^{13}C of the residual DIC and precipitated carbonate (Hollander and McKenzie, 1991; O'Leary, 1988). Carbonates enriched in ^{13}C have been reported from freshwater lakes with high levels of photosynthetic activity (Hollander and McKenzie, 1991; Thompson et al., 1997). Conversely, heterotrophic decomposition of organic matter results in little fractionation, leading to the formation of CO_2 similar in isotopic composition to the source organic matter (Abraham et al., 1998; Blair et al., 1985). Thus, heterotrophic degradation of ^{13}C-depleted organic matter inputs correspondingly low $\delta^{13}C$ CO_2 leading

to a ^{13}C-depletion in the DIC and precipitated carbonate (Andres et al., 2006; Breitbart et al., 2009).

1.4. Investigations of modern analogues

Modern microbialites represent an opportunity to gain further understanding of mechanisms of their formation and the microbial metabolic processes that may leave preserved biosignatures. Some of the earliest identified microbialites of sizes and shapes analogous to ancient structures were in Hamelin Pool, a hypersaline zone within Shark Bay, Australia that hosts microbialites up to ~ 0.5 m in size (Logan, 1961; Reid et al., 2003). Additional examples of modern microbialites of varying sizes and morphologies have been discovered in a number of different environments and locations around the world including the Bahamas (e.g. Dravis, 1983), Lake Van, Turkey (e.g. Kempe et al., 1991), Mexico (e.g. Breitbart et al., 2009) and Canada (Ferris et al., 1997; Laval et al., 2000). These modern examples tend to exist in environments that preclude extensive grazing activity (e.g. hypersaline). Modern microbialites are considered analogues of Precambrian stromatolite forming communities that existed prior to the rise of metazoan grazers (Awramik, 1992; Riding, 2000).

Biogenic origins have been proposed for modern microbialites found at Shark Bay (Burns et al., 2004), Highborne Cay, Bahamas (Andres et al., 2006; Macintyre et al., 2000) and Cuatro Ciénegas, Mexico (Breitbart et al., 2009) where clear evidence for abiotic processes is not observed. The most extensively studied modern microbialite systems are the marine environment examples from Shark Bay, Australia and the Exuma

Cays, Bahamas. In contrast to the hypersaline conditions of Hamelin Pool, Bahamian microbialites are the only known example of modern structures forming in an open marine environment (Dill et al., 1986; Reid et al., 1995). In Highborne Cay, lithification is the result of interactions between the various members of the surface microbial mat community whereby filamentous cyanobacteria trap suspended sediment while heterotrophic processes, specifically sulfate reduction promote *in situ* calcification that cements the particles together (Reid et al., 2000; Visscher et al., 2000; Visscher et al., 1998). Support for heterotrophic influences on *in situ* carbonate precipitation comes from identification of carbonate with $\delta^{13}C$ values lower than expected for equilibrium precipitation in Highborne Cay, Bahamas (Andres et al., 2006) and also in freshwater, geothermal spring microbialites from Cuatro Ciénegas, Mexico (Breitbart et al., 2009).

Although photosynthetic processes have been invoked to explain observed ^{13}C-enrichements in ancient microbialites (Burne and Moore, 1987) and small, modern stromatolites and thrombolites (Ferris et al., 1997), few indications of photosynthetically induced precipitation have been identified in modern, large scale microbialites. However, as carbonates enriched in ^{13}C have been identified in freshwater lakes with high levels of photosynthetic activity (Ferris et al., 1997; Hollander and McKenzie, 1991; Thompson et al., 1997) additional isotopic investigations of modern, lacustrine microbialite systems associated with photosynthetic microbial communities are warranted.

Questions arise regarding variation in microbial activity including potential changes in the relative dominance of heterotrophy versus autotrophy and effects on microbialite morphology or associated biosignatures. Microbial activity changes with

environmental parameters such as light level or temperature (Bebout and Garcia-Pichel, 1995; Fritsen and Priscu, 1998; Jorgensen et al., 1988) implying that autotrophic or heterotrophic influences on precipitation may not be consistently present. Investigations of the microbial community associated with Bahamian microbialites demonstrate that it remains relatively unchanged over different seasonal periods (Reid et al., 2000). Morphological variation in Highborne Cay microbialites has been linked to physical environmental parameters such as accommodation space and sediment depth (Andres and Reid, 2006). However, environmental conditions within these systems are relatively stable. No studies have addressed the extent of variability in the dominance of autotrophy or heterotrophy and associated biosignatures within a microbialite system that undergoes significant seasonal and spatial temperature and light fluctuations or within individual microbialite structures.

Microbial mats

In addition to modern microbialite environments, microbial mat systems have also been extensively studied as analogues of Precambrian stromatolite-forming communities (Canfield and Des Marais, 1993; Des Marais et al., 1989; Schidlowski et al., 1992). Microbial mats are layered microbial communities that were once common in marine environments, but now are typically confined to extreme environments (high temperature, salinity and pH) that prevent predation and destruction of the mats by grazing organisms (Paerl et al., 2000; Pinckney and Paerl, 1997). Modern microbial mats form complex, typically layered communities in which major biochemical metabolic pathways exist in

close proximity and over short vertical distances (Canfield and Des Marais, 1993; Paerl and Pinckney, 1996; van Germerden, 1993). Cyanobacteria are typically the dominant primary producers in most microbial mat systems, supporting a host of other organisms employing different metabolisms. Other major groups may include aerobic heterotrophs, green and purple sulfur bacteria, colourless sulfur bacteria, sulfate reducing bacteria and methanogens (Bebout et al., 2004; Paerl et al., 2000; Stal et al., 1985). The interactions of these different microbial groups create a biologically stratified mat with variations in pH, redox potential, nutrient concentrations occur over relatively small distances e.g. (Jørgensen et al., 1983; Revsbech et al., 1983). Microbial metabolic activity within the mat may result in the creation of a mat microenvironment that differs from the overlying water column (Bissett et al., 2008).

Modern microbial mats studied as analogues to ancient ecosystems are typically organic rich with high rates of productivity such as those from Guerrero Negro and Solar Lake (Des Marais, 1992; Des Marais et al., 1989; Schidlowski et al., 1992; Schidlowski et al., 1994). Hot spring microbial mats composed of cyanobacteria and anoxygenic phototrophs as primary producers from Yellowstone National Park have also been extensively studied as analogues of early ecosystems (Estep, 1984; van der Meer et al., 2005; van der Meer et al., 2000). Microbial mats have been noted to produce organic matter with highly enriched $\delta^{13}C$ values typically ranging from -10 to -18 ‰, but up to -5 ‰ in some cases (Des Marais et al., 1989; Scherf and Rullkötter, 2009; Schidlowski et al., 1984; Schouten et al., 2001). The ^{13}C-enriched organic matter is often attributed to CO_2 limitation during photosynthesis as net fractionation decreases during photosynthesis due

to increased quantitative conversion resulting in a discrimination between DIC (inorganic) and organic carbon pools ($\Delta^{13}C_{inorg-org} = \delta^{13}C_{inorg} - \delta^{13}C_{org}$) of less than ~ 25 ‰ (Hollander and McKenzie, 1991; O'Leary, 1988). Increased salinity and reduced CO_2 solubility coupled with high carbon demand in non-lithifying, organic rich mats has been attributed to CO_2 limitation (Des Marais et al., 1989). However, other factors must also influence limitation as no significant trends have been noted in microbial mats at varying salinities (Scherf and Rullkötter, 2009; Schidlowski et al., 1994).

Increased organic values in hot spring environments are typically attributed to the presence of microbial metabolisms that do not use the Calvin cycle for CO_2 fixation (Preuß et al., 1989; van der Meer et al., 2000; van der Meer et al., 1998). Hot spring microbial mats from Yellowstone National Park that are composed of cyanobacteria and anoxygenic photosynthetic bacteria (van der Meer et al., 2000; Ward et al., 1998). Anoxygenic photosynthetic bacteria have been shown to use carbon fixation pathways, such as the 3-hydroxypropionoate pathway and reversed tricarboxylic acid (TCA) cycle that have small fractionations between inorganic carbon and bulk organic biomass relative to the Calvin cycle (Preuß et al., 1989; Quandt et al., 1977) leading to ^{13}C-enriched biomass (van der Meer et al., 2000; van der Meer et al., 1998).

In contrast to the ^{13}C-enriched bulk organic values typically observed in modern microbial mats, organic matter $\delta^{13}C$ values from the geologic record are closer to -26 ‰ (Eichmann and Schidlowski, 1975; Schidlowski, 1988; Schidlowski et al., 1994) typical of C_3 photosynthesis. These observations have been used to propose higher atmospheric CO_2 concentrations during the Precambrian allowing for non-CO_2 limited photosynthesis

(Des Marais et al., 1992; Schidlowski, 1985). However, carbonate rich microbial mats have not been as extensively studied and have been suggested to represent important analogues to ancient stromatolite-forming communities (Des Marais, 1992). Carbonate rich microbial mats provide a significant comparative system to non-lithifying, organic rich and hot spring mats in which to investigate isotopic discriminations due to potential differences in microbial community structure, productivity levels, and close association with carbonate precipitates and related isotopic discriminations.

1.5. Research objectives

The value of microbialite and microbial mat research is the fundamental contribution to understanding the role of microbes in modern carbon cycling, and also the ability to apply that knowledge to understanding how early microbial ecosystems interacted with their environment and what signatures are preserved in the rock record. Due to the challenges associated with the identification and interpretation of biosignatures, any further contributions to our understanding of microbial influences on carbonate precipitation and isotope signatures are important. The discovery of microbialites of varying morphologies in association with photosynthetic microbial communities in freshwater Pavilion Lake (Laval et al., 2000; Lim et al., 2009) in British Columbia, Canada and the presence of nearby carbonate rich microbial mats on the Cariboo Plateau presents an opportunity to investigate microbialite formation in a distinct environment and further scientific understanding of microbial influences on carbonate precipitation and associated biosignatures.

Pavilion Lake is a relatively small lake (5.7 km x 0.8 km, 65 m deep) located in the Marble Canyon, British Columbia. In contrast to other modern microbialite systems, Pavilion has not been readily identified as an extreme environment. Pavilion Lake is a groundwater fed lake in a region dominated by karst hydrology with creeks flowing down the sides of the Marble Canyon that disappear underground before reaching Pavilion and enter the lake at unknown locations. Surficial geology is primarily glacial till overlying bedrock that includes Permian to Jurassic chert and argillite, Permian to Upper Triassic aged limestone and marble and Late Jurassic granodioritic intrusions (Figure 2.1). Pavilion is a freshwater lake with a mean pH of ~ 8.3, and is dimictic with a summer thermocline depth of ~ 9 m and experiences annual ice cover reaching a thickness of ~ 50 cm (Lim et al., 2009). It is characterized as ultra-oligotrophic (mean total phosphorus 3.3 μg/L) with low sedimentation rates and little evidence of carbonate precipitation within the water column. The lake is clear with Secchi depths of ~ 15 m and optical transmission of 95 % of pure water for most of the water column. Microbialites present in the lake are estimated to be younger than 12,000 years based on uranium series dating and their situation within silts that bury postglacial clastic sediments (Laval et al., 2000). The microbialite structures are distributed throughout the lake at depths of ~ 5 to 55 m and range in size from several centimeters to meters in height with varying morphologies. These structures were originally classified into four depth categories based on morphology, however although these morphological differences do show a trend with depth, the exact physical, chemical or biological factors that contribute to the morphology are unknown (Laval et al., 2000). The processes that led to the formation of these

structures at these lakes are not currently known. In contrast to marine microbialites that tend to exist in relatively shallow waters, the depth and seasonal variation in light and temperature in Pavilion Lake permit the investigation of many of the unknown questions related to influences of seasonality, or spatial changes in temperature and light on the complexity of microbialite systems and associated biosignature genesis.

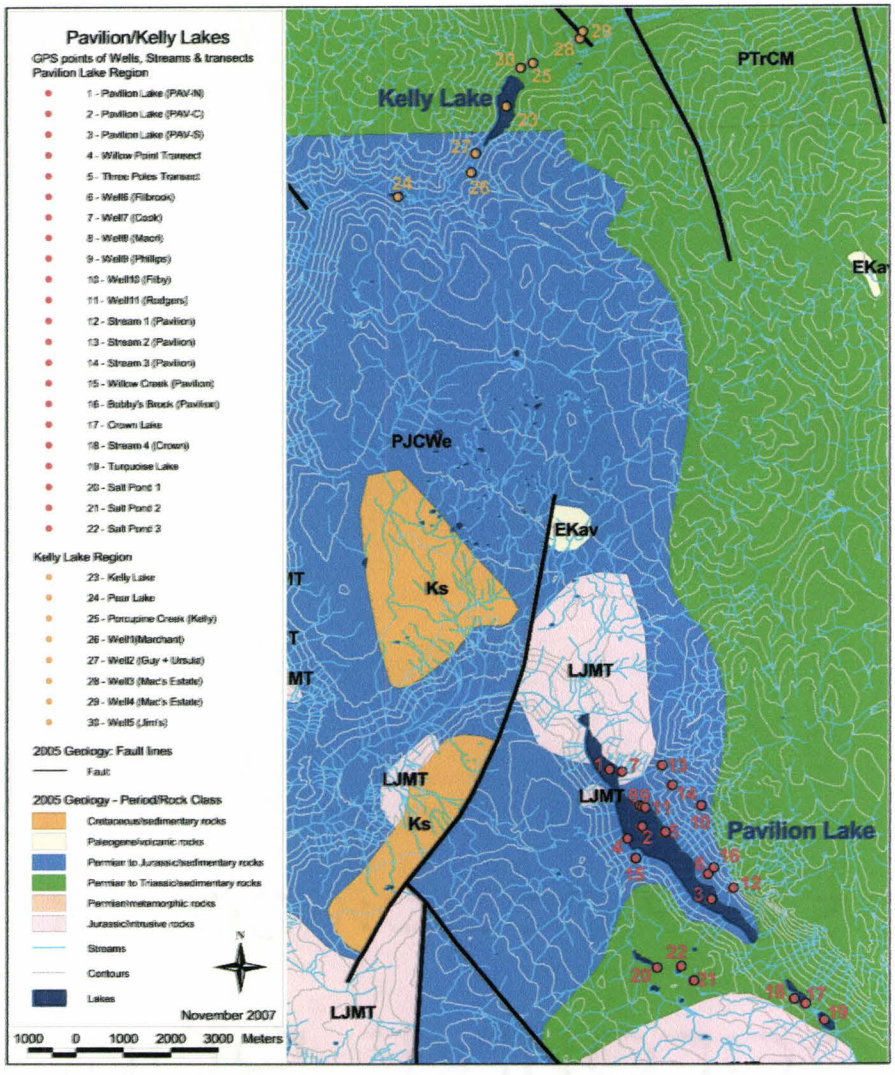

Figure 1.2: Location and geology map of Pavilion Lake region (as in Lim et al. 2009).

The carbonate rich microbial mats located on the nearby Cariboo Plateau likewise represent an opportunity to contribute to our understanding of microbial influences on carbonate precipitation and associated biosignatures through examination of a distinct system. The Cariboo Plateau is an arid region located in the interior of British Columbia that is underlain by Miocene and Pliocene basalt flows and glacial till 1 to 5 m thick.

Several hundred saline lakes exist in this area ranging from large freshwater lakes to small hypersaline lakes that occupy depressions within glacial deposits (Renaut, 1990; Renaut and Long, 1989). Freezing of the lakes, spring precipitation and summer evaporation leads to variability in the salinity of the water (Renaut, 1990; Renaut and Long, 1989). Extensively developed cyanobacteria dominated microbial mats with high amounts of carbonate (> 60 %), but no large microbialite structures are present within the lakes chosen for this study. Investigation of these extensively developed, high carbonate content microbial mats presents an opportunity to examine variability in microbial influences on organic and inorganic biosignatures in contrast to other non-lithifying systems.

Within this dissertation, biosignatures of microbial influence on carbonate precipitation within the microbialites and microbial mats was investigated using natural abundance ^{13}C and ^{14}C analysis of DIC, carbonate, bulk cell and microbial phospholipid fatty acids (PLFA). Natural abundance ^{14}C analysis was used in combination with ^{13}C analysis to further constrain the source of carbon in the microbialites and to the microbial community. ^{14}C analysis has been demonstrated to distinguish between modern and more ancient carbon sources as atmospheric CO_2 is enriched in ^{14}C while in contrast, carbon older than ~ 60, 000 years will be ^{14}C-depleted (Petsch et al., 2001; Slater et al., 2005).

Microbial PLFA analysis was used to characterize the diversity within the microbial communities present at the lakes and to link microbial groups to specific carbon sources and growth conditions. Phospholipids degrade rapidly upon death (White et al., 1979) and the ability to link PLFA to specific microbial groups has been used to

assess microbial diversity within environmental systems (Fang et al., 2006; Navarrete et al., 2000; Rajendran et al., 1992; Scherf and Rullkötter, 2009). The relationship between PLFA $\delta^{13}C$ values and bulk cell $\delta^{13}C$ values provides a further understanding of carbon assimilation pathways in these microbial communities as cyanobacteria PLFA are depleted by ~ 7 – 9 ‰ (Jahnke et al., 2004; Sakata et al., 1997) and heterotrophic PLFA are depleted by ~ 2 – 4 ‰ (Abraham et al., 1998; Blair et al., 1985; Teece et al., 1999).

1.6. Dissertation structure

In order to validate the use of modern microbialites and microbial mats as analogues to ancient stromatolites and early microbial ecosystems, we need to understand the role of biotic processes in carbonate precipitation, the potential signatures of microbial influence and the preservation of these signatures. Therefore, the overarching hypotheses tested in this research were:

1. biology plays a role in the formation of Pavilion Lake microbialites;
2. isotopic biosignatures of microbial activity exist in association with the microbialites and are related to environmental parameters;
3. microbial activity and influence on carbonate precipitation are reflected in isotopic biosignatures present in carbonate rich Cariboo Plateau microbial mats.

The Cariboo Plateau microbial mats are examined as distinct from the organic rich mats previously studied, and can be related to the processes occurring in Pavilion Lake. These hypotheses led to the development of specific research objectives that form the basis of the papers presented in this thesis. While the previous sections represent a

general overview of the concepts and unknowns associated with this research area, specific background information, objectives and discussion are included in the chapters that comprise the main body of work.

Chapter 1 presents an overview of the importance of analogue research, the proposed mechanisms of microbialite formation and associated isotopic biosignatures. This chapter also highlights the focus of previous investigations on marine microbialite environments and organic rich and hot spring microbial mats in contrast to the freshwater microbialite and carbonate-rich microbial mat systems explored in this research.

Chapter 2 describes the use of the ^{13}C and ^{14}C isotopic composition of dissolved inorganic carbon (DIC), precipitated carbonate and microbial biomass as natural abundance tracers of carbon source and processing to identify carbon sourcing and microbialite growth rates. The hypotheses tested in this study were:

a. microbialites are precipitating abiotically due to locally elevated carbonate saturation from mixing of groundwater and lake water

This hypothesis is related to the overarching hypothesis 1 and represents the negative of this hypothesis, that microbialites are biologically formed. Identification of abiotic precipitation negates hypothesis 1.

b. if precipitation of microbialites is abiotic it will result in $\delta^{13}C$ and $\Delta^{14}C$ values of the microbialites reflecting that of the groundwater carbon influx

A comparison of the isotopic composition of the carbonate and associated microbial communities to potential DIC sources, tests the hypothesis that groundwater DIC is the source of microbialite carbonate carbon.

c. microbialites are actively growing

This hypothesis is related to both overarching hypotheses regarding microbialite formation and the ability to detect biosignatures in a modern system as it tests the underlying assumption that the processes are actively occurring. Testing the veracity of this assumption is crucial to supporting the use of this system as a modern analogue and the ability to relate observed signatures to microbial or abiotic processes.

Chapter 3 explores evidence for photosynthetically driven carbonate precipitation in association with Pavilion Lake microbialites. The hypotheses tested in this study were:

a. microbial photosynthetic activity is altering the geochemistry within the nodule

b. precipitation is occurring *in situ* within the microenvironment of the nodule

These study hypotheses are related to the overarching hypotheses 1 and 2 as they examine the potential for photosynthetic activity to influence the local geochemistry, promoting carbonate precipitation.

c. photosynthetic influence on carbonate precipitation within the nodules will result in a biosignature of enriched carbonate $\delta^{13}C$ values and depleted organic $\delta^{13}C$ values with a discrimination between inorganic and organic carbon pools consistent with photosynthetic fractionation

If photosynthesis is influencing carbonate precipitation, and altering the isotopic composition of the local DIC, the associated carbonate $\delta^{13}C$ values are expected to reflect the ^{13}C-enriched DIC.

Chapter 4 examines the potential for changes in environmental parameters to influence microbial activity and biosignatures. Hypotheses explored in this study were:

a. distinct coloured zones on individual microbialites correspond to zones of autotrophic and heterotrophic dominance within the surface microbial mat with distinct isotopic biosignatures

This hypothesis is related to hypothesis 2 and tests the influence of environmental parameters on the development of autotrophic and heterotrophic dominated regions within the microbialites.

b. organic biomarkers and inorganic and organic isotopic signatures would reflect distinct autotrophic and heterotrophic communities

This hypothesis relates to hypothesis 2 as it tests the ability to distinguish biosignatures of autotrophy versus heterotrophy, important for identifying microbial processes related to carbonate precipitation. Examination of the surface microbial mat in this Chapter provides a contrast to the results outlined in Chapter 3 that focused on small, sporadic nodules located on the surface of the microbialites.

c. seasonal and spatial changes in light and temperature would affect the relative autotrophic and heterotrophic dominance and corresponding isotope biosignatures

This hypothesis relates primarily to the overarching hypothesis 2 that microbial activity and associated isotopic biosignatures are related to environmental parameters such as light and temperature. The study outlined in Chapter 4 examines the relationship between these parameters and associated organic biomarkers and isotopic biosignatures.

Chapter 5 describes carbon cycling and associated biosignatures within the Cariboo Plateau microbial mats. The main hypotheses addressed were:

a. photosynthetic activity will result in enriched carbonate $\delta^{13}C$ values and large discrimination between organic and inorganic carbon reservoirs

b. organic biomarkers would reflect a largely autotrophic microbial community

This paper relates primarily to hypothesis 3 and identifies isotopic signatures associated with microbial activity. It provides information regarding the range of annual and seasonal variation in inorganic and organic isotope biosignatures and compares results to other non-lithifying systems. Mechanisms for observed differences and implications for interpretation of biosignatures in the rock record are discussed.

Chapter 6 is a summary of the significant findings and general conclusions that have evolved from this body of work including directions for future research.

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CHAPTER 2

CONSTRAINING CARBON SOURCES AND GROWTH RATES OF FRESHWATER MICROBIALITES IN PAVILION LAKE USING ^{14}C ANALYSIS

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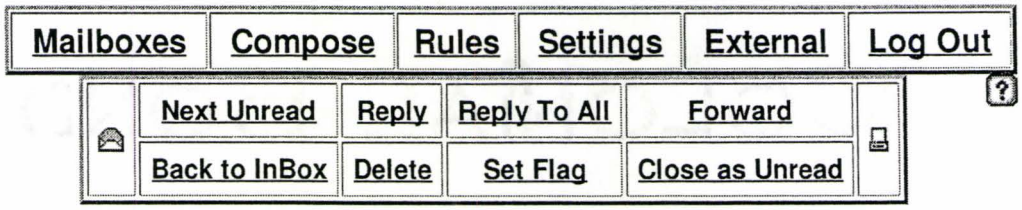

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ABSTRACT

This study determined the natural abundance isotopic compositions (^{13}C, ^{14}C) of the primary carbon pools and microbial communities associated with modern freshwater microbialites located in Pavilion Lake, British Columbia, Canada. The $\Delta^{14}C$ of dissolved inorganic carbonate (DIC) was constant throughout the water column and consistent with a primarily atmospheric source. Observed depletions in DIC ^{14}C values compared to atmospheric CO_2 indicated effects due either to DIC residence time and/or inputs of ^{14}C-depleted groundwater. Mass balance comparisons of local and regional groundwater indicate that groundwater DIC could contribute a maximum of 9 – 13 % of the DIC. ^{14}C analysis of microbial phospholipid fatty acids (PLFA) from microbialite communities had $\Delta^{14}C$ values comparable to lake water DIC, demonstrating that lake water DIC was their primary carbon source. Microbialite carbonate was also primarily derived from DIC. However, some depletion in microbialite carbonate ^{14}C relative to lake water DIC occurred, due either to residence time or mixing with a ^{14}C-depleted carbon source. A detrital branch covered with microbialite growth was used to estimate a microbialite growth rate of 0.05 mm per year for the past thousand years, faster than previous estimates for this system. These results demonstrate that the microbialites are actively growing and that the primary carbon source for both microbial communities and recent carbonate is DIC originating from the atmosphere. While this data cannot conclusively differentiate between abiotic and biotic formation mechanisms, the evidence for minor inputs of groundwater derived DIC is consistent with the previously hypothesized biological origin of the Pavilion Lake microbialites.

2.1 INTRODUCTION

Stromatolites and putative microbial fossil remains recovered from the geologic record are often cited as evidence for early life, despite the fact that their biogenic origins are debated (Schopf, 1993 ; Brasier *et al.*, 2002). Stromatolites are lithified layered organo-sedimentary structures found throughout the geologic record as far as ~ 3.4 billion years ago (Walter *et al.*, 1980 ; Riding, 2000 ; Turner *et al.*, 2000). The term 'microbialites' is used more generally for organo-sedimentary structures, including stromatolites, that are hypothesized to have been constructed under biological influences (Burne & Moore, 1987). Microbes, in particular photosynthetic microbial communities, are thought to play a significant role in the formation of microbialite structures through trapping and binding and/or influences on carbonate precipitation (Merz, 1992 ; Golubic & Seong-Joo, 1999 ; Merz-Preiß, 2000 ; Riding, 2000 ; Arp *et al.*, 2001 ; Altermann *et al.*, 2006). However, abiotic processes resulting from changes in geochemical parameters and/or sedimentation have also been proposed as mechanisms of microbialite precipitation (Council & Bennett, 1993 ; Grotzinger & Rothman, 1996). A clear understanding of the mechanisms of microbialite formation is required for interpreting the geologic record and evidence for life on early earth. Distinguishing between these potential abiotic and biotic imprints is crucial for many areas of research as biosignatures are a valuable tool for reconstructing ancient global carbon cycling and palaeo-CO_2 concentrations (Hollander & McKenzie, 1991 ; Des Marais, 1997 ; Schidlowski, 2001 ;

Andrews, 2006), and may be further applied as a basis of knowledge to the search for evidence of life on other planets (Kazmierczak & Kempe, 2003 ; Chekroun *et al.*, 2004).

The presence of modern microbialites in association with photosynthetic microbial communities in Pavilion Lake, British Columbia, Canada (50°51' N, 121°44' W) presents an opportunity to investigate on-going microbialite formation (Laval *et al.*, 2001). While Laval et al. (2000) hypothesized a biogenic origin for these structures, the potential for abiogenic formation via groundwater mixing, such as has been observed in Mono Lake (Council & Bennett, 1993), cannot be discounted. The goal of the present study was to determine the carbon source(s) for the microbialites and associated microbial communities to determine whether there is evidence of groundwater dissolved inorganic carbon (DIC) contributing to their formation.

The ^{13}C and ^{14}C isotopic composition of dissolved inorganic carbon (DIC), precipitated carbonate and microbial biomass may be used as natural abundance tracers of carbon source and processing (Abraham *et al.*, 1998 ; Sumner, 2001 ; Slater *et al.*, 2005). Stable carbon isotope analysis has been widely applied to understand inorganic and organic carbon sources (Coffin *et al.*, 1989 ; Shi *et al.*, 2001 ; Gu *et al.*, 2004 ; Pelz *et al.*, 2005). However, in many cases, $\delta^{13}C$ values are not sufficiently distinct to allow for definitive interpretations. In systems where sufficiently large natural abundance variations occur, ^{14}C analysis can overcome this limitation by providing an additional tool to differentiate carbon sources. Atmospheric CO_2 is enriched in ^{14}C which decays to ^{14}N with a half-life of ~ 5730 years (Libby, 1961 ; Godwin, 1962). Modern atmospheric carbon in the northern hemisphere currently has a $\Delta^{14}C$ value of ~ + 55 ‰, (Turnbull *et*

al., 2007). In contrast, carbon more than ~ 60,000 years old, such as that making up the carbonate minerals in the bedrock below Pavilion Lake, will contain virtually no ^{14}C ($\Delta^{14}C$ = -1000 ‰, Deevey Jr. *et al.*, 1954). This extreme difference in $\Delta^{14}C$ provides a natural abundance tracer of these two types of carbon in an environmental system. In lacustrine environments, lake water in isotopic equilibrium with the atmosphere will have a $\Delta^{14}C$ value reflecting modern atmospheric levels while groundwater is expected to have much lower $\Delta^{14}C$ values due to long residence times or interactions with the surrounding geology (Deevey Jr. *et al.*, 1954).

This same natural abundance tracer approach can be used to investigate the primary carbon source used by microbes by determining the $\Delta^{14}C$ of the microbial cellular components (Pearson *et al.*, 2001 ; Petsch *et al.*, 2001 ; Slater *et al.*, 2005 ; Slater *et al.*, 2006). The isotopic composition of microbial phospholipid fatty acids (PLFA) provides an effective manner in which to examine *in situ* the carbon sources to the metabolically active microbial community in an environmental system (Vestal & White, 1989 ; Abraham *et al.*, 1998). Phospholipids are membrane components that are known to degrade rapidly, within days to weeks, upon death (White *et al.*, 1979) therefore they represent the viable microbial community at a site. Further, specific PLFA have been shown to be linked to certain microbial groups (Vestal & White, 1989 ; Zelles, 1999 ; Boschker & Middelburg, 2002). PLFA can be isolated and collected by Preparative Capillary Gas Chromatography (PCGC) (Eglinton *et al.*, 1996 ; Pearson *et al.*, 2001) thereby removing any inputs from detrital organics and providing a direct measure of the ^{14}C content of the viable microbial community.

In this study we used the carbon isotopic composition (^{13}C, ^{14}C) of inorganic and organic carbon in Pavilion Lake in order to determine the source of carbon to the microbialites and the associated microbial communities in order to elucidate the role of biogenic versus abiogenic formation processes.

2.2. SAMPLING AND ANALYTICAL METHODS

2.2.1. Study Site

Pavilion Lake (5.7 km x 0.8 km, 65 m deep) is located in the Marble Canyon, British Columbia, Canada at an altitude of 823 m above sea level. It is an ultra-oligotrophic, dimictic, freshwater lake with a mean pH of 8.3, surface mixing layer temperature of 19°C in the summer, and DIC concentrations of 36.2 mg L^{-1} at the surface and 37.6 mg L^{-1} at 35 m (Lim *et al.*, 2009). Pavilion Lake hosts microbialites ranging from several centimeters to meters in height (e.g. Figure 2.1) at depths ranging from 5 m to 55 m.

The microbialites are estimated to be younger than 12,000 years based on uranium series dating and their location above silts that bury postglacial clastic sediments (Laval *et al.*, 2000). Pavilion Lake is located in a relatively arid region with an annual rainfall of 200 mm (Lim *et al.,* 2009) with no perennial surface water inputs and is believed to be groundwater fed although the extent of groundwater inputs is currently unknown. Estimating hydraulic residence time is complicated by uncertainty in the nature and quantity of groundwater inputs, lack of perennial surface water inputs and

ungauged surface outflow, and forms the basis of ongoing research. However, hydraulic residence time can be constrained to be less than 60 years based on recent measurements of summer draw down (accounting for evaporation and precipitation) of the lake level for downstream irrigation (PLRP unpublished data).

2.2.2. Field Sampling and Analysis

Data for this study are based on samples collected over the course of three field seasons occurring between 2004 and 2006.

2.2.2.1. Water and Solid Samples

Water samples for DIC ^{14}C and ^{13}C analysis were collected in the summer of 2006 at depths of 3 m (hereafter referred to as "surface water", above thermocline) and 59.5 m (deep, below thermocline, 1 m above bottom) using a Niskin water sampler. The pH and clarity of Pavilion Lake, as well as previous sampling, has indicated no particulate carbonate has been observed in the water (Lim *et al.,* 2009), thus all carbonate in these samples was assumed to be dissolved. Summer 2006 light level data were collected using a LI-COR light measurement instrument attached to a CTD (conductivity-temperature-depth) profiler. Water samples representative of potential groundwater inputs to the lake were collected from a natural spring (Soda Spring) located approximately 20 km southeast of Pavilion Lake that was assumed to represent a regional groundwater end-member. This spring is constantly flowing and was therefore not purged prior to sampling. A second sample was collected from a drinking-water well

adjacent to the lake representing a local groundwater source end-member. The well was purged for a period of ~ 5 minutes before water samples were collected. Samples for ^{14}C analysis were collected in glass bottles, fixed using mercuric chloride and sealed with ground glass stoppers with no headspace until further analysis. Water samples were sent to the National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) facility at Woods Hole Oceanographic Institution. Samples were acidified to convert all DIC to CO_2 which was cryogenically purified and then graphitized for DIC ^{14}C analysis by accelerator mass spectrometry (AMS). All measurements are normalized to $\delta^{13}C$ values of -25 ‰ and are reported as $\Delta^{14}C$ values (Stuiver & Polach, 1977). Analytical precision on DIC $\Delta^{14}C$ was better than 5 ‰. Ages are reported as calibrated radiocarbon ages and have been calibrated using the program OxCal version 4.0 (IntCal04) (Bronk Ramsey, 1995 ; Bronk Ramsey, 2001).

Water samples for ^{13}C analysis were collected from the same locations as for ^{14}C in crimp sealed glass serum bottles with no headspace and fixed with mercuric chloride. Samples were acidified to convert all DIC to CO_2 analyzed using an automated continuous-flow system at G.G. Hatch Laboratory in Ottawa (St-Jean, 2003). All $\delta^{13}C$ values are reported in standard delta notation in reference to PeeDee Belemnite.

Microbialite samples were hand collected by Pavilion Lake Research Project (PLRP) SCUBA divers during 2004, 2005 and 2006. Representative samples were collected from depths ranging from 20 m to 45 m. The depths of individual microbialites sampled are listed in Table 2.1.

These samples included a 6 cm thick section of microbialite that had grown over a detrital branch (PAV1) that was sampled in order to determine microbialite growth rate (Figure 2.2). Microbialite pieces were frozen on-site and transported to McMaster University on dry ice.

Growth Rate Sampling

Using a solvent rinsed scalpel, the outer surface of the branch was scraped lightly to remove surface contact contamination and a directly underlying piece of wood was removed for ^{14}C analysis and dating. Carbonate was sampled from a cross section of the microbialite growing on the detrital branch. A sample representing the initial carbonate deposition on the branch was collected from a region adjacent to the stick (PAV2 21.5 m "inner"). Carbonate four centimeters away from this inner edge (PAV3 21.5 m "outer") was sampled as representative of more recent precipitation (Figure 2.2). Carbonate was not sampled at a distance of six centimeters directly at the outer surface due to uneven surface morphologies and sample size requirements for ^{14}C analysis.

Carbonate Sampling

Microbialite carbonate representative of recent mineral precipitation was sampled from the outer surface of the samples PAV4 20 m, PAV5 23 m, PAV6 26 m, PAV7 29 m, PAV8 33 m and PAV9 45 m and placed into acid-cleaned glass vials. Carbonate sample size requirements were 8 – 12 mg. Carbonate was sampled as close to the outer surface as possible but uneven surface morphology and sample size requirements sometimes

necessitated that samples be taken from ~ 0.5 cm from the surface of the carbonates. Carbonate and detrital wood samples were sent to NOSAMS for ^{14}C analysis. The wood sample was treated with acid and base to remove inorganic carbon prior to combustion to CO_2 at 850°C. Carbonate samples were acidified and evolved CO_2 was cryogenically purified prior to graphitization and analysis by AMS. Analytical precision on all carbonate $\Delta^{14}C$ analysis was better than 5 ‰. Microbialite carbonate was also analyzed for $\delta^{13}C$ values at NOSAMS during ^{14}C analysis. Precision on carbonate $\delta^{13}C$ analysis was better than 0.2 ‰.

2.2.2.2. Microbial Phospholipid Fatty Acid Extraction

In order to obtain sufficient microbial PLFA for ^{14}C analysis, approximately 500 g microbialite samples were extracted according to a modified Bligh and Dyer method (Bligh & Dyer, 1959) and purified using silica gel chromatography to separate lipids into non-polar, neutral and polar fractions. Phospholipids recovered from the polar fraction were subjected to a mild alkaline methanolysis and converted to fatty acid methyl esters (FAMEs) (Guckert *et al.*, 1985). FAMEs were identified and quantified using gas chromatography mass spectrometry (GC/MS) on an Agilent GC-MS with HP-5MS 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). Identification of PLFA was made based on the retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc.). PLFA are named according to the number of carbon atoms present and the number of double bonds.

Cyclopropyl PLFA are denoted by Δ. In all samples, $C_{i15:0}$, $C_{a15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:2}$, $C_{18:1}$, $C_{\Delta 17:0}$ and $C_{\Delta 19:0}$ were the most abundant and could provide sufficient carbon for ^{14}C analysis.

Individual or groups of PLFA were collected using preparative capillary gas chromatography (PCGC) as per Slater *et al.*, 2005. Collection of the purified, individual lipids was made using repeated injections (~ 80) of concentrated FAME solutions into an Agilent GC equipped with an autosampler, a Gerstel CIS-3 cooled injection system and a Gerstel preparative trapping device. 10 % of effluent passes to the flame ionization detector (FID) and the remaining 90 % is collected by a series of U-shaped tube traps that were cooled to < 0°C. FAMEs were separated on a DB-5MS capillary column (60 m x 0.54 mm I.D. x 0.25 μm film thickness) using a GC temperature program of 50°C (1 min.), 10°C/min to 160°C, 1.5°C/min to 180°C (10 min), 1.5°C/min to 250°C, 20°C/min to 320°C.

A minimum of 40 micrograms of carbon was collected for PLFA $\Delta^{14}C$ analysis. Where concentrations allowed, individual PLFA were analyzed for ^{14}C. In cases where there was insufficient mass for individual analysis or when isomers eluted too closely for reliable chromatographic separation, similar compounds were combined to obtain sufficient material for analysis. Compounds were eluted from U-traps using 2:1 hexane:DCM directly into GC vials. Collected PLFA were analyzed by GC/MS to ensure purity. Purified PLFA were submitted to NOSAMS for analysis. PLFA were combusted to CO_2 by closed tube combustion at 850°C with cupric oxide. The evolved CO_2 was cryogenically purified and graphitized for ^{14}C analysis by AMS. The methanol

used during methanolysis was characterized for ^{14}C and FAME $\Delta^{14}C$ values were corrected for methyl carbon via the relationship:

$$\Delta^{14}C_{FAME} = [(N+1)^* \Delta^{14}C_{meeasured} - \Delta^{14}C_{MeOH}]/N \quad (1)$$

where N is the number of carbon atoms. The accuracy and precision for small sample AMS analysis was considered to be better than 20 ‰ based on Pearson et al. (2001).

2.3. RESULTS

2.3.1. Isotopic Composition of Dissolved Inorganic Carbon

The results of DIC ^{14}C and ^{13}C analysis of the water samples are shown in Table 2.1. The Pavilion Lake (3 m, above thermocline) surface water and deep water (59.5m, below thermocline, 1 m above bottom) had a $\Delta^{14}C$ value of -45 ‰, indicating that the lake DIC is well mixed with respect to ^{14}C (Figure 2.3). The representative regional groundwater sample (Soda Spring) had a DIC $\Delta^{14}C$ value of -997 ‰, equivalent to the analysis blank making it essentially ^{14}C free. Local groundwater DIC represented by the drinking-water well adjacent to the lake had a $\Delta^{14}C$ value of -126 ‰ (Figure 2.3).

The $\delta^{13}C_{DIC}$ value of the surface water (3 m) sample was -1.9 ‰. In contrast, the 59.5 m sample $\delta^{13}C_{DIC}$ value was -4.4 ‰. The $\delta^{13}C_{DIC}$ value of the regional groundwater was -1.2 ‰. The local well groundwater $\delta^{13}C_{DIC}$ value was -9.9 ‰. Results of surface DIC isotopic analysis from 2005 – 2008 (mean $\delta^{13}C$ -1.2 ± 1.4 ‰, n = 7 PLRP unpublished data) are consistent with the 2006 surface DIC $\delta^{13}C$ value of -1.9 ‰ indicating that it is representative of the lake surface water. $\delta^{13}C$ values of deepwater

samples from below the thermocline over the same time period have ranged from -1.9 to the -4.4 ‰ observed during this study. This indicates an input of ^{13}C-depleted carbon affecting deepwater DIC to varying extents.

2.3.2. Isotopic Composition of Microbialite Carbonate

Microbialite carbonate and detrital wood $\Delta^{14}C$ and $\delta^{13}C$ results are shown in Table 1. The microbialite carbonate $\Delta^{14}C$ value ranged from -148 ‰ (PAV6 26 m) to -655 ‰ (PAV9 45 m) (Figure 2.3). The detrital branch, PAV1, had a $\Delta^{14}C$ value of -148 ‰, which converted to an age estimate of 1,230 ± 55 years using the program Oxcal version 4.0 (https://c14.arch.ox.ac.uk) (Bronk Ramsey, 1995 ; Bronk Ramsey, 2001). The inner sample collected from microbialite growth over a detrital branch, PAV2 had a $\Delta^{14}C$ value of -254 ‰ while the outer sample, PAV3 had a $\Delta^{14}C$ value of -186 ‰. The fact that the carbonate directly associated with the detrital branch was more ^{14}C depleted than the branch indicates inputs of ^{14}C depleted carbon to the microbialite carbonate must have occurred in the past 1200 years. Microbialite carbonate $\delta^{13}C$ values ranged from -1.0 to +1.1 ‰ with a mean of +0.2 ± 0.7 ‰ (n = 8).

2.3.3. Isotopic Composition of Microbial Phospholipid Fatty Acids

The microbial PLFA composition of the microbialite samples PAV4 20 m, PAV5 23 m and PAV6 26 m are listed in Table 2.2. Monounsaturated PLFA comprised the majority of the total PLFAs present in all samples (> 37 mole %) with $C_{16:1}$ and $C_{18:1}$ as the major components detected in the three samples. Saturated straight chain PLFA

ranged from C_{14} to C_{18} and comprised 25.4 – 27.8 mole % of the total with the most abundant, C_{16} comprising 17.9 to 20.8 mole % of the three samples. Branched chain saturated PLFA were also present, specifically *iso*- and *anteiso*-$C_{15:0}$ that represented on average 2.5 and 3.3 mole % respectively of the total PLFA. Cyclopropyl PLFA were also detected ($C_{\Delta 17:0}$ and $C_{\Delta 19:0}$ represented on average 7.4 and 2.2 mole % respectively). Polyunsatured PLFA were detected in some of the samples and ranged from 0.6 to 7.5 mole % of total PLFA.

The results of the AMS ^{14}C analysis of specific microbial phospholipid fatty acids are listed in Table 2.3. The error on PLFA ^{14}C analysis by AMS is assumed to be 20 ‰ as reported in Pearson et al. (2001). With one exception, the microbial PLFA $\Delta^{14}C$ values were consistent with the lake water $\Delta^{14}C$ (Figure 2.3). PLFA from sample PAVPLFA2 23 m have $\Delta^{14}C$ values that range from -36 ‰ ($C_{18unsats}$) to -25 ‰ ($C_{16:1}$). Microbial PLFA collected from sample PAVPLFA3 26 m have $\Delta^{14}C$ values that range from -61 ‰ ($C_{i\text{-}a15:0}$) to -22 ‰ ($C_{16:1}$). The single PLFA sampled from PAVPLFA1 20 m was $C_{16:0}$ and was the most depleted sample collected with a $\Delta^{14}C$ value of -109 ‰.

2.4. DISCUSSION

2.4.1. Primary Carbon Sources to Pavilion Lake Dissolved Inorganic Carbon

Atmospheric CO_2 is the primary carbon source to Pavilion Lake DIC with ^{14}C-depleted carbon from regional or local drinking-well groundwater DIC making less than a 13 % contribution based on ^{14}C and ^{13}C analyses. The $\Delta^{14}C$ value of North American

atmospheric CO_2 is estimated as +55 ± 5 ‰ as of January 2006 (Turnbull *et al.*, 2007). Lake water DIC in equilibrium with atmospheric CO_2 as the sole carbon input should have a $\Delta^{14}C$ value close to this modern CO_2 value (Deevey Jr. *et al.*, 1954). The fact that the lake water DIC is only ~ 100 ‰ depleted in ^{14}C relative to the atmosphere indicates that atmospheric CO_2 is the primary source of DIC in the lake but that contributions from this source are being affected by either inputs of ^{14}C-depleted carbon and/or *in situ* radioactive decay of DIC during its residence time within the lake.

Stable isotope analysis supports that atmospheric CO_2 is the primary carbon input to Pavilion Lake DIC. Pavilion Lake has a mean pH of 8.3 that remains stable on a yearly basis and throughout the water column and results in bicarbonate (HCO_3^-) being the dominant DIC species (Lim *et al.*, 2009). Based upon the fractionation factor of Mook *et al.*, (1974) and the measured $\delta^{13}C$ of atmospheric CO_2 at Pavilion Lake ($\delta^{13}C$ = -9.6 ± 0.2 ‰), the expected $\delta^{13}C$ value of the DIC (~ 90 % HCO_3^-) in the lake is -1.3 ‰, in close agreement with the measured surface (3 m) DIC $\delta^{13}C$ of -1.9 ‰. Some difference between the predicted and measured values is expected as DIC $\delta^{13}C$ values will be affected by seasonal temperature variations. A surface water temperature range of 0 – 20°C would result in equilibrium DIC $\delta^{13}C$ values ranging from +1.1 ‰ to -1.3 ‰. Additional DIC $\delta^{13}C$ values obtained over 3 years for Pavilion Lake surface water (mean $\delta^{13}C$ -1.2 ± 1.4 ‰, n = 7 PLRP unpublished data) are similar to the $\delta^{13}C$ value obtained in August 2006 and generally fall within this predicted seasonal range confirming that the lake DIC is consistently at or near isotopic equilibrium with atmospheric CO_2.

The $\delta^{13}C$ of the deepwater sample was depleted with respect to the surface water. This indicates either greater inputs of local groundwater DIC to the water below the thermocline, or to DIC inputs from respiration of organic matter (see discussion below).

2.4.1.1. Mechanisms for Observed ^{14}C Depletion of DIC

The two primary potential mechanisms that may account for the observed depletion of surface water $\Delta^{14}C$ DIC with respect to equilibrium with the atmosphere are *in situ* radioactive decay or mixing with ^{14}C-depleted carbon. If the ^{14}C-depletion in lake water DIC is assumed to be entirely due to radioactive decay, this would imply a mean age of 440 ± 45 years for the DIC. As discussed previously, there is currently no firm estimate of hydraulic residence time of Pavilion Lake; however ongoing research suggests that it is maximally 60 years. The DIC residence time is expected to be even shorter than the hydraulic residence time due to the additional factor of exchange with atmospheric CO_2. Based on these arguments, it is unlikely that the DIC has an age of 440 years.

The observed ^{14}C-depletion of surface water DIC indicates addition of a ^{14}C-depleted source of carbon. Dissolution by the lake water of the surrounding limestone (hard-water effect) or of the microbialites would be one possible direct source for such ^{14}C-depleted carbon to Pavilion DIC (Deevey Jr. *et al.*, 1954 ; Rea & Colman, 1995). However, Pavilion Lake is saturated with respect to calcium carbonate (Lim *et al.*, 2009) and the ongoing formation of the carbonate microbialites indicate that it is unlikely that extensive dissolution or isotopic exchange is occurring (Gonfiantini & Zuppi, 2003).

Rather, it is more likely that any inputs of ^{14}C-depleted DIC are coming from groundwater sources containing ^{14}C-depleted carbon (Deevey Jr. *et al.*, 1954 ; Rea & Colman, 1995). The Soda Spring, considered representative of the regional groundwater end-member, had a $\Delta^{14}C$ value of -997 ‰, essentially ^{14}C free. This is likely due to dissolution of ancient carbonates through which this groundwater is flowing, or extended isolation of the bicarbonate in this water from atmospheric CO_2. The local groundwater end-member measured from the drinking-water well near the shore of Pavilion Lake had a $\Delta^{14}C$ value of -126 ‰. This local drinking-well groundwater was assumed to be representative of what is flowing through the unconsolidated scree/till slope on the sides of the lake that is utilized by the local residents. The observed ^{14}C depletion in this sample is likewise likely due to a combination of residence time in the groundwater system and inputs of ^{14}C-depleted carbon during transit. Based on the concurrent isotopic depletion in ^{13}C ($\delta^{13}C_{DIC}$ -9.9 ‰), the most likely cause of this depletion is microbial respiration of soil organic carbon depleted in both ^{14}C and ^{13}C relative to atmospheric sources however, there could also be additional inputs from some carbonate dissolution.

2.4.1.2. Mass Balance Estimation of Potential Groundwater Associated Inputs

Assuming that the two groundwater samples are representative endmembers, an isotopic mass balance can be used to constrain the potential maximum contributions of each carbon source to lake DIC in combination with atmospheric CO_2 (Figure 2.3). The formula used for mass balance mixing using two endmembers (in this case atmospheric CO_2 and groundwater DIC) is shown in Equation 2.

$$\Delta^{14}C_{lakeDIC} = (f)*\Delta^{14}C_{atm} + (1-f)*\Delta^{14}C_{gwDIC} \quad (2)$$

*f = fraction of carbon derived from each source

Based on a mass balance mixing of atmospheric CO_2 ($\Delta^{14}C$ +55 ‰) (Turnbull *et al.*, 2007) and the regional groundwater DIC end-member ($\Delta^{14}C$ -997 ‰), the maximum ^{14}C contribution of the regional groundwater DIC to the lake water DIC is estimated to be 9 %. If local groundwater end-member ($\Delta^{14}C$ -126 ‰) is used as the source of ^{14}C-depleted carbon input to the lake DIC in combination with atmospheric CO_2 a maximum contribution of 55 % is estimated.

The estimated contribution of the two end-member groundwater sources may be further refined using a mass balance based on the stable carbon isotope data. The $\delta^{13}C$ value of the regional groundwater was -1.2 ‰ which is the same as that observed for the Pavilion Lake surface water and therefore cannot be used to constrain the extent of this input. However, an isotopic mass balance using the expected equilibrium $\delta^{13}C$ value of the lake DIC (-1.3 ‰), the measured DIC $\delta^{13}C$ value (-1.9 ‰) and the drinking-well DIC $\delta^{13}C$ value (-9.9 ‰) limits the potential contribution of local drinking-well groundwater DIC to lake surface water DIC to a maximum of 7 %. While variations in ^{13}C content of local groundwater may affect the precision of this estimate, any decrease in $\delta^{13}C$ values due to increased respiration inputs would decrease this maximum further.

For deepwater DIC (below the thermocline), while the depleted $\delta^{13}C$ might suggest increased inputs of local groundwater DIC mixing with the surface DIC, the consistent $\Delta^{14}C$ between the two water masses constricts this input to less than 13 % in order not to change the DIC $\Delta^{14}C$ value outside of the +/- 5 permil error associated with

each $\Delta^{14}C$ value. Such an input of local groundwater would only account for a 1 permil depletion in $\delta^{13}C$ DIC of deepwater. The remaining $\delta^{13}C$ depletion in the deepwater can be explained by inputs from respiration or organic matter in the lake. Due to the lack of surface water inputs to the lake (Lim *et al.*, 2009), the primary source of organic carbon within the lake is autochthonous production. Respiration of aquatic plant derived organic matter ($\delta^{13}C$ assumed to have a maximum depletion of -25 ‰) or microbial communities associated with the microbialites (bulk cell mean $\delta^{13}C$ value = -25.2 ± 3.5 ‰, n = 22, Chapter 3) would also result in inputs of ^{13}C-depleted carbon into the surface water. Assuming respiration as the primary carbon source, an estimated input of 13 % carbon with a $\delta^{13}C$ value of -25 ‰ would be required to shift the expected $\delta^{13}C$ value of the DIC (> 90 % HCO_3^-) in the lake bottom waters from -1.3 ‰ to the measured value of -4.4 ‰. Because this organic matter is assumed to be photosynthesized from lake DIC, it would not affect the $\Delta^{14}C$ values of the DIC.

Thus, in all cases, the primary source of DIC to the lake is atmospheric CO_2. Inputs of DIC from groundwater are limited to a maximum of 9 % to surface waters and a maximum of 13 % to the deepwater. Any additional inputs from respiration to the deepwater would further reduce potential groundwater DIC inputs to this deepwater DIC, with an input of 13 % being sufficient to account for the observed depletion in $\delta^{13}C$ values.

2.4.2. Primary Carbon Sources to the Pavilion Lake Microbialites

The primary question to be addressed regarding the formation of the Pavilion Lake microbialites is whether they are being produced by abiotic processes or biotic processes as hypothesized by Laval et al (2000). An inherent component of this question is determining the source of carbon that makes up the microbialite carbonate structures. The $\delta^{13}C$ and $\Delta^{14}C$ values of the microbialite carbonates and microbial communities were compared to the DIC values to determine their primary carbon sources (Figure 2.3).

2.4.2.1. Primary Carbon Source to Microbialite Associated Microbial Community

A more detailed investigation of PLFA profiles of the microbialite communities is ongoing (Chapter 4). For the purposes of this study it was noted that observed PLFA were consistent with a predominantly cyanobacterial community as observed by Laval et al (2000), but also with the presence of a broadly bacterial microbial community (Table 2.2). Beyond the ubiquitous saturated PLFA, monenoic PLFA are produced by Gram-negative bacteria (Guckert *et al.,* 1985 ; Vestal & White, 1989) including cyanobacteria (Nichols & Wood, 1968 ; Grimalt *et al.,* 1992). Cyclopropyl PLFA have been shown to indicate aerobic bacteria (Parkes and Taylor, 1983) and could result from cyanobacteria or aerobic heterotrophs. The presence of other bacteria was indicated by PLFA such as *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$ that are characteristic for Gram-negative bacteria but are less common in cyanobacteria (Jahnke *et al.,* 2004). Specific biomarkers for sulphate-reducing bacteria such as $10MeC_{16:0}$ were also observed (Londry *et al.,* 2004). Long chain PLFA characteristic of eukaryotic organisms (Volkman *et al.,* 1980) were not detected. Based on sample size requirements, five groups of microbial PLFA were

extracted from microbial communities living on microbialite samples from Pavilion Lake ranging in depth from 20 to 26 m and selected for ^{14}C analysis (Table 2.3). The suite of PLFA collected included branched-chain (*iso*- and *anteiso*-) $C_{15:0}$ PLFA characteristic of bacteria (Kaneda, 1991) as well as the ubiquitous $C_{16:0}$ and $C_{18:0}$ that are produced by numerous organisms, including eukaryotic algae (Green & Scow, 2000).

Based on the $\Delta^{14}C$ values of the PLFA (Table 2.3, Figure 2.3), the primary carbon source to microbial communities present on the microbialites is the surface water DIC. All of the PLFA with one exception have $\Delta^{14}C$ values that are the same within the error of the analysis (± 20 ‰, Pearson *et al.*, 2001) and are consistent with the $\Delta^{14}C$ values of the surface water DIC. These PLFA are significantly ^{14}C-enriched relative to the potential groundwater sources and therefore indicate no significant role for groundwater DIC in the growth of the microbialite communities. The single PLFA $C_{16:0}$ sampled from the depth of 20 m did have a $\Delta^{14}C$ value of -109.5 ‰, lower than the measured $\Delta^{14}C$ value of the lake water DIC. This finding suggests that this microbial community may be receiving a greater input of ^{14}C depleted carbon. However, it is unclear whether this input is coming from groundwater or from some minor dissolution of the carbonate with which it is associated. In either case, the $\Delta^{14}C$ value indicates that the primarily source of DIC for this community is the lake water DIC with minimal evidence of groundwater inputs contributing DIC to the microbialite microbial communities.

2.4.2.2. Primary DIC Inputs to Microbialites

The results of mass balance estimations for ^{14}C-depleted carbon input of regional groundwater to Pavilion Lake microbialite carbonate are shown in Table 2.4. The ^{14}C-content of the microbialite surface carbonate indicates that the primary source of carbon is ambient lake water DIC. However, if this were the sole source of carbon, the $\Delta^{14}C$ of the surface carbonates would be expected to be in closer agreement with lake water DIC. Instead, the surface carbonate samples were depleted in $\Delta^{14}C$ relative to the lake water DIC by -103 ‰ to -610 ‰. Similar to the observed depletions of lake DIC with respect to the atmosphere, these carbonate ^{14}C depletions could be either the result of radioactive decay after precipitation, mixing with ^{14}C-depleted DIC inputs and/or factors associated with the sampling resolution. Based on the results in Tables 2.1 and 2.3, microbialite samples fall into two groups. The samples above 29 m are all highly similar whereas the two samples from below 33 m show significantly different behaviors and will be discussed separately.

i. Samples Above 29 m

The samples above 29 m show similar offsets between surface water DIC and microbialite carbonate $\Delta^{14}C$ values of 103 to 156 ‰. These offsets are relatively small and correspond to an age difference of 800 to 1270 years from the estimate for the lake water DIC. However, these ages cannot be true ages because the sample for the inner carbonate associated directly with the detrital branch microbialite would then be older than the branch itself (Table 2.1). This observation requires that there must be some inputs of ^{14}C-depleted carbon to the microbialite carbonate. There are three possible

contributors to the observed offset in $\Delta^{14}C$. The first is variations in the $\Delta^{14}C$ of lake water DIC over time. If the offset between atmospheric CO_2 and lake surface water DIC did not remain constant at approximately -100 ‰ this would result in concurrent changes in ^{14}C content of the carbonates over time. A second potential source of the offset is variations due to sampling resolution. Due to uneven surface morphologies and sample size requirements, microbialite carbonate samples for ^{14}C analysis were generally taken from < 0.5 up to 2 cm (in the case of microbialite growing on the detrital branch) from the surface of the microbialites. Based on the growth rate calculated for the microbialite associated with the detrital branch, these variations in sampling location correspond to a range of 100 to 400 years or a $\Delta^{14}C$ of up to -100 ‰. Such sampling artifacts could account for some of the observed offset. However, such a growth rate estimate was only completed for the sample associated with the detrital branch where the estimate could be compared to the age of the branch. Therefore, the extent of this contribution cannot be fully constrained.

The third alternative explanation for the observed offsets is varying contributions of ^{14}C-depleted DIC to the site of precipitation. For the samples above 29 m, a ^{14}C mass balance indicates that the maximum contribution of depleted DIC from regional groundwater is 10 – 16 %. The potential input of local, respiration impacted groundwater DIC is limited by the measured range in $\delta^{13}C$ values of the microbialites of -1.0 to +1.1 ‰ (i.e. significantly ^{13}C-depleted DIC coming from local groundwater would not produce the observed carbonate $\delta^{13}C$ values). Although there is no way to completely resolve these two potential contributions, the relatively small value of the observed

offsets and the potential sampling resolution contributions indicate that the primary source of carbon to the microbialite carbonates is surface water DIC.

ii. Samples Below 33 m

The situation is somewhat different for the two deepest samples. Here the offset from the surface water DIC is greater, 360 ‰ at 33 m and 610 ‰ at 45 m. Due to the well-mixed nature of the lake with respect to $\Delta^{14}C$ DIC, these increased offsets either indicate that these samples are growing significantly more slowly than the samples in the zone above 29 m or alternatively, they indicate that there are much greater localized inputs of regional groundwater DIC to these systems of 38 and 64 % respectively. Although there is no way to precisely differentiate these possibilities, these greater ^{14}C offset occur at depths where summer light levels in Pavilion decrease to 1 % of surface photosynthetically active radiation (PAR) at depths below 32 m (Figure 2.4). Light limitation has been demonstrated to affect photosynthetic activity in microbial mats under experimental conditions (Garcia-Pichel *et al.,* 1994 ; Bebout & Garcia-Pichel, 1995). The observed decrease in light levels observed below 32 m at Pavilion suggests that photosynthetic activity may be decreased at these depths. If this process is driving the formation of the microbialites as originally hypothesized, their growth rates below this depth would be expected to be lower. Such a decrease in growth rate could account for the increased offsets in $\Delta^{14}C$ of the microbialites.

2.4.3. Determination of Microbialite Growth Rate

Using measured $\Delta^{14}C$ values of microbialite growing over a detrital branch (Figure 2.2), a minimum constant growth rate of 0.05 mm per year was determined for the last thousand years. Two separate approaches to estimate the microbialite growth rate yielded the same result for this sample. The first estimate used the age of the branch as the starting point for growth. The branch was dated to be 1,230 ± 55 years old. Since there was six centimeters of microbialite crust covering the branch, assuming a constant growth rate gives an estimate of 0.05 mm per year. The second estimate used the difference in age between the inner and outer carbonate samples from the microbialite covering the branch. The difference in ages for the two carbonate samples was 830 years. Since the samples were taken four centimeters apart, this converts to a constant growth rate of 0.05 mm per year, consistent with the estimate based on the age of the branch. Thus, notwithstanding the inputs of ^{14}C-depleted carbon that must have occurred to make the $\Delta^{14}C$ of these carbonates more depleted than the branch, the two growth rate estimates are consistent.

2.4.3.1. Variation in Microbialite Growth Rates

This growth rate estimate of 0.05 mm per year is faster than the previous Pavilion estimate of 0.025 – 0.030 mm per year (Laval *et al.*, 2000). But compared to calculated growth rates for modern stromatolites from Belize that have been estimated to have an average accretion rate ranging from 0.25 mm per year to 0.65 mm per year (Rasmussen *et al.*, 1993) this growth rate is relatively slow.

However, it is clear that there are significant variations in microbialite growth rates within Pavilion Lake. This can be demonstrated by comparing the growth rate estimates to the time available for microbialite growth. Pavilion Lake was last glaciated approximately 10,000 years ago. At a growth rate of 0.025 to 0.05 mm per year, this would allow for the formation of microbialites ranging from 25 to 50 cm in height. However, there are numerous microbialites in the lake that exceed this size significantly. These microbialites must have grown at significantly faster rates than the samples that have currently been studied. Even for the sample in this study, the growth rate is a minimum rate assuming constant growth. Any reduction/interruption in growth would imply faster subsequent growth rates. These observations are consistent with previous studies that also suggest that variations in growth rate exist for modern stromatolites (Rasmussen *et al.*, 1993).

2.4.4. Implications to the Origins of the Pavilion Lake Microbialites

As stated, the primary question concerning the Pavilion Lake microbialites is whether the proposed hypothesis concerning their biological origin is indeed correct. The results of this study characterize the isotopic compositions of the carbon pools in this system and demonstrate minimal groundwater derived carbon inputs to the DIC, carbonate structures or associated microbial communities. Mixing at the groundwater/surface water interface resulting in carbonate supersaturation and precipitation is suggested to be the mechanism of formation for Mono Lake and other sites (Kempe *et al.*, 1991 ; Council & Bennett, 1993 ; Omelon *et al.*, 2001). Based on

the fact that groundwater DIC concentrations are higher than surface water DIC at Pavilion Lake (Lim *et al.,* 2009), it would be reasonable to expect that groundwater DIC would make up a large proportion of the precipitated carbonate if mixing and precipitation were the mechanism of formation. The lack of such an observation suggests that this process is not the primary mechanism of microbialite formation.

The fact that mixing at the groundwater/surface water interface does not appear to be the mechanism of formation of the microbialites is consistent with the original hypothesis of Laval et al. (2000) that biology is playing a role in microbialite formation. Further if the observed decreases in $\Delta^{14}C$ values below 32 m are due to slower growth rates, the fact that light levels at these depths fall below 1% PAR is consistent with a role of photosynthetic influences on precipitation.

2.5. CONCLUSIONS

In this study, ^{14}C and ^{13}C isotope analysis determined that the primary carbon source to the Pavilion Lake microbialites is atmospheric CO_2 via precipitation from ambient lake water DIC with relatively minor inputs from groundwater. Regional and/or local groundwater DIC inputs were constrained to a maximum of 9 –13 % of carbon input to ambient water DIC.

Microbial PLFA ^{14}C analysis shows that the microbial community is using ambient lake water DIC and there is minimal evidence of uptake of ^{14}C-depleted carbon consistent with groundwater inputs. These data support microbial use of ambient lake water DIC rather than DIC from upwelling groundwater.

Microbialite carbonate ^{14}C content also indicates that groundwater DIC makes a minor contribution to the carbon in the microbialite structures. Microbialites found in the range of 20 to 29 m show minimal inputs of groundwater derived carbon on the order of 10 to 16 %. Deep (> 32 m) microbialite samples showed greater ^{14}C depletion. While the precise extent of these contributions cannot be differentiated from the contributions of radioactive decay, the fact that carbonates associated with the detrital branch appear older than the branch itself indicate that some input of ^{14}C depleted carbon must be occurring.

The carbonate encrusted branch allowed for two independent estimates of a microbialite growth rate of 0.05 mm per year over the last ~ 1,200 years. This demonstrates that the microbialites are actively growing in the lake. However, variation in the size of the microbialites currently found within Pavilion Lake suggests that this growth rate is a conservative estimate and factors may have existed in the past that supported periods of more rapid growth needed to create structures of the large sizes observed at Pavilion.

The results of this study demonstrate that Pavilion Lake microbial communities on the surface of the microbialites are using DIC from the ambient lake water and that microbialites are not precipitating with groundwater inputs acting as the primary carbon source. These observations are consistent with proposed biological influences on carbonate precipitation and microbialite formation but do not rule out a role for abiotic processes in microbialite formation.

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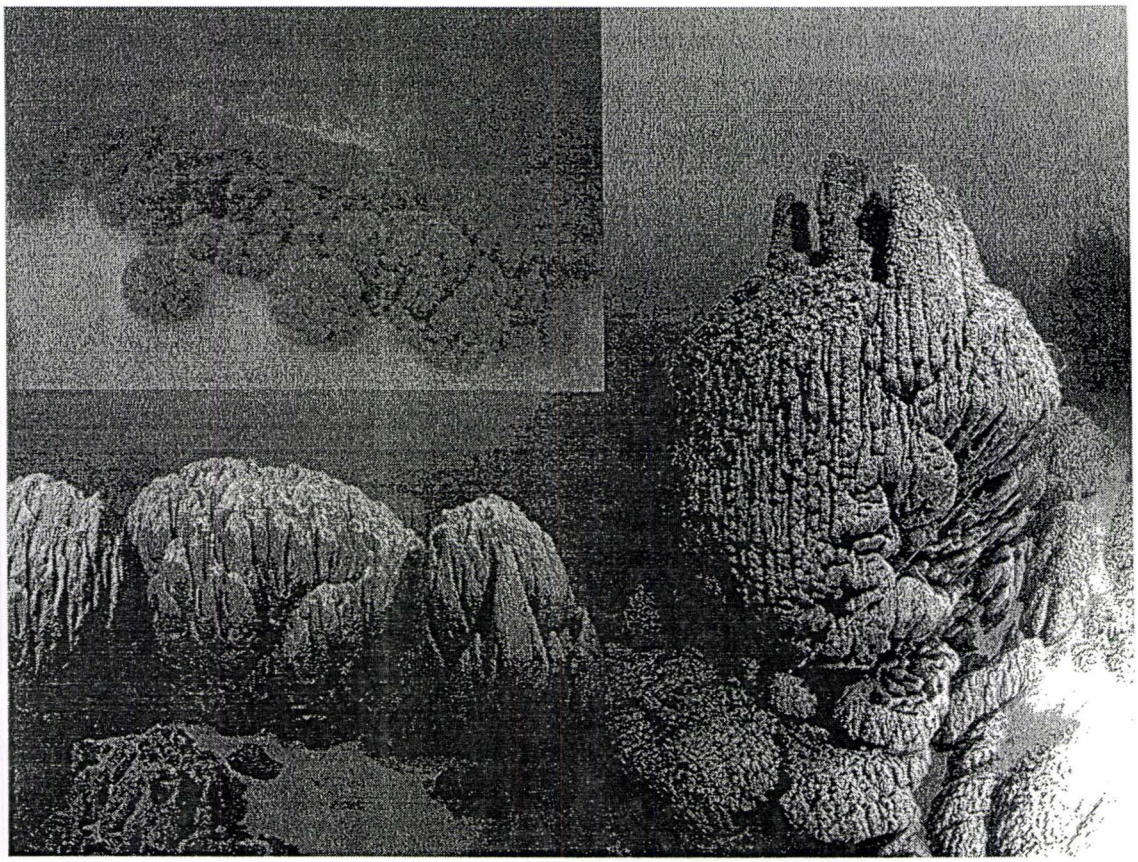

Figure 2.1: Examples of Pavilion Lake microbialites from varying depths. Images are courtesy of Donnie Reid.

Figure 2.2: Image showing carbonate growth over detrital branch from 21.5 m depth used for estimation of growth rate.

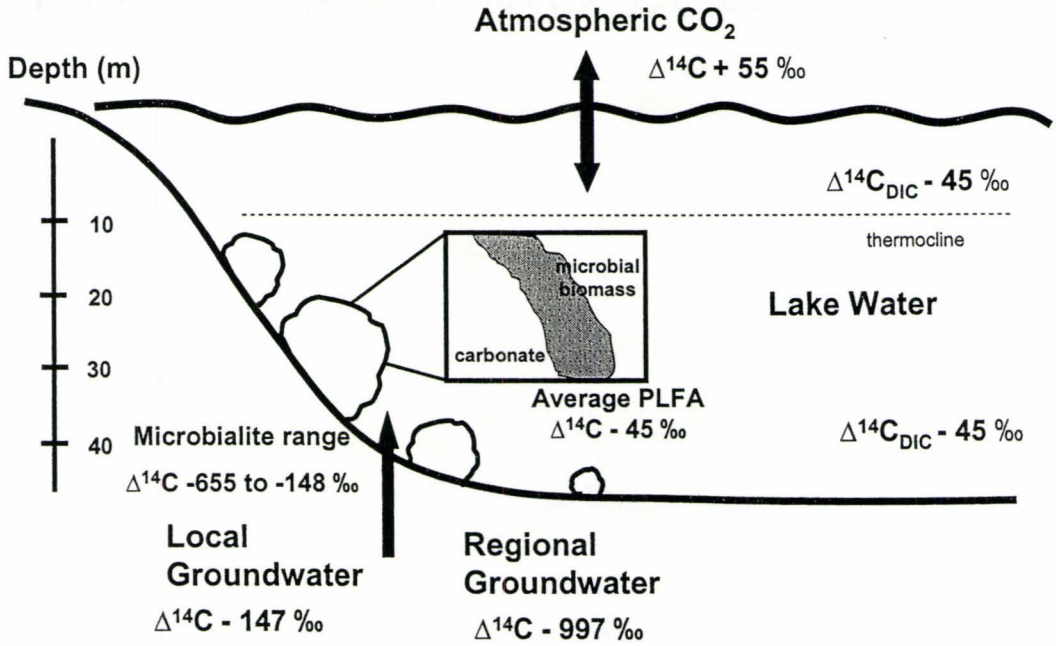

Figure 2.3: Schematic of Pavilion Lake illustrating example sampling locations and results of ^{14}C analysis for various DIC inputs, microbialite carbonate and microbial PLFA.

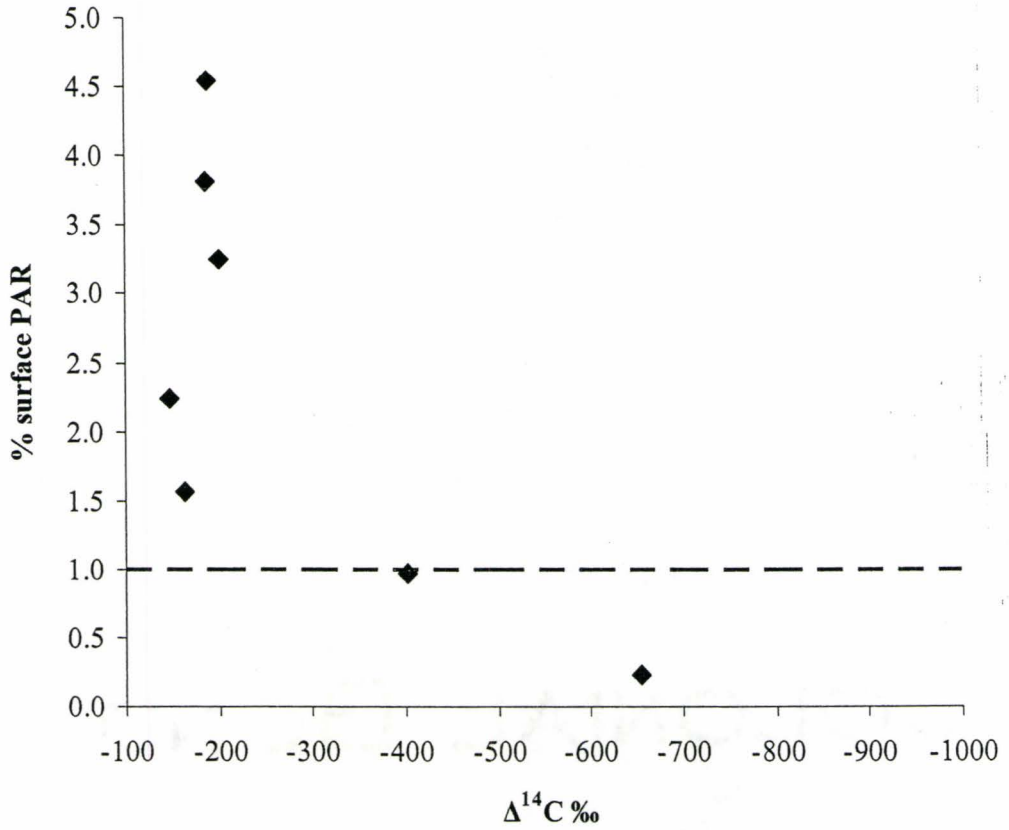

Figure 2.4: Graph of microbialite $\Delta^{14}C$ values and light levels as percent of surface PAR demonstrating a trend between decreasing light and ^{14}C content. The dashed line highlights the 1 % of surface PAR level that occurs at a depth of 32 m in August 2006.

Table 2.1: $\Delta^{14}C$ ‰ and $\delta^{13}C$ isotope values for water DIC samples, solid carbonate and organic wood.

Sample	Date Collected	$\Delta^{14}C$ ‰	Age[a]	Age Error	$\delta^{13}C$ ‰ PBD
Pavilion surface water (3 m) DIC	08/2006	-45	440	45	-1.9[b]
Pavilion deep water (59.5 m) DIC	08/2006	-45	440	45	-4.4[b]
Local groundwater DIC (drinking water well)	08/2006	-126	1000	25	-9.9[b]
Regional groundwater DIC (Soda Spring)	08/2006	-997	45300	1700	-1.2[b]
Pavilion air sample (CO_2)	08/2007	n/a	n/a	n/a	-9.6 ± 0.2, n = 4
PAV1 21.5 m detrital branch	08/2005	-148	1230	55	-20.8
PAV2 21.5 m "inner crust"	08/2005	-254	2360	65	0.6
PAV3 21.5 m "outer crust"	08/2005	-186	1530	40	1.1
PAV4 20 m $CaCO_3$	08/2005	-188	1560	50	0.4
PAV5 23 m $CaCO_3$	08/2005	-201	1710	50	-0.6
PAV6 26 m $CaCO_3$	08/2004	-148	1240	50	-1.0
PAV7 29 m $CaCO_3$	08/2006	-164	1360	20	0.1
PAV8 33 m $CaCO_3$	08/2006	-402	4650	100	0.6
PAV9 45 m $CaCO_3$	08/2005	-655	9550	30	0.0

[a] Calibrated age using OxCal (IntCal04)
[b] $\delta^{13}C$ value provided by G.G. Hatch

Table 2.2: Microbial PLFA detected in Pavilion Lake microbialite samples from 20, 23 and 26 m presented in mol %.

PLFA I.D.	PAV4 20 m	PAV5 23 m	PAV6 26 m
me13:0	0.9	0.7	1.6
14:0	2.8	3.6	5.2
15:1	2.5	0.9	3.1
i-15:0	2.3	3.1	2.2
a-15:0	3.8	3.9	2.2
15:0	0.8	0.7	1.5
me15:0	n.d.	n.d.	1.4
i-16:0	1.2	1.0	1.6
16:3	n.d.	n.d.	n.d.
16:2	0.8	0.9	1.9
16:1	14.8	15.8	16.5
16:0	18.4	20.8	17.9
10me16:0	1.2	1.5	1.3
me16:0	1.6	0.8	3.2
i-17:0	0.9	0.8	1.3
a-17:0	1.1	1.1	1.4
17:0 Δ	9.6	9.7	2.9
17:0	0.9	0.6	1.3
me17:0	1.2	n.d.	n.d.
18:2	4.5	5.2	7.5
18:1	19.4	21.0	17.6
18:0	1.9	1.7	1.5
me18:0	3.0	2.4	n.d.
19:0 Δ	2.4	2.7	1.5
19:1	n.d.	n.d.	1.3
20:4	1.6	0.8	1.2
20:3	0.6	n.d.	n.d.
20:1	1.3	n.d.	1.4
20:0	0.6	0.5	n.d.

n.d., not detected

Table 2.3: Results of microbial PLFA ^{14}C analysis with MeOH corrected $\Delta^{14}C$ ‰ values.

Sample (PLFA)	Date Collected	Corrected $\Delta^{14}C$ (‰)[a]
PAV PLFA1 20 m $C_{16:0}$	08/2005	-109
PAV PLFA2 23 m $C_{16:1}$	08/2005	-25
PAV PLFA3 23 m $C_{16:0}$	08/2005	-36
PAV PLFA4 23 m $C_{18\ unsats}$[b]	08/2005	-36
PAV PLFA5 23 m $C_{\Delta 17:0,\ \Delta 19:0}$	08/2005	-25
PAV PLFA6 26 m $C_{i\text{-}a15:0}$	08/2004	-61
PAV PLFA7 26 m $C_{16:1}$	08/2004	-21
PAV PLFA8 26 m $C_{\Delta 17:0,\ \Delta 19:0}$	08/2004	-50
PAV PLFA9 26 m $C_{18\ unsats}$[b]	08/2004	-42

[a]corrected for carbon added during methanolysis

[b]dominated by 18:1 with 18:2 a minor component (~ 5 %)

Table 2.4: Estimated inputs of regional groundwater carbon to microbialite carbonate as determined using $\Delta^{14}C$ values and mass balance.

Sample	$\Delta^{14}C$ ‰	input regional g.w. (%)
PAV3 21.5 m "outer crust"	-186	15
PAV4 20 m $CaCO_3$	-188	15
PAV5 23 m $CaCO_3$	-201	16
PAV6 26 m $CaCO_3$	-148	11
PAV7 29 m $CaCO_3$	-164	13
PAV8 33 m $CaCO_3$	-402	38
PAV9 45 m $CaCO_3$	-655	64

g.w., groundwater

CHAPTER 3

PHOTOSYNTHETIC ISOTOPE BIOSIGNATURES IN LAMINATED MICRO-STROMATOLITIC AND NON-LAMINATED NODULES ASSOCIATED WITH MODERN, FRESHWATER MICROBIALITES IN PAVILION LAKE, B.C.

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ABSTRACT

The influence of microbial activity on carbonate precipitation was investigated within micro-stromatolitic nodules associated with modern, freshwater microbialites located in Pavilion Lake, B.C. Observed carbonate $\delta^{13}C$ values enriched by up to +3.6 ‰ as compared to predicted abiotic carbonate $\delta^{13}C$ values from measured dissolved inorganic carbon (mean -1.2 ‰, n = 13) were consistent with microbial photosynthetic influence on in situ precipitation within the nodule microenvironment. Estimated carbonate precipitation temperatures within the nodules based on $\delta^{18}O$ were consistent with recorded summertime temperatures, indicative of precipitation during the period of highest levels of photosynthetic activity. Low $\delta^{13}C$ values of organic matter within the nodules (-30.6 to -21.1 ‰) and an average inorganic to organic carbon $\Delta\delta^{13}C$ value of 26.8 ‰ reflected the preferential uptake of ^{12}C during non-CO_2 limited photosynthesis, supporting the generation of ^{13}C-enriched DIC. Microelectrode profiles through the nodules showed oxygen supersaturation of up to ~ 275 %, elevated pH compared to ambient water and a lack of any observable sulphide further indicating photosynthetic activity was the predominant metabolic process within the nodule. Microbial phospholipid fatty acid profiles of the nodule communities were indicative of bacteria rather than eukaryotes and PLFA $\delta^{13}C$ values were depleted relative to the bulk cell by 2.6 – 6.6 ‰, consistent with a predominance of photosynthetic microbes. Scanning electron microscopy images of the relationship between carbonate minerals and filaments indicated that carbonate precipitation had occurred in situ due to microbial influences on the geochemistry within the nodule microenvironment rather than due to cell surface

effects or trapping and binding. The observation of photosynthetically induced ^{13}C-enrichment of in situ precipitated carbonate within the nodule microenvironment is thus a biosignature of the activity of these surface communities and is consistent with the hypothesized role of biology in the formation of microbialites.

3.1. INTRODUCTION

Resolving biological signatures (biosignatures) from abiotic signatures of carbonate formation remains an important component of interpretation of the early rock record and the debate concerning the timing of the rise of life (Awramik, 1992; Kempe et al., 1991; Lowe, 1994; Merz-Preiß and Riding, 1999; Walter, 1996). Differentiating between biotic and abiotic processes of carbonate formation on the basis of mineralogical and morphological data remains challenging and undoubtedly some systems comprise both biotic and abiotic mechanisms (Chafetz and Guidry, 1999). Putative microfossils and stromatolites have been cited as evidence for early life (Schopf, 1993; Tyler and Barghoorn, 1954), however clear evidence for the biologic origin of such fossils is debated (Brasier et al., 2002; Grotzinger and Rothman, 1996). Stromatolites are laminated lithified structures that are considered amongst the earliest purported evidence for life on Earth (see Riding, 2000 for overview). 'Microbialite' is a more general term used to describe organo-sedimentary structures, including stromatolites, formed through the trapping and binding of sediment and/or calcification of microbes resulting in a layered fabric (Burne and Moore, 1987). The three main hypotheses regarding biogenic mechanisms of microbialite formation are; 1) trapping and binding of sediment by

microbial communities (Burne and Moore, 1987; Reid et al., 2000); 2) microbial cell surfaces acting as nucleation sites for crystal growth (Bosak and Newman, 2003; Schultze-Lam et al., 1996) and/or 3) promotion of calcification via alteration of the local geochemical environment through metabolic activity (Merz-Preiß, 2000; Merz-Preiß and Riding, 1999; Stolz et al., 2001; Thompson and Ferris, 1990). Alternatively, abiotic processes resulting from changes in geochemical conditions and/or sedimentation patterns have also been proposed as mechanisms for the formation of microbialites (Council and Bennett, 1993; Grotzinger and Rothman, 1996; Walter, 1996).

Modern microbialites have been discovered in a number of different environments and locations around the world including Australia (Awramik and Riding, 1988; Logan, 1961), the Bahamas (Andres et al., 2006; Reid et al., 1995; Visscher et al., 1998), Mexico (Breitbart et al., 2009), Lake Van (Kempe et al., 1991) and Canada (Ferris et al., 1997; Laval et al., 2000). These modern microbialites have been suggested as analogues to ancient structures and opportunities to gain insight into mechanisms of their formation and identification of microbial biosignatures. The discovery of microbialites in association with photosynthetic microbial communities in freshwater Pavilion Lake, British Columbia, Canada (50°51' N, 121°44' W) presents one such opportunity. Our study focuses on understanding biological influences on microbialite formation and associated isotope biosignatures that have the potential to be preserved in the geologic record.

3.1.1. Microbial influences on carbonate precipitation and isotopic composition

If biology is playing a role in the formation of microbialite structures, it is often unclear whether this is through trapping and binding of externally precipitated material or through direct microbial influence causing in situ precipitation. Of the three proposed mechanisms of biological formation of microbialites, the potential for microbial metabolic activity to change local aqueous geochemistry and promote the precipitation of calcium carbonate minerals (Merz, 1992; Shiraishi et al., 2008; Shiraiwa et al., 1993) represents the greatest potential for the generation of a biosignature. These microbially induced changes in geochemistry, and any associated biosignatures, can be substantial enough to result in system-wide effects in lacustrine environments (Hollander and McKenzie, 1991; Thompson et al., 1997). Alternatively, microbial effects on geochemical conditions may be restricted to microenvironments associated with the cells, particularly if these cells are generating a distinct microenvironment, as occurs in microbial mats (Andres et al., 2006; de Beer et al., 1997; Jørgensen et al., 1983; Revsbech et al., 1983). Microbial influences on the concentration and isotopic composition of the dissolved inorganic carbon (DIC) and precipitated carbonate occur during both autotrophic and heterotrophic metabolisms. Both of these processes can generate biosignatures that may be incorporated into carbonate and preserved in the geologic record (Andres et al., 2006; Breitbart et al., 2009; Merz, 1992).

In most natural environments, the pH is high enough (~ 7 – 8) that most dissolved CO_2 is in the form of bicarbonate (HCO_3^-). During photosynthesis, HCO_3^- is taken into the cell, converted into CO_2 and OH^- by the enzyme carbonic anhydrase for use in the ribulose-1,5-bisphosphate carboxylase enzyme (RUBISCO) (Miller and Colman, 1980;

Paneth and O'Leary, 1985; Thompson and Ferris, 1990). The CO_2 is incorporated into the cell biomass while the OH^- is expelled, resulting in an increased pH in the microenvironment around the cell and therefore a shift towards a higher CO_3^{2-} concentration. This shift can result in a corresponding increase in the calcium carbonate saturation index (SI) leading to precipitation (Ludwig et al., 2005; Merz, 1992; Revsbech et al., 1983; Schultze-Lam et al., 1992; Thompson and Ferris, 1990).

Heterotrophic metabolic activity may also have significant but contrasting influence on carbonate precipitation. Sulfate-reducing bacteria (SRB) oxidize ^{13}C-depleted organic matter to hydrogen sulphide and CO_2. Input of this CO_2 into the medium leads to an increase in total DIC and an increase in the SI (Altermann et al., 2006; Baumgartner et al., 2006; Visscher et al., 2000). Other heterotrophic activity may have similar effects on ion concentration during oxidation of organic matter. Furthermore, Ca^{2+} and Mg^{2+} ions stored in cyanobacteria extracellular polymeric substance (EPS) are released during decomposition, increasing the SI and promoting precipitation of $CaCO_3$ (Altermann et al., 2006; Paerl et al., 2001).

Either of the microbial influences above has the potential to induce deviations in measured $\delta^{13}C$ carbonate values from the predicted equilibrium values thereby providing a biosignature. The direction of these deviations indicate autotrophic or heterotrophic dominant processes (Ferris et al., 1997; Hodell et al., 1998; McConnaughey, 1989; McConnaughey et al., 1997; Merz, 1992; Thompson et al., 1997). Biological preference for ^{12}C during photosynthesis leads to incorporation of the lighter isotope into cell biomass and a corresponding enrichment in ^{13}C of the residual DIC (O'Leary, 1988). In

contrast, heterotrophic activity results in an input of ^{13}C-depleted CO_2 from degradation of organic matter, leading to a ^{13}C-depletion in the residual DIC. When carbonate precipitates from DIC that has been affected by either of these processes, the precipitated carbonates record the $\delta^{13}C$ value, including any microbial effects on the isotopic composition (Burne and Moore, 1987; Guo et al., 1996; Sumner, 2001; Thompson et al., 1997). Carbonates enriched in ^{13}C have been reported from a variety of environments including saline ponds, shallow lakes with high methane production, hot spring travertines (Guo et al., 1996; Valero-Garcés et al., 1999), and small carbonate precipitates from freshwater lakes with high levels of photosynthetic activity (Hollander and McKenzie, 1991; Thompson et al., 1997). Carbonate with $\delta^{13}C$ values lower than expected for equilibrium precipitation has been used in recent studies to infer influence of heterotrophic metabolisms on carbonate precipitation in freshwater microbialites from Cuatro Ciénegas, Mexico (Breitbart *et al.*, 2009) and in modern marine microbialites from Highborne Cay, Bahamas (Andres et al., 2006).

In addition to the isotopic composition of the inorganic component, examination of the isotopic composition of the organic material associated with microbialites provides insight into dominant microbial metabolic processes. The isotopic composition of microbial phospholipid fatty acids (PLFA) offers insight into the metabolisms of the active in situ microbial community in an environmental system (Abraham et al., 1998; Sakata et al., 1997). Phospholipids are membrane components that are known to degrade rapidly, within days to weeks, upon death (White et al., 1979) therefore they represent the viable microbial community at a site. Further, specific PLFA have been shown to be

linked to certain microbial groups and may be used to identifying changes in microbial community (Green and Scow, 2000; Rajendran et al., 1995; Vestal and White, 1989; Zelles, 1999). The PLFA profiles and associated isotopic signatures provide additional information about the activity of the dominant microbial community present within the nodules. Biosynthesis of PLFA in heterotrophic microbes leads to PLFA with $\delta^{13}C$ values that are depleted relative to the bulk cell biomass by 3 – 4 ‰ (Blair et al. 1985; Monson and Hayes, 1982) while cyanobacteria lipid $\delta^{13}C$ values are depleted relative to bulk biomass by 7 – 9 ‰ (Sakata et al., 1997).

This study employs stable isotope analysis of laminated micro-stromatolitic and non-laminated nodules located on the surface of Pavilion Lake microbialites in combination with geochemical analysis and imaging of associated microbial communities to investigate the potential for identifying photosynthetically influenced carbonate precipitation.

3.2. SAMPLING AND ANALYTICAL METHODS

3.2.1. Study Site

Pavilion Lake is located in south-central British Columbia, Canada approximately 450 km North-East of Vancouver at an altitude of 823 m above sea level. It is a small (5.7 km x 0.8 km and 65 m deep) freshwater, ultra-oligotrophic lake with a pH of ~ 8.3 and hosts microbialites ranging from several centimetres to meters in height with varying morphologies (Laval et al., 2000; Lim et al., 2009). The microbialites are estimated to be younger than 12,000 years based on uranium series dating and their location above silts

that bury postglacial clastic sediments (Laval et al., 2000). Photosynthetic cyanobacteria such as *Synechococcus* sp. and *Oscillatoria* sp. are known to be associated with these freshwater microbialites (Laval et al., 2000).

3.2.2. Microbialite and water chemistry collection and characterization

Sampling of microbialites was performed along transects running perpendicular to the shoreline from 7 to 24 m depth as described further in (Lim et al., 2009). Microbialite samples were collected along these transects by SCUBA divers during field seasons conducted two to four times a year from spring to autumn between 2006 and 2008. Microbialite pieces were frozen on-site and transported to McMaster University on dry ice for further analysis. Green laminated micro-stromatolitic and pink/purple non-laminated nodules recovered from the surface of microbialites are the focus of the current study (Figure 3.1). Selected samples for investigation using scanning electron microscopy (SEM) were preserved in a 2.5 % glutaraldehyde solution with 0.45 μm pore size filtered lake water.

Water samples for ^{13}C analysis were collected in crimp sealed glass serum bottles with no headspace and fixed with mercuric chloride to prevent further microbial activity. Water samples for ^{18}O analysis were also collected in crimp sealed glass serum bottles with no headspace.

In situ water temperature was measured along the aforementioned transects 10 cm above the lake bed at nominal water depths of 10 m and 18 m (lake level varies by about 1 m over seasonal time scales) using SCUBA diver deployed Onset WaterTempPro v2

water temperature data loggers (resolution and calibrated accuracy 0.2°C). The sampling interval was 30 minutes.

3.2.3. Phospholipid fatty acid analysis of microbialite nodules

Due to the small size/mass of the individual nodules, multiple green nodules were combined into a representative sample from each of the two field seasons in which sufficient sample was collected for PLFA analysis (April 2006 and April 2008). Microbial PLFA were extracted from micro-stromatolitic nodules according to a modified Bligh and Dyer method (Bligh & Dyer, 1959) and purified using silica gel chromatography to separate lipids into non-polar, neutral and polar fractions. Phospholipids recovered from the polar fraction were subjected to a mild alkaline methanolysis and converted to fatty acid methyl esters (FAMEs) (Guckert et al., 1985). Microbial FAMEs were separated using gas chromatography mass spectrometry (GC/MS) on an Agilent GC-MS (Agilent Technologies Inc., Santa Clara, California, USA) with DB-XLB capillary column (30 m x 0.32 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). Identification of PLFA were made based on the retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc., Pleasant Gap, Pennsylvania, USA). PLFA are named according to the number of carbon atoms present and the number of double bonds.

3.2.4. Stable isotope analysis

DIC isotopic composition was determined by acidification and conversion to CO_2 analyzed by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa (St-Jean, 2003). All $\delta^{13}C_{DIC}$ values are reported in standard delta notation in reference to PeeDee Belemnite (PDB). Water oxygen values were determined by CO_2-water equilibration at 25°C prior to analysis on a Gasbench and Finnigan MAT Deta Delta^{Plus} XP. $\delta^{18}O$ values are reported in standard delta notation in reference to Vienna Standard Mean Ocean Water (VSMOW).

Surface nodules were removed from microbialite samples using solvent rinsed tweezers. 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 gave precisions of less ± 0.7 ‰ (1 σ) for $\delta^{13}C_{carb}$ and ± 0.2 ‰ (1 σ) for $\delta^{18}O$ analyses. Samples for bulk organic carbon analysis were dried and treated with 1 M HCl to remove carbonate. Bulk organic isotopic analyses were conducted on an EA-Delta XL at McMaster University. Triplicate analyses gave a precision of less ± 1.2 ‰ (1 σ) for $\delta^{13}C_{org}$ values of all nodules. All carbonate $\delta^{13}C$ and $\delta^{18}O$ values are reported in standard delta notation relative to PeeDee Belemnite (PDB).

Aliquots of microbial FAMEs were injected into a split/splitless injector set to splitless mode at 300°C prior to separation using gas chromatography mass spectrometry (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 combusted to CO_2 as they eluted

from the column via a combustion oven set at 960°C. Evolved CO_2 was analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS).

The methanol used during methanolysis was characterized for ^{13}C and FAME $\delta^{13}C$ values were corrected for the added methyl carbon via the relationship:

$$\delta^{13}C_{FAME} = [(N+1)* \delta^{13}C_{meeasured} - \delta^{13}C_{MeOH}]/N$$

where N is the number of carbon atoms. All $\delta^{13}C$ values are reported in standard delta notation relative to PeeDee Belemnite (PDB). Individual samples were analyzed in triplicate and data are reported as mean ± one standard deviation (s.d.). Precision on triplicate $\delta^{13}C_{PLFA}$ values for individual PLFA was less than ± 0.7 ‰.

3.2.5. Voltammetry

Microsensor measurements were conducted on freshly collected nodules and on nodules in situ. Measurements on freshly collected nodules incubated in Pavilion lake water were performed using glass Au-Hg amalgam electrodes (with tips drawn to ~500 micron diameter) constructed in the lab according to methods published in Brendel and Luther (1995) and lowered vertically in 1-5 mm increments using a micromanipulator. A sequence of ten cyclic voltammograms (-0.1 to -1.8 V vs. Ag/AgCl at 1V/sec with 2 second deposition at -0.1 V) was obtained from each electrode at each depth using DLK-60 (AIS Instruments) software. The current response for signals of the last 5 scans of each sequence were measured and averaged (AIS Instruments DLK-60 Analysis program). The instrumental variability between measurements is extremely small (typically less than 1 %). Oxygen peaks exist at -1.3V and -0.3V (O_2 and H_2O_2), Mn^{2+} at

-1.6V, Fe(II) at -1.4V, Fe(III) at -0.6 V, and HS^- at -0.8 V. The electrodes were calibrated using 2-point O_2 calibrations (air-saturated and N_2 purged), standard additions of freshly prepared Na_2S*9H_2O, and standard additions of MnCl to N_2 purged water, with calibration for other ions, relative to Mn^{2+}, accomplished using the pilot ion method (Brendel and Luther, 1995). In situ oxygen and pH measurements were conducted on green and purple nodules at depths of 17 – 19 m using a Unisense oxygen and pH electrode connected to a Unisense underwater picoammeter/mV meter. Electrodes were 100 μm in diameter and lowered in 200 μm steps via a micromanipulator fixed to a stand. The pH electrode had an external reference electrode. Profiling was performed from the bulk solution into and through any boundary layer that may have existed at the surface of the nodules. Placement of the microelectrode above the nodular surface was aided by visual observation. Green nodules were penetrated to a maximum depth of 3 mm below the surface of the nodule, crossing laminations present within these samples while the purple nodules were penetrated to a maximum depth of 5 mm. In all cases, great care was taken to minimize disruption to the boundary layer.

3.2.6. Scanning electron microscopy

To examine the internal structure of the microbialites, subsamples preserved in 2.5 % glutaraldehyde were prepared following the procedure of Omelon et al. (2006). Specifically, nodule were sectioned vertically using sterile razor blades and washed in 0.1 M sodium cacodylate buffer (pH 7.3), postfix stained in 0.1% osmium tetroxide/0.1 M sodium cacodylate buffer, washed in 0.1 M sodium cacodylate buffer, dehydrated

through a graded alcohol series (70 %, 90 %, 95 %, 100 % ETOH), and embedding in LR White acrylic resin in 1" diameter plastic moulds. These were subsequently ground, polished, and platinum coated. All samples were viewed on a LEO (Zeiss) 1540XB scanning electron microscope equipped with both quadrant back scattering and X-ray spectroscopic (EDS) detectors.

3.3. RESULTS

The nodules present on the surface of Pavilion Lake microbialites were dominated by filamentous bacterial communities that ranged in size (up to 1 cm in diameter) and were either green or pink/purple in colour (Figure 3.1b). Sectioning of the green spherical nodules revealed internal laminations of dark green organic-rich bands with lighter coloured carbonate rich bands of up to 1 mm thickness that were observable with the naked eye (Figure 3.1c). The purple nodules had a less organized internal structure composed of microbial filaments and randomly distributed carbonate precipitates (Figure 3.1d).

3.3.1. Isotopic composition of DIC and nodule carbonate

DIC $\delta^{13}C$ and H_2O $\delta^{18}O$ values for Pavilion Lake surface water samples that were collected between 2005 and 2008 are presented in Table 3.1. DIC $\delta^{13}C$ values average -1.2 ± 1.3 ‰ (n = 13) and water $\delta^{18}O$ values average -11.1 ± 0.2 ‰ (n = 10). Pavilion Lake has a mean pH of 8.3 that remains stable on a yearly basis and throughout the water column and results in bicarbonate (HCO_3^-) being the dominant DIC species (Lim et al.,

2009). Based upon the fractionation factor of Mook et al., (1974) and the measured $\delta^{13}C$ of atmospheric CO_2 at Pavilion Lake ($\delta^{13}C$ = -9.6 ± 0.2 ‰), a temperature range of 0 – 20°C would result in equilibrium DIC $\delta^{13}C$ values ranging from +1.1 ‰ to -1.3 ‰. DIC $\delta^{13}C$ values average -1.2 ± 1.3 ‰ (n = 13) and generally fall within this predicted seasonal range confirming that the lake DIC is consistently at or near isotopic equilibrium with atmospheric CO_2, in agreement with previous results based on ^{14}C analysis (Brady et al., 2009). Previous studies have shown that calcite is enriched in ^{13}C by +1.0 ± 0.2 ‰ above the bicarbonate from which it precipitates (Romanek et al., 1992). Thus, abiotic precipitation of microbialites would result in carbonate $\delta^{13}C$ values generally within 1 ‰ of the bulk measured DIC $\delta^{13}C$ values. Based on this, the average predicted carbonate equilibrium $\delta^{13}C$ value from the average measured Pavilion Lake DIC is -0.2 ± 1.3 ‰.

Nodule carbonate $\delta^{13}C$ and $\delta^{18}O$ values are listed in Table 3.2. The measured $\delta^{13}C$ values from green micro-stromatolitic and purple non-laminated nodules range from +1.1 to +3.4 ‰ (mean +2.3 ± 0.5 ‰, n = 27), enriched above the prediction based on equilibrium precipitation (Figure 3.2). $\delta^{18}O$ values ranged from -10.7 to -9.2 ‰ (mean -9.5 ± 0.3 ‰, n = 27). No correlation was observed between carbonate $\delta^{13}C$ and $\delta^{18}O$ values for either the green (R^2 = 0.13) or purple nodules (R^2 = 0.00) (Figure 3.3).

3.3.2. Organic matter carbon isotope composition of microbial communities

Bulk organic $\delta^{13}C$ values of the micro-stromatolitic nodules are listed in Table 3.2. All of the nodule samples combined have an average $\delta^{13}C$ value of -28.0 ± 2.1 ‰ (n = 19) with a range of -30.6 to -21.1 ‰. The green nodules averaged -27.5 ± 2.0 ‰ and the

purple nodules had an average $\delta^{13}C$ value of -30.0 ± 1.1 ‰. The difference between these means was statistically significant (Mann-Whitney Rank Sum test, $p = 0.011$, $\alpha = 0.05$). The average offset between mean inorganic (DIC) and organic $\delta^{13}C$ values ($\Delta\delta^{13}C$) for all nodule samples is -26.8 ± 1.1 ‰ (n = 21). For green nodules the average offset was -26.3 ± 2.0 ‰ (n = 15) and the purple nodules had a mean offset of -28.8 ± 1.1 ‰ (n = 4).

3.3.3. Microbial phospholipid fatty acid analysis

The microbial PLFA composition of the micro-stromatolitic nodule communities are listed in Table 3.3. Saturated straight chain PLFA ranged from C_{14} to C_{18}, comprised 27 – 36 % of the total with the most abundant, C_{16} comprising 28.1 and 22.5 % in April 2006 and April 2008. Monounsaturated PLFA comprised the majority of the total PLFAs present in both samples (total > 45 %) with $C_{16:1}$ and $C_{18:1}$ as the major components detected in the two samples (10.7 – 19.8 % and 31.4 – 34.6 % respectively). Branched chain saturated PLFA were also present, specifically *iso*- and *anteiso*-$C_{15:0}$ that represented on average 1.8 and 1.9 % respectively of the total PLFA. *Iso*- and *anteiso*-$C_{17:0}$ PLFA were also observed in the nodule sample from April 2008 (< 2 %). Cyclopropyl PLFA $C_{17:0\Delta}$ was also detected in small amounts (1.5 – 1.6 %) in both samples. Polyunsatured PLFA $C_{18:3}$ was identified in the April 2006 sample at 4.1 mol % but was not detected in the April 2008 sample.

3.3.4. Microbial phospholipid fatty acid carbon isotope composition

The small nature of the nodules necessitated the combination of multiple nodules to have sufficient material for GC/MS and $\delta^{13}C$ analysis of individual PLFA. Bulk $\delta^{13}C_{org}$ values of individual nodules were consistent, indicating that combined nodules would give a representative PLFA profile. PLFA $\delta^{13}C$ values from nodules collected in April 2006 and April 2008 are listed in Table 3.3. The $\delta^{13}C$ values from April 2006 ranged from -34.0 to -31.5 ‰ and from April 2008 ranged from -35.0 to -31.4 ‰. PLFA were depleted relative to the mean bulk organic $\delta^{13}C$ values by on average -5.0 and -4.9 ‰ respectively. The largest offsets are seen in saturated straight chain PLFA (C_{14} and C_{16}) with values ranging from -5.9 to -6.6 ‰. Branched-chain PLFA *iso*- and *anteiso*-$C_{15:0}$ from the April 2008 sample were depleted relative to the average April 2008 bulk organic $\delta^{13}C$ value of -28.0 ‰ by -4.0 ‰.

3.3.5. Voltammetry

Microelectrode profiling of oxygen, sulphide and pH were used to characterize the chemical composition of the boundary layer and within the nodular structures (Figure 3.4). In the green nodules, oxygen showed an increase in concentration as compared to the bulk solution, reaching the maximum concentration observed in all nodules of 35.2 mg/L (275 % saturation) within the region of strongest green/white banding in the initial 1.5 – 2 mm from the surface of the nodule. O_2 concentrations generally began to decline at depths of 2 mm within the green nodules reaching a low of 7.1 mg/L or 78 % saturation at 3 mm in one nodule. Within the purple nodules, oxygen concentrations were at saturation or supersaturated through the nodule with one exception. Generally, oxygen increased from an average saturation of 14.2 mg/L (112 % saturation) at the

nodule surface to an observed maximum of 34.2 mg/L (267 % saturation) at a depth of 2.8 mm. However, one purple nodule showed a maximum oxygen level of 100 % saturation at a depth of 1 mm from the surface with the concentration declining to 0 % at a depth of 3.5 mm (Figure 3.4a). Differences in oxygen concentrations between nodules profiled on the surface and in situ likely resulted from differences in temperature, and corresponding oxygen solubility, or ambient light levels during profiling. No sulphide was detected in either the green or purple nodules.

The average pH value for the ambient water at a maximum distance of 3 mm from the surface of the nodules was 8.9 (n = 3), slightly higher than previously measured values from Pavilion (Lim et al., 2009) but possibly resulting from photosynthetic influences within the nodules extending from the nodule surface into the boundary layer (Jørgensen and Des Marais, 1990). All nodules showed an increase in pH with increased depth from the surface of the nodule (Figure 3.4b). The purple nodules reached a peak of 9.5 at a depth of 1.5 – 2 mm from the surface with one sample reaching 9.6 and the green nodule reached a maximum of 9.5 at a depth of 1.8 mm.

3.3.6. Scanning electron microscopy

SEM examination of the internal structure of the green nodules showed filamentous cyanobacteria oriented perpendicular to the microbialite surface with carbonate aggregates present within this biomass as laminated bands (Figure 3.5a). Purple nodules also had filamentous cyanobacteria oriented perpendicular to the microbialite surface but carbonate precipitates were not organized in distinct laminations

(Figure 3.5b). Cyanobacteria filaments were embedded in the carbonate matrix and within the green nodules crossed through the carbonate laminations, maintaining a vertical growth orientation. The carbonate present within both the green and purple nodules showed no evidence for precipitation within cyanobacteria sheaths or in direct association with cell surfaces, but rather within the surrounding EPS matrix. Structures within these carbonates include void spaces of similar shape and morphology to extant microbes, as well as secondary infilling of void spaces within the mineral matrix (Figure 3.6).

3.4. DISCUSSION

3.4.1. Carbonate isotope biosignatures

All of the nodules sampled from Pavilion Lake microbialites had $\delta^{13}C$ values enriched above the average predicted abiotic value of -0.2 ± 1.3 ‰ from bulk Pavilion Lake DIC (mean $\delta^{13}C$ = -1.2 ± 1.3 ‰ (n = 13) over 4 years) with values ranging from +1.1 to 3.4 ‰ (+1.3 to 3.6 ‰ above expected) (Figure 3.2). These findings are consistent with shifts in carbonate isotopic composition typically on the order of 2 – 5 ‰ induced by microbial photosynthetic activity (Guo et al., 1996; Merz, 1992; Thompson et al., 1997) and are similar to, albeit lower than, enrichments of 4.6 – 5.2 ‰ observed in small stromatolites and thrombolites recovered in nearby Kelly Lake (Ferris et al., 1997). The observation of nodule carbonate with $\delta^{13}C$ values enriched over values predicted for precipitation from the bulk lakewater DIC was consistent with microbial metabolic influences on the geochemistry and isotopic composition of the DIC within the local zone

of influence (microenvironment) of the microbes, either surrounding the microbial cells or within the environment of the nodule as a whole (Beveridge, 1988; Schultze-Lam et al., 1992). It indicated that the rate of preferential ^{12}C utilization by microbial photosynthetic activity within the microenvironment of the nodule was sufficiently greater than the rate of exchange with the bulk water such that it resulted in a ^{13}C-enrichment of the DIC within this environment but did not affect the bulk lake water. This observed isotopic enrichment of the carbonate is therefore a biosignature of microbial photosynthetic activity affecting the local geochemistry within the nodule.

Cyanobacteria blooms and extensive photosynthetic activity in the water column are capable of resulting in precipitation of ^{13}C-enriched carbonate in whiting events such as those observed in Green Lake, NY (Thompson et al., 1997). These whiting events significantly affect water clarity and induced positive shifts in the Green Lake DIC $\delta^{13}C$ values of ~ 3 ‰ over the period of increased photosynthetic activity. The fact that over a period of four years Pavilion Lake $\delta^{13}C$ DIC fell within the range predicted for equilibrium with the atmosphere (predicted range of $\delta^{13}C$ = +1.1 to -1.3 see Table 3.1) and the lack of any observation of whiting events or other changes in water clarity by year round residents of the lake further indicate that the observed isotopic enrichments are confined to the microenvironment within the nodules.

Concurrent with this observation of photosynthetic effects on the nodule carbonate, nodule bulk organic $\delta^{13}C$ values were ^{13}C-depleted (mean $\delta^{13}C$ = -28.0 ± 2.1 ‰) compared to DIC, consistent with microbial photosynthesis and the uptake of ^{12}C. The discrimination between the average bulk Pavilion Lake DIC $\delta^{13}C$ value (-1.2 ± 1.3 ‰, n

= 13) and the mean bulk organic $\delta^{13}C$ value for all nodules was -26.8 ± 2.1 ‰ (n = 19) close to the maximum isotopic discrimination expected for C_3-photosynthesis under non-limiting CO_2 conditions (O'Leary, 1988). The purple nodules had a slightly greater offset than the green nodules, likely resulting from differences in the microbial community (Estep et al., 1978). Expression of this isotopic discrimination during DIC uptake from the local DIC pool within the nodule microenvironment explains a ^{13}C-enrichment in the DIC and therefore the observed enrichment of the carbonates.

The lack of correlation between micro-stromatolitic nodule carbonate $\delta^{13}C$ and $\delta^{18}O$ values ($R^2 = 0.13$ for green nodules and $R^2 = 0.00$ for purple nodules) (Figure 3.3) further supports that the observed ^{13}C-enrichment was due to microbial activity rather than physical processes such as evaporation. Microbial metabolic (photosynthetic) effects on carbon isotope values have been shown to have no significant influence on oxygen values (McConnaughey, 1989). In contrast, correlation between $\delta^{13}C$ and $\delta^{18}O$ values has been observed in previous studies where physical processes such as evaporation affect both isotope systems and can lead to ^{13}C- and ^{18}O-enrichment in precipitates (Andrews et al., 1993; Léveillé et al., 2007; Valero-Garcés et al., 1999). Therefore, a lack of correlation between $\delta^{13}C$ and $\delta^{18}O$ values can be used to infer biological influences on carbonate precipitation (Burne and Moore, 1987; Kremer et al., 2008; Lojen et al., 2004).

The $\delta^{18}O$ values of the nodule carbonates can also be used to estimate the temperature of formation as they are a function of the $\delta^{18}O$ value in the water and the temperature of precipitation (Epstein and Mayeda, 1953). Pavilion Lake water $\delta^{18}O$

values show minor variation over 3 years (-11.1 ± 0.2 ‰) suggesting that the majority of the observed variation within the carbonate $\delta^{18}O$ values is the result of temperature fluctuations during precipitation. Using the measured $\delta^{18}O$ values of the ambient water, and established temperature-dependent fractionation factors and equations (Kim and O'Neil, 1997), the $\delta^{18}O$ values of the carbonates within the nodules correspond to an estimated temperature range of 8.2 to 13.6°C (Figure 3.7). The estimated temperatures of formation were higher for the samples from 10 m than those from 18 m, consistent with the observed differences in annual mean water temperature at those depths (Figure 3.7). However, the estimated temperatures of formation were also generally higher than the recorded water temperatures at the time of sampling. For the 10 m samples, the estimated temperatures were consistent with water temperatures recorded in early summer (circa July) and late fall (October). For the 18 m samples, the estimated temperatures of formation were consistent with recorded water temperatures in late summer (circa August) to late fall (October – November). This observation suggests that carbonate precipitation is occurring during summer to late fall when photosynthetic rates are expected to be the highest.

The agreement between recorded water temperatures and estimated temperatures of formation changes when the effects of pH on $\delta^{18}O$ $CaCO_3$ were taken into account. DIC $\delta^{18}O$ values are known to decrease with increasing pH due to changes in the proportions of the carbonate species resulting in an increase in the proportion of relatively ^{18}O-depleted CO_3^{2-} (Zeebe, 1999). Such a decrease in $\delta^{18}O$ values would correspond to an increase in the estimated temperature of formation. Since our results indicate that

carbonate precipitation is taking place in a microenvironment within the nodule that has a measured increase in pH of up to 0.7 pH units, the estimated temperatures of formation were recalculated using the distribution of carbonate species and expected corresponding DIC $\delta^{18}O$ at this elevated pH. Estimates generated using the maximum observed pH increase of 0.7 units corresponded to estimated temperatures of formation that were an average of 3.6 ± 0.1°C lower than the estimates using bulk lake water at ambient pH values (Figure 3.7). These estimated temperatures of formation were now consistent with carbonate precipitation at temperatures recorded circa June and October at 10 m and the period circa May to October at 18 m depth. The implication of these estimates is that there appears to be a seasonal period of carbonate precipitation within the nodules that is consistent with summertime temperatures in Pavilion Lake. Since this is also the time when photosynthetic activity is expected to be highest (Fritsen and Priscu, 1998; McConnaughey et al., 1994), this is consistent with a photosynthetically driven precipitation process.

3.4.2. Photosynthetic activity within the nodules

The ^{13}C enrichment in the nodule carbonate indicated that biological influences on carbonate precipitation occurred within the microenvironment of the nodules. Within the nodules elevated oxygen (up to 275 % saturation) and pH levels that increased by 0.7 pH units as compared to the ambient water demonstrated that photosynthesis was the dominant microbial metabolism influencing the local geochemistry (Figure 3.4). Microbial photosynthetic activity resulting in elevated oxygen and pH values have been

observed in numerous saline microbial mats (Jørgensen et al., 1983; Ludwig et al., 2005; Revsbech et al., 1983; Vasconcelos et al., 2006) and mats from the North Sea (Stal et al., 1985).

PLFA analysis further supported photosynthetic activity within the nodules as the results indicated that nodular communities were dominated by prokaryotic microbes and eukaryotic organisms contribute little (below detection) of the community. PLFA profiles of the two green micro-stromatolic nodule samples contained biomarkers common to many microbial groups including *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, monoenoics and straight chain saturates ranging from C_{14} to C_{18} (Vestal and White, 1989). In particular, *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$ are characteristic for Gram-negative and Gram-positive bacteria but are less common in cyanobacteria indicating that some heterotrophic organisms are present that could be contributing to carbonate precipitation (Jahnke et al., 2004). Specific biomarkers for sulfate-reducing bacteria such as 10me16:0 were not observed suggesting that sulphate-reduction was not a dominant process contributing to carbonate precipitation (Londry et al., 2004). High concentrations of monoenoic PLFA are typically found in Gram-negative bacteria (Guckert et al., 1985; Vestal and White, 1989). However, cyanobacteria have likewise been shown to produce monoenoic fatty acids in significant quantities (Grimalt et al., 1992; Nichols and Wood, 1968) suggesting that they may have contributed to the significant proportion of these PLFA. Cyclopropyl PLFA have been shown to indicate aerobic bacteria (Parkes and Taylor, 1983) and could result from cyanobacteria or aerobic heterotrophs present in the nodules. Long chain and PLFA characteristic of eukaryotic organisms (Volkman et al., 1980) were not detected but

polyunsatured $C_{18:3}$ was detected within the April 2006 sample. Polyenoic PLFA are typically associated with eukaryotes but have been shown to be produced by cyanobacteria (Kenyon, 1972; Kenyon et al., 1972). These findings are consistent with previous characterizations of the Pavilion Lake microbialites that found surface communities composed of diatoms and cyanobacteria including *Synechococcus* sp., *Pseudoanabaena* sp. and purple pigmented *Oscillatoria* sp. in addition to heterotrophic organisms (Laval et al., 2000). The presence of heterotrophic organisms could be responsible for the decrease in oxygen observed within some of the nodules, however, no sulphide was detected in either nodule and PLFA profiles support the presence of phototrophic cyanobacteria with no evidence of sulphate-reducing bacteria within the nodules suggesting that this process was not contributing significantly to carbonate precipitation. Nonetheless, the absence of observable sulphide could have been due to profiles only being conducted during the day. Previous microelectrode characterizations of microbial mat systems found that oxygen dominated during the day when photosynthesis was active with no sulphide detection until the dark period when respiration dominated (Revsbech et al., 1983). However, if significant sulphate reduction was occurring during the night that promoted carbonate precipitation, the nodule carbonate $\delta^{13}C$ values would be expected to be lower than those predicted for abiotic precipitation from the DIC and this was not the case.

PLFA $\delta^{13}C$ values likewise supported the presence of a photosynthesis dominated community within the nodules with some evidence of heterotrophs that are using photosynthetically produced organic matter as their substrate. $\delta^{13}C$ values from both

April 2006 and April 2008 samples ranged from -34.0 to -31.5 ‰ and -35.0 to -31.4 ‰ respectively (Table 3.3). Saturated PLFA from the two samples, in particular $C_{14:0}$ and $C_{16:0}$ which are the most abundant are depleted relative to the bulk cell $\delta^{13}C$ value by 5.9 to 6.6 ‰, offsets of this size are more characteristic of cyanobacteria (Sakata et al., 1997). Other PLFA such as *iso*- and *anteiso*-$C_{15:0}$ that are indicative of heterotrophic microbes had smaller offset (~ 4 ‰) that are characteristic of heterotrophic metabolisms (Abraham et al., 1998; Blair et al., 1985).

As Pavilion Lake microbial communities were not solely dominated by cyanobacteria there was likely some heterotrophic input of ^{13}C-depleted carbon to the DIC within the nodules. However, as the isotopic biosignature within the carbonate nodules showed an overall enrichment, autotrophic photosynthetic activity was the dominant process influencing carbonate precipitation.

3.4.3. Evidence for in situ carbonate precipitation

Microbial photosynthetic influence on carbonate precipitation within the nodule microenvironment was further supported by SEM analysis that indicated carbonate build-up occurred via in situ precipitation rather than through processes of trapping and binding of allochthonous particles. Filaments in both the green and purple nodules showed an organized direction of growth that did not appear to be affected by the presence of carbonate precipitates (Figure 3.5). Within the nodules the filaments were present within the mineral matrix suggesting precipitation around individual filaments, a state unlikely to occur via trapping and binding but that would occur if precipitation occurred within the

nodule. Void spaces within the matrix of similar size and morphology to adjacent extant microbes is consistent with in situ precipitation and infilling of void spaces indicated active precipitation within the nodules (Figure 3.6). In situ carbonate precipitation could be the result of microbial influences on the local geochemical environment and/or microbial cells and associated EPS acting as nucleation sites for mineral growth. Cyanobacteria are known to produce the EPS for attachment and protection (Dittrich and Obst, 2004) and there is strong evidence in the literature for microbial cells acting as nucleation sites for heterogeneous precipitation of carbonate (Bosak and Newman, 2003; Chekroun et al., 2004; Thompson and Ferris, 1990). Carbonate precipitated on bacteria would have had the same isotopic composition as the ambient DIC if this were the sole mechanism however this was not the case within the nodules. Additionally, carbonate precipitates were not observed within the sheaths of cyanobacteria filaments, suggesting that direct surface contact with cells was not a prerequisite for carbonate formation in this environment.

In situ precipitation due to microbial influences on nodule microenvironment geochemistry is consistent with the lack of significant sedimentation within the lake. Although Pavilion Lake is saturated with respect to calcite, dolomite and aragonite (0.72, 1.39 and 0.57 respectively) (Lim et al. 2009), sedimentation rates within the lake were low compared to surrounding lakes with a maximum of 0.07 g of sediment (estimated to be ~ 2 – 5 % $CaCO_3$) collected from sediment traps after a period of one year (Lim et al., 2009). Given this low rate of sedimentation, it is unlikely that trapping and binding of carbonate precipitates is the dominant mechanism of accretion, however this mechanism

cannot be wholly discounted on this basis alone. In particular, the effect of benthic sediment dynamics and slope characteristics on microbialite development has yet to be explored.

3.5. CONCLUSIONS

Elevated carbonate $\delta^{13}C$ values within green and purple nodules present on the surface of Pavilion Lake microbialites represent biosignatures formed via photosynthetic influences on the geochemistry of the microenvironment within the nodules. High levels of oxygen saturation and elevated pH values within the nodules demonstrated that microbial photosynthetic activity influenced the geochemistry of the microenvironment without affecting the bulk lake water. Biological uptake of ^{12}C was recorded by the low $\delta^{13}C$ values of the bulk organic material and PLFA profiles and $\delta^{13}C$ values supported the dominance of photosynthetic activity within the nodules. The corresponding ^{13}C-enrichment of the residual DIC resulted in precipitation of carbonate within the nodules with $\delta^{13}C$ values on average 1 – 2 ‰ higher than expected for abiotic precipitation. The lack of correlation with carbonate $\delta^{18}O$ values further supported that the observed ^{13}C-enrichment was due to microbial activity rather than physical processes. Estimates of formation temperatures based on carbonate $\delta^{18}O$ values further supported summer as opposed to winter precipitation consistent with precipitation during the period of highest photosynthetic activity. SEM imaging showing cyanobacteria filaments entombed within the mineral matrix supports in situ carbonate precipitation and microbial influences on microenvironment geochemistry and isotopic composition. The observation of microbial influences on carbonate precipitation within the nodules suggests that microbes are in

some part playing a role in the precipitation of the carbonate in Pavilion Lake microbialites.

These findings represent the first evidence of photosynthetic influences on micro-environmental geochemistry and isotopic composition associated with large-scale modern freshwater microbialites. Results are consistent with a hypothesized role of biology in the formation of microbialites and the observed ^{13}C-enrichment in photosynthetically influenced carbonates represents a biosignature of microbial activity for recent carbonate precipitation on the surface of the microbialites. However, research is ongoing to determine the extent to which these surface processes are responsible for overall microbialite growth and the potential for the biosignatures of photosynthetic activity observed in this study to be preserved within the bulk structures. These findings give insight into potential formation mechanisms of modern microbialites and the recognition of potential biosignatures that may be preserved throughout geologic time increasing our understanding of microbialite genesis.

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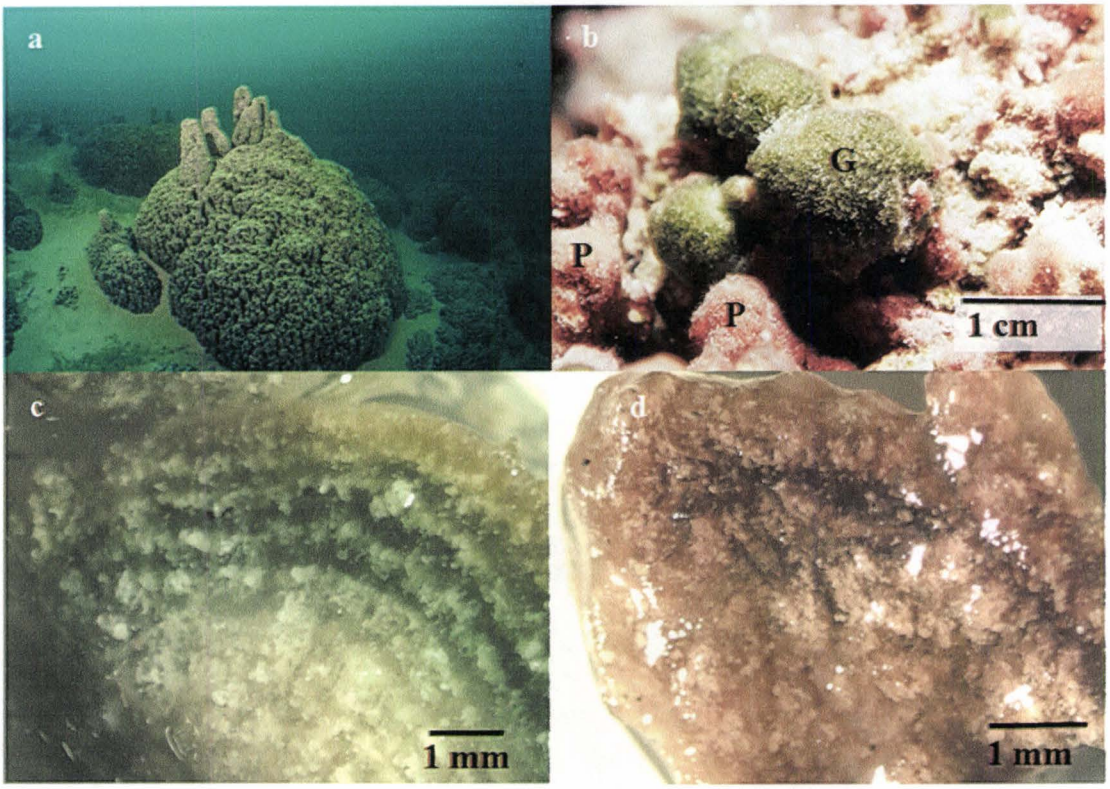

Figure 3.1: Representative images of the samples examined in this study. a) Pavilion Lake microbialite approximately 1 m in height (image courtesy D. Reid). b) Green (G) and purple (P) nodules on the surface of a microbialite showing range in size (image courtesy D. Reid). c) Cross-section of green nodule illustrating internal dark, green organic rich laminations of filamentous cyanobacteria alternating with light coloured carbonate rich lamination at a distance of approximately 1 mm apart. d) Cross-section of purple nodules showing random distribution of carbonate among filaments.

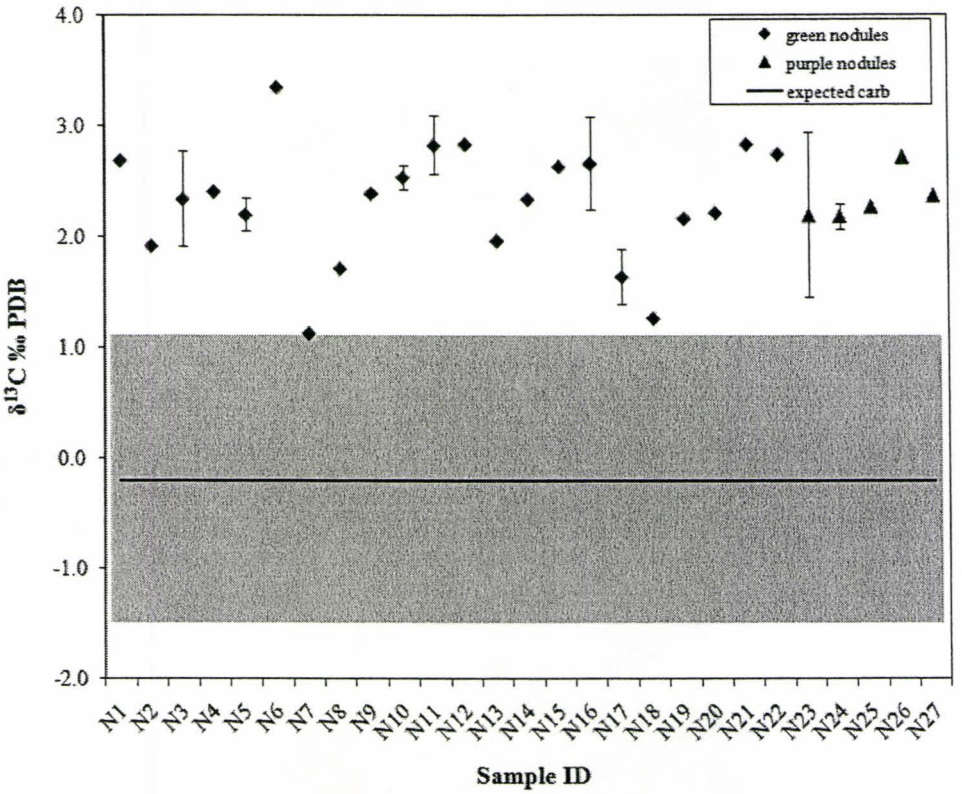

Figure 3.2: Relationship between carbonate $\delta^{13}C$ values of individual green (♦) and purple (▲) nodules to predicted carbonate $\delta^{13}C$ values precipitated under abiotic conditions with no biological influence from the mean DIC $\delta^{13}C$ value in Pavilion Lake. The shaded area illustrates the range in predicted abiotic precipitation $\delta^{13}C$ values. Green and purple nodules carbonate $\delta^{13}C$ are elevated above the range expected for abiotic precipitation

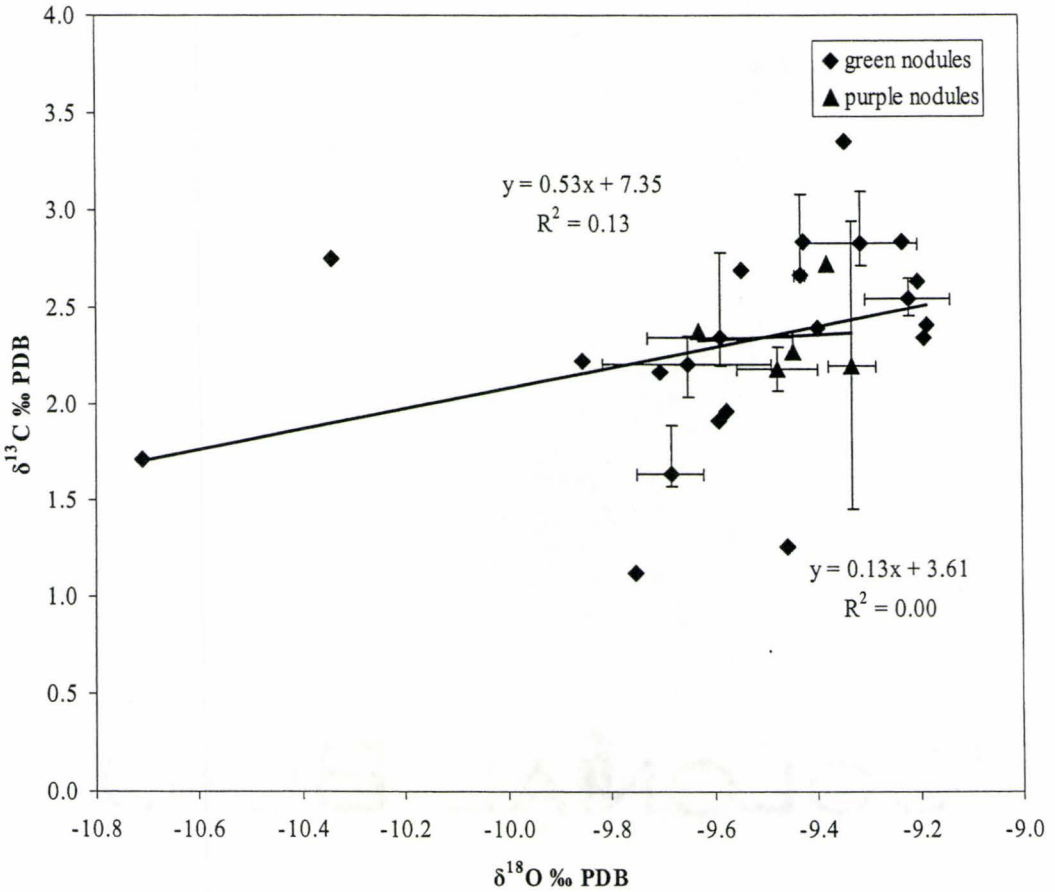

Figure 3.3: Carbon versus oxygen isotope composition for green (♦) and purple (▲) nodule carbonate. The lack of correlation between carbonate $\delta^{13}C$ and $\delta^{18}O$ values for green and purple nodules suggests no significant non-biological enrichment effects.

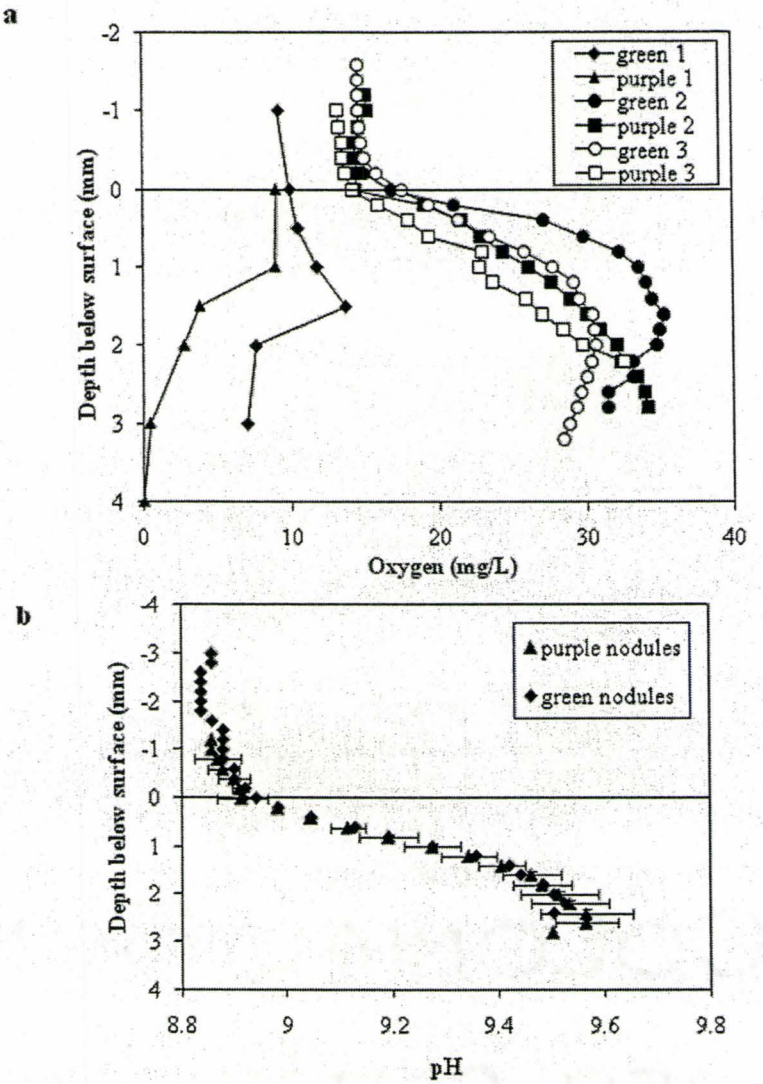

Figure 3.4: Oxygen and pH microelectrode profiles through green and purple nodules. a) Oxygen profiles demonstrating increased oxygen concentrations within the nodules, green 1 and purple 1 represent samples profiled on the surface in Pavilion Lake water while green 2,3 and purple 2,3 were profiled in situ. b) pH profiles demonstrating increased pH values within the nodules consistent with photosynthetic influences on the nodule geochemistry. Error bars represent one standard deviation on triplicate measurements in the purple nodules.

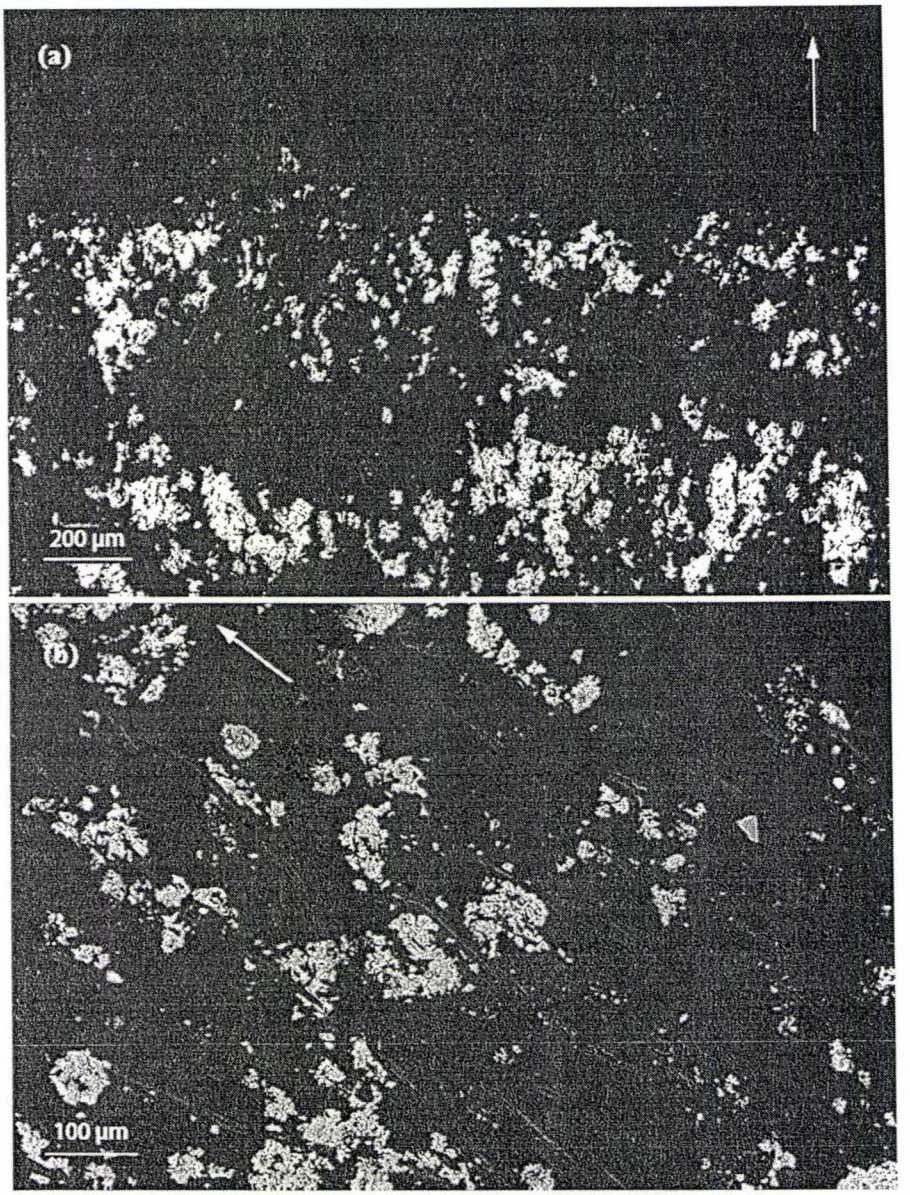

Figure 3.5: SEM back-scattered electron (BSE) micrographs of microbial nodules in cross section. (a) Green-pigmented nodule, with the outermost (exposed) surface of the nodule at top of image. Filamentous phototrophic bacteria grow outward from a central point or region, observed as cell trichomes with distinct organized orientation (see arrow). Note carbonates in horizons perpendicular to microbial growth within the biomass framework. (b) Purple-pigmented nodule showing filamentous bacteria in the presence of carbonates. Filaments retain their growth orientation despite the presence of carbonate minerals, suggesting in situ nucleation and crystal growth.

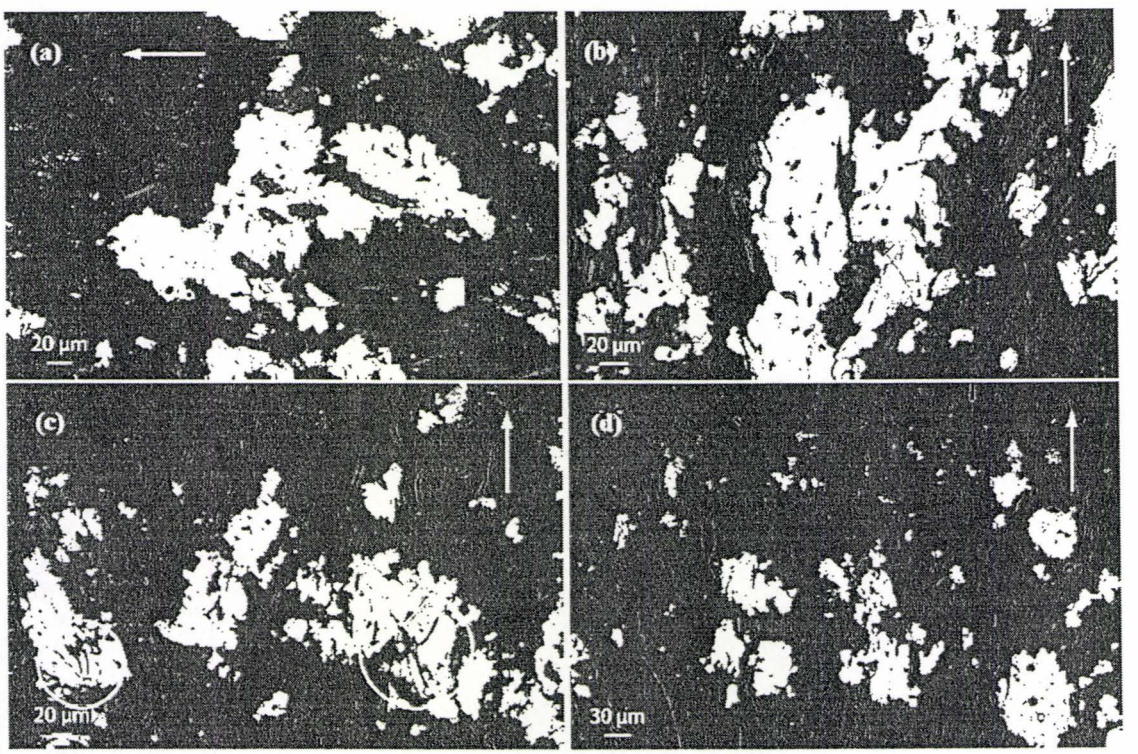

Figure 3.6: SEM-BSE micrographs of microbialite nodules in cross section. Predominant growth orientation shown by arrows. Evidence for in situ carbonate precipitation: (a) filamentous microorganisms entombed in carbonate minerals, suggesting mineral growth around individual filaments; (b) void spaces within carbonates with similar size and morphology to adjacent extant microorganisms; (c) in addition to having coccoid morphologies, void spaces within carbonates resemble filamentous shapes that mimic the morphology of the dominant microbial population; (d) carbonate minerals also reveal secondary infilling of void spaces, further suggesting active carbonate precipitation within the microbialite nodules.

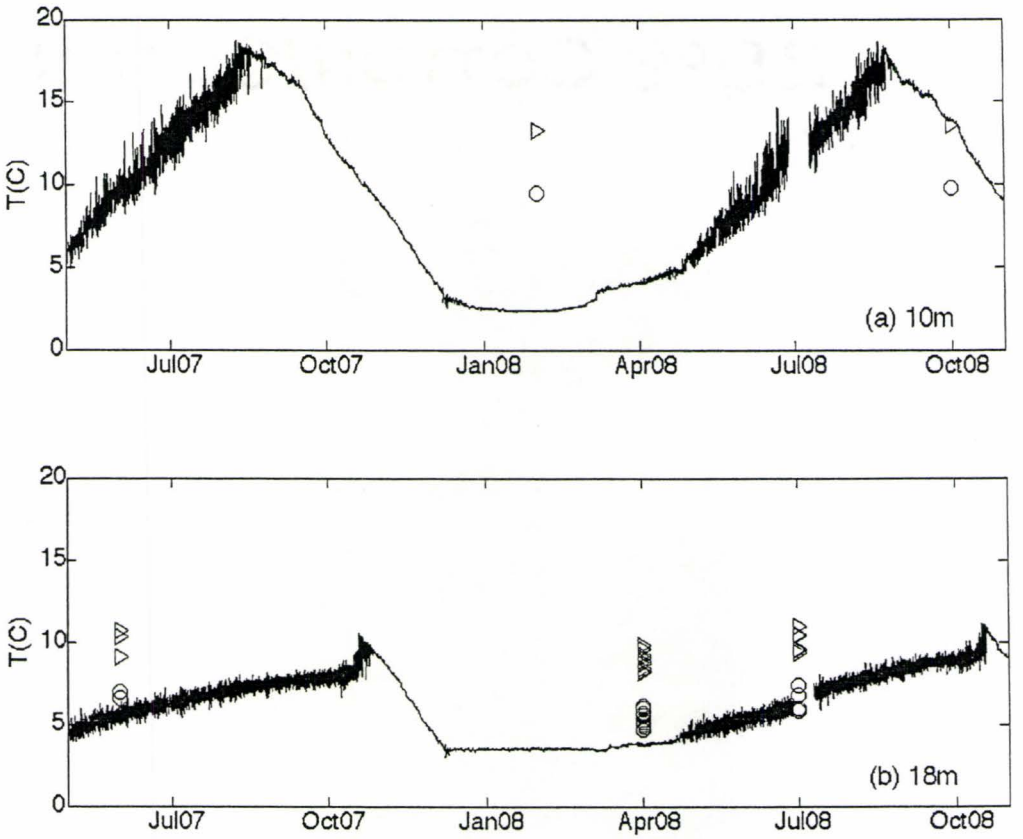

Figure 3.7: Measured water temperature at a) 10 m and b) 18 m depth along transect in Pavilion Lake. Temperature of nodule carbonate formation predicted from $\delta^{18}O$ are plotted as triangles. Circles represent predicted temperature of formation adjusted for pH effects.

Table 3.1: $\delta^{13}C$ value of DIC and $\delta^{18}O$ values of water in Pavilion Lake from 2005 – 2008.

Sample	$\delta^{13}C_{DIC}$ ‰ (PDB)	$\delta^{18}O$ ‰ (VSMOW)
02/2005	-2.5	-
02/2005	-2.4	-
08/2005	-1.6	-
06/2006	-3.7	-11.1
08/2006	-1.9	-10.7
06/2007	-1.0	-11.1
08/2007	-1.1	-10.9
10/2007	-1.7	-11.0
02/2008	-0.3	-11.4
04/2008	0.6	-11.2
06/2008	0.4	-11.2
07/2008	0.3	-11.1
10/2008	-0.2	-10.9
mean	-1.2	-11.1
s.d.	1.3	0.2

-, not determined

Table 3.2: Depth, collection date and inorganic and organic isotopic composition of green and purple nodules. Results are for triplicate analysis.

I.D.	Description	Date Collected	$\delta^{13}C_{carb}$ ‰	$\delta^{18}O_{carb}$ ‰	Predicted T°C	$\delta^{13}C_{org}$ ‰	$\Delta^{13}C_{inorg}$ - $^{13}C_{org}$ ‰
N1	Green, depth 18 m	04/2006	2.7	-9.5	10.0	-27.1	25.9
N2	Green, depth 18 m	04/2006	1.9	-9.6	10.2	-28.0 ± 1.2	26.8
N3	Green, depth 18 m	04/2006	2.3 ± 0.4	-9.6 ± 0.1	10.2	-27.1	25.9
N4	Green, depth 21 m	04/2006	2.4	-9.2	8.7	-	-
N5	Green, depth 18 m	06/2007	2.2 ± 0.1	-9.6 ± 0.2	10.3	-26.6	25.4
N6	Green, depth 18 m	06/2007	3.4	-9.3	9.1	-	-
N7	Green, depth 18 m	06/2007	1.1	-9.8	10.7	-28.5 ± 0.7	27.3
N8	Green, depth 10 m	02/2008	1.7	-10.7	13.3	-	-
N9	Green, depth 18 m	04/2008	2.4	-9.4	9.0	-28.5 ± 0.7	27.3
N10	Green, depth 18 m	04/2008	2.5 ± 0.1	-9.2 ± 0.1	8.3	-	-
N11	Green, depth 18 m	04/2008	2.8 ± 0.3	-9.3 ± 0.1	8.7	-28.3 ± 0.6	27.1
N12	Green, depth 18 m	04/2008	2.8	-9.2	8.4	-29.6	28.4
N13	Green, depth 18 m	04/2008	2.0	-9.6	9.6	-	-
N14	Green, depth 18 m	04/2008	2.3	-9.2	8.2	-	-
N15	Green, depth 18 m	04/2008	2.6	-9.2	8.2	-	-
N16	Green, depth 18 m	07/2008	2.7 ± 0.4	-9.4 ± 0.0	9.5	-27.3 ± 1.2	26.1
N17	Green, depth 18 m	07/2008	1.6 ± 0.3	-9.7 ± 0.1	10.4	-28.1 ± 0.4	26.1
N18	Green, depth 18 m	07/2008	1.3	-9.5	9.6	-27.1	25.9
N19	Green, depth 18 m	07/2008	2.2	-9.7	10.5	-27.5	26.3
N20	Green, depth 18 m	07/2008	2.2	-9.9	11.0	-29.7	28.5
N21	Green, depth 18 m	07/2008	2.8	-9.4	9.4	-28.1 ± 0.8	26.8
N22	Green, depth 10 m	10/2008	2.7	-10.3	13.6	-21.1 ± 0.7	19.9
N23	Purple, depth 18 m	04/2008	2.2 ± 0.7	-9.3 ± 0.0	8.7	-30.6	29.4
N24	Purple, depth 18 m	04/2008	2.2 ± 0.1	-9.5 ± 0.1	9.2	-	-
N25	Purple, depth 18 m	04/2008	2.3	-9.4	9.1	-28.4	27.2
N26	Purple, depth 18 m	04/2008	2.7	-9.4	8.9	-30.4 ± 0.9	29.2
N27	Purple, depth 18 m	04/2008	2.4	-9.6	9.8	-30.6	29.4

Table 3.3: PLFA distribution in mol % and $\delta^{13}C$ values for April 2006 and April 2008 nodule samples.

PLFA I.D.	April 2006	$\delta^{13}C$ ‰ PDB	April 2008	$\delta^{13}C$ ‰ PDB
br14:0	0.0		1.1	-31.4 ± 0.6
14:0	2.0		1.9	-34.7 ± 0.1
i-15:0	1.7		1.8	-32.8 ± 0.7
a-15:0	1.9		2.0	
i-16:0	0.0		2.2	
16:1	10.7	-31.5 ± 0.7	19.8	-34.4 ± 0.3
16:0	28.1	-34.0 ± 0.1	22.5	-35.0 ± 0.3
i-17:0	0.0		1.5	
a-17:0	0.0		1.6	
17:0Δ	1.6		1.5	
18:3	4.1		0.0	
18:2	8.7		5.9	
18:1	31.4	-31.7 ± 0.6	34.6	-33.7 ± 0.3
18:0	5.7		2.7	
19:1	4.2		0.0	
20:1	0.0		0.9	

CHAPTER 4

RELATIONSHIP BETWEEN SEASONAL AND SPATIAL ENVIRONMENTAL CHANGES AND MICROBIAL INFLUENCES ON CARBONATE PRECIPITATION AND ASSOCIATED BIOSIGNATURES IN MODERN, FRESHWATER MICROBIALITES

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ABSTRACT

Seasonal and spatial variations in surface mat microbial community phospholipid fatty acid (PLFA) profiles and potential isotopic biosignatures associated with freshwater microbialites of varying morphologies were investigated in Pavilion Lake, B.C. In general the PLFA profile associated with the microbialites was dominated by saturated and monoenoic microbial phospholipid fatty acids with lower levels of polyenoic and cyclopropyl PLFA consistent with a predominantly prokaryotic community. Microbialite bulk organic $\delta^{13}C$ values with a mean of -25.0 ± 3.4 ‰ and a mean discrimination between inorganic and organic carbon pools of 23.8 ‰ were consistent with non-CO_2 limited photosynthesis. Individual microbialites showed evidence of distinct autotrophic and heterotrophic dominated zones as observed through comparatively higher biomass estimates in green coloured regions, with saturated and monoenoic PLFA $\delta^{13}C$ values depleted relative to the bulk cell by 7 – 14 ‰, consistent with photosynthetic organisms. In contrast, brown coloured regions had lower total biomass estimates and higher proportions of heterotroph associated PLFA with smaller $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values of 3 – 4 ‰, consistent with heterotrophic synthesis. Notwithstanding the presence of both autotrophic and heterotrophic dominated regions, the PLFA profiles of the surface microbial mat communities were primarily consistent with photosynthetic dominance and were stable over seasonal and depth profiles to 33 m. In addition, no shift towards heterotrophic dominance as measured by PLFA distribution or isotopic composition was observed after up to one month during light limitation experiments. In contrast to samples recovered from above 33 m, dark coloured microbialites recovered from below

46 m had comparatively low biomass and contained higher proportions of branched PLFA including the sulfate reducing bacteria biomarker 10me16:0 and negligible levels of polyenoic PLFA indicative of a heterotrophic dominated community containing sulfate reducing bacteria.

Carbonate $\delta^{13}C$ values associated with surface microbial mats from all regions had a mean value of 0.7 ± 0.8 ‰, within the range for abiotic equilibrium with dissolved inorganic carbon. Carbonate $\delta^{13}C$ values did not show significant trends with depth or season below 20 m. However, surface microbial mats associated with microbialites recovered from 11 m had carbonate $\delta^{13}C$ values enriched relative to the mean predicted abiotic equilibrium value by up to 2 ‰ and increased surface biomass during summer sampling periods. Estimated carbonate precipitation temperatures based on $\delta^{18}O$ were consistent with measured late summer water temperatures. These findings are consistent with microbial photosynthetic influences on precipitation during periods of high photosynthetic activity at these shallower depths which may be related to the friable and porous nature of microbialites at these depths. These results indicate that variation in microbial activity levels related to light and temperature fluctuations, rather than significant changes in microbial community as reflected in PLFA distributions, influence isotope biosignatures in shallow microbialite samples.

4.1. INTRODUCTION

Biology has been proposed to play an important role in microbialite formation through trapping and binding of sediment by microbial communities (Stolz et al. 2001);

microbial cells acting as nucleation sites for crystal growth (Bosak and Newman 2003) and/or microbial influences on geochemical conditions causing *in situ* precipitation (Merz-Preiß 2000; Merz-Preiß and Riding 1999; Reid et al. 2000). However, abiotic models of microbialite formation have also been proposed (Grotzinger et al. 2000) and there is a lack of agreement over the role of biology in microbialite formation, in both modern and ancient settings due to the difficulty associated with identifying unambiguous biosignatures of microbial activity. Understanding the role of biology in the formation of microbialites and identifying the relative importance of microbial autotrophy versus heterotrophy in modern microbialite systems is important to the interpretation of ancient structures and associated biosignatures preserved in the geologic record.

4.1.1. Autotrophic and heterotrophic influences on carbonate precipitation

Primary production via photosynthesis is the driving force of microbial communities that are associated with modern microbialites from marine and hypersaline environments (Reid et al. 2000). Photosynthesis has been demonstrated to influence carbonate precipitation in nodular microbial communities associated with the surface of Pavilion Lake microbialites (Chapter 3) and in small, stromatolites and thrombolites from nearby Kelly Lake (Ferris et al. 1997). However, both photosynthesis and heterotrophy have been linked to carbonate precipitation (Ludwig et al. 2005; Merz 1992; Visscher et al. 2000) and to microbialite formation in other systems. In the case of photosynthetically driven carbonate precipitation, uptake of HCO_3^- and conversion into

CO_2 via carbonic anhydrase for use in ribulose-1,5-bisphosphate carboxylase, releases OH^- into the microenvironment surrounding the cell (RUBISCO) (Miller and Colman 1980; Paneth and O'leary 1985). The resulting increase in pH increases the calcium carbonate saturation index (S.I.) leading to precipitation of calcite and microbialite structure growth (Merz 1992; Schultze-Lam et al. 1992; Thompson and Ferris 1990). Alternatively, heterotrophic metabolic activity, such as sulfate reduction that is responsible for the majority of organic matter mineralization within microbial mat systems, can also result in carbonate precipitation (Canfield and Des Marais 1993; Visscher et al. 2000). Sulfate reduction and concurrent oxidation of organic matter leads to an increase in the total dissolved inorganic carbon (DIC) and a corresponding increase in the S.I. (Baumgartner et al. 2006; Visscher and Stolz 2005). Furthermore, Ca^{2+} and Mg^{2+} ions stored in cyanobacteria extracellular polymeric substance (EPS) are released during decomposition, also contributing to increasing the S.I. and promoting precipitation of carbonate (Altermann et al. 2006; Paerl et al. 2001; Visscher and Stolz 2005).

During both autotrophic and heterotrophic metabolic activities, microbes can influence the isotopic composition of DIC and precipitation of carbonate from this DIC can preserve the isotopic effects as a biosignature of the microbial activity (Chapter 3) (Andres et al. 2006; Merz 1992; Sumner 2001). In order to be resolvable, the biosignature of microbial activity must induce deviations in measured carbonate $\delta^{13}C$ values from the range of values predicted for equilibrium precipitation. The direction of these deviations indicates either autotrophic or heterotrophic dominant processes (Ferris et al. 1997; Hodell et al. 1998; Mcconnaughey 1989; Mcconnaughey et al. 1997; Merz

1992; Thompson et al. 1997). In the case of autotrophy, biological preference for ^{12}C during photosynthesis leads to net incorporation of the lighter isotope into cell biomass and a corresponding enrichment in ^{13}C of the residual DIC (O'leary 1988). Such biosignatures of high levels of photosynthetic activity in some lakes have been recognized in DIC and carbonates enriched in ^{13}C in Pavilion Lake (Chapter 3) and others (Hodell et al. 1998; Hollander and Mckenzie 1991; Thompson et al. 1997). In contrast, input of ^{13}C-depleted CO_2 into the aqueous microenvironment derived from oxidation of ^{13}C-depleted organic matter by sulfate-reducing bacteria (SRB) leads to an overall decrease in the ^{13}C-content of DIC and associated carbonate (Altermann et al. 2006; Baumgartner et al. 2006; Visscher et al. 2000).

Microbial phospholipid fatty acids (PLFA) provide another means to investigate microbial activity associated with microbialites. PLFA degrade rapidly, within days to weeks, upon death of the cell, and therefore extracted PLFA may be used to estimate total cell biomass at the time of sampling. Further, individual or groups of PLFA have been linked to certain microbial groups (Green and Scow 2000; Rajendran et al. 1995; Vestal and White 1989; White et al. 1996; Zelles 1999). Thus, assessing the distribution and concentrations of PLFA associated with microbialites, and different components thereof, provides a means to characterize the extent of microbial growth and potentially the types of predominant organisms associated with the structure. The isotopic composition of PLFA and the relationship to the isotopic composition of bulk cell organic matter can further provide insight into the metabolisms of the active *in situ* microbial community in an environmental system (Abraham et al. 1998; Blair et al. 1985; Jahnke et al. 2004;

Londry et al. 2004; Sakata et al. 1997). PLFA representative of cyanobacteria have $\delta^{13}C$ values that are depleted relative to bulk biomass by ~ 7 – 9 ‰ (Jahnke et al. 2004; Sakata et al. 1997) whereas biosynthesis of PLFA in heterotrophic microbes results in smaller fractionations, leading to PLFA with $\delta^{13}C$ values that are depleted relative to the bulk cell biomass by ~ 2 – 4 ‰ (Abraham et al. 1998; Blair et al. 1985; Teece et al. 1999).

Modern microbialites represent analogue systems in which to investigate biotic mechanisms of formation and biosignatures of autotrophy and heterotrophy. Microbial mats associated with the surface of modern microbialites are diverse communities composed of autotrophic and heterotrophic microbes including cyanobacteria, aerobic heterotrophs and sulfate reducing bacteria (Burns et al. 2004; Foster et al. 2009; Reid et al. 1995). Previous studies of modern microbialites have identified heterotrophy as an important mechanism contributing to the overall formation of freshwater microbialites from Cuatro Ciénegas, Mexico (Breitbart et al. 2009) and in marine microbialites from the Exuma Cays, Bahamas (Andres et al. 2006; Visscher et al. 2000; Visscher et al. 1998). In contrast, autotrophy has been demonstrated to be an important process within surface nodular communities associated with the Pavilion Lake microbialites (Chapter 3). However, since the nodular communities make up only a relatively small component of the microbial communities associated with the Pavilion Lake microbialites, and since microbial activity changes with environmental parameters such as light level or temperature (Bebout and Garcia-Pichel 1995; Fritsen and Priscu 1998; Jorgensen et al. 1988), autotrophic or heterotrophic influences on precipitation may not be consistently present. Variations in the microbial community over a spatial or seasonal scale may lead

to the formation of both autotrophic and heterotrophic isotopic biosignatures in different structures within a microbialite system or within an individual microbialite. Understanding the variation in isotopic biosignatures resulting from changes in autotrophic and heterotrophic activity provides important information for interpreting similar signatures preserved in ancient stromatolite systems.

Microbialites found in Highborne Cay, Bahamas showed little variation in the surface microbial mat diversity between seasons over a two year sampling period (Reid et al. 2000), but to our knowledge, no studies have addressed the extent of variability in the dominance of autotrophy or heterotrophy and associated biosignatures within a microbialite system that undergoes significant seasonal and spatial temperature and light fluctuations or within individual microbialite structures. Freshwater microbialites in Pavilion Lake, B.C. that exist at varying depths and exhibit morphological and surface colour variation represent an opportunity to address questions of the dominance of autotrophy versus heterotrophy within surface microbial mats associated with the microbialites. Specifically, this study investigates potential seasonal, depth and intra-sample changes in the role of autotrophy versus heterotrophy as indicated by variation in PLFA profiles and associated organic and inorganic isotope biosignatures.

4.2. SAMPLING AND ANALYTICAL METHODS

4.2.1. Pavilion Lake microbialites

Pavilion Lake is located in south-central British Columbia, Canada approximately 450 km North-East of Vancouver at an altitude of 823 m above sea level. Pavilion Lake

is a temperate, dimictic lake that experiences annual ice cover reaching a thickness of ~ 50 cm (Lim et al. 2009). It is a small (5.7 km x 0.8 km and 65 m deep) freshwater and ultra-oligotrophic lake with a pH of ~ 8.3 and hosts microbialites ranging from several centimetres to meters in height with varying morphologies (Laval et al. 2000; Lim et al. 2009). Although microbialite morphological variation shows a trend with depth, it is not yet clear what factors, physical, chemical or biological, influence the morphology of the structures (Figure 4.1) (Laval et al. 2000; Lim et al. 2009). Previous research outlined in Chapter 3 has identified a biosignature of photosynthetic influence in carbonate precipitation associated with nodular microbial communities on the surface of the microbialites, however, these communities represent a relatively small component of the surface microbial communities. The majority of the microbial community is present as surface microbial mats approximately 5 mm thick composed of photosynthetic cyanobacteria including *Synechococcus* sp. and *Oscillatoria* sp. and heterotrophic microbes that covers most of the microbialite surface (Laval et al. 2000). This study focuses on changes in this surface microbial mat and associated isotope signatures as compared to a previous study that examined the distinct, but less common surface nodules composed predominantly of filamentous cyanobacteria that had an associated biosignature of photosynthesis (see Chapter 3).

4.2.2. Microbialite and water chemistry collection and characterization

Sampling of microbialites was performed SCUBA divers two to five times a year between February and October from 2004 to 2008. Several sets of microbialite samples

were collected in order to assess variability in microbial community and isotopic parameters over seasonal, depth profiles and between visually distinct colour zones on individual microbialites. To determine the extent of variability in PLFA distribution between individual microbialites recovered from the same depth, triplicate samples of adjacent microbialites were collected from 11 m during a single sampling period. In order to assess seasonal variations, a temporal series of microbialite samples were collected at two depths (11 and 26 m) that were representative of two of the major microbialite morphologies observed in the lake (Figure 4.2) (Laval et al. 2000). Samples were collected maximally 5 times a year, Winter (W), Spring (Spr, April), Early Summer (ES, early June), Late Summer (LS, late July or early August) and Fall (F, October). In addition, microbialites samples were collected during single seasonal periods from depths ranging from 11 to 52 m to investigate variability associated with depth. Individual microbialite samples were collected that exhibited visually distinct regions of colour hypothesized to represent separate communities dominated by autotrophic or heterotrophic bacteria to examine intra-microbialite variability. This colour zonation included an upper green region that covered the majority of the structure that was exposed to light, a brown region near the sediment water interface (SWI) and a grey region below the SWI (Figure 4.3). To assess the effects of light limitation and subsequent decrease in photosynthesis on PLFA profiles and associated isotopic biosignatures an experiment was conducted whereby adjacent microbialites from a depth of 24 m were subjected to light limitation *in situ* by covering for a period of two weeks or four weeks. The time periods were based on previous demonstrations of decreased

cyanobacteria photosynthetic activity in modern microbialites during *in situ* burial after two weeks (Perkins et al. 2007). These light limited samples were compared to an adjacent sample from the same depth that had not been subject to light limitation. After sampling, microbialite pieces were frozen on-site and transported to McMaster University on dry ice prior to lyophilisation and further analysis.

Water samples for ^{13}C analysis were collected in crimp sealed glass serum bottles with no headspace and fixed with mercuric chloride to prevent further microbial activity. Water samples for ^{18}O analysis were also collected in crimp sealed glass serum bottles with no headspace.

Summer light level data were collected using a LI-COR light measurement instrument attached to a CTD (conductivity-temperature-depth) profiler. *In situ* water temperature was measured at sampling intervals of 30 minutes at nominal water depths of 10 and 18 m at 10 cm above the lake bed (lake level varies by about 1 m over seasonal time scales) using SCUBA diver deployed Onset WaterTempPro v2 water temperature data loggers (resolution and calibrated accuracy 0.2°C).

4.2.3. Phospholipid fatty acid analysis of microbialites

A modified Bligh and Dyer method (Bligh and Dyer 1959) was used to extract PLFA from microbialites and purified using silica gel chromatography separated lipids into non-polar, neutral and polar fractions. Polar fraction phospholipids were converted to fatty acid methyl esters (FAMEs) via a mild alkaline methanolysis (Guckert et al. 1985). Microbial FAMEs were separated using gas chromatography mass spectrometry

(GC/MS) on an Agilent GC-MS (Agilent Technologies Inc., Santa Clara, California, USA) with DB-XLB capillary column (30 m x 0.32 mm I.D. x 0.25 μm film thickness) using a temperature program of 50°C (1 min.), 20°C/min to 130°C, 4°C/min to 160°C, 8°C/min to 300°C (5 min). PLFA identification was based on the retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc., Pleasant Gap, Pennsylvania, USA). PLFA are named as follows; total number of carbon atoms:number of double bonds, followed by the position (Δ) of the double bond from the carboxyl end of the molecule. *Iso-* and *anteiso-* branching are denoted by the prefixes i or a respectively. Other methyl branching is indicated as the position of the additional methyl carbon from the carboxylic end. Br indicates a branch in an unknown location followed by the total number of carbon atoms. Cy indicates cyclopropyl PLFA. Double bond position was determined using dimethyl disulfide as in (Nichols et al. 1986). Viable cell estimates were made from PLFA data using a conversion factor of 2.6×10^4 cells/pmol PLFA (Franzmann et al. 1996).

4.2.4. Stable isotope analysis

DIC isotopic composition was determined by acidification and conversion to CO_2 analyzed by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa (St-Jean 2003). All DIC $\delta^{13}C$ values are reported in standard delta notation in reference to PeeDee Belemnite (PDB). Water oxygen values were determined by CO_2-water equilibration at 25°C prior to analysis on a Gasbench and

Finnigan MAT Deta Delta^{Plus} XP. $\delta^{18}O$ values are reported in standard delta notation in reference to Vienna Standard Mean Ocean Water (VSMOW).

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 gave precisions of less ± 0.2 ‰ (1 σ) for $\delta^{13}C_{carb}$ and ± 0.3 ‰ (1 σ) for $\delta^{18}O$ analyses. Bulk organic carbon samples were dried and treated with 1 M HCl to remove carbonate before analysis on an EA-Delta XL at McMaster University. Triplicate analyses gave a precision of less ± 2.2 ‰ (1 σ) for $\delta^{13}C_{org}$ values of all microbialites sampled. Carbonate $\delta^{13}C_{carb}$ and $\delta^{18}O$ values are reported in standard delta notation relative to PeeDee Belemnite (PDB).

For compound specific PLFA $\delta^{13}C$ analysis, aliquots of microbial FAMEs were injected into a split/splitless injector set to splitless mode at 300°C prior to separation using gas chromatography mass spectrometry (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 combusted to CO_2 as they eluted from the column via a combustion oven set at 960°C. Evolved CO_2 was analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS). As PLFA i15:0 and a15:0 were not completely resolved the $\delta^{13}C$ value reported represents both peaks. FAME $\delta^{13}C$ values were corrected for methyl carbon added during methanolysis using the ^{13}C characterized methanol via the relationship:

$$\delta^{13}C_{FAME} = [(N+1)* \delta^{13}C_{meeasured} - \delta^{13}C_{MeOH}]/N$$

where N is the number of carbon atoms. All PLFA $\delta^{13}C$ values are reported in standard delta notation relative to PeeDee Belemnite (PDB). Individual samples were analyzed in triplicate and data are reported as mean ± one standard deviation (s.d.). Precision on triplicate $\delta^{13}C_{PLFA}$ values for individual PLFA was less than ± 0.8 ‰.

4.3. RESULTS

4.3.1. Isotopic composition of DIC and carbonate

DIC $\delta^{13}C$ and H_2O $\delta^{18}O$ values for Pavilion Lake surface water samples that were collected at between 2005 and 2008 are presented in Table 4.1. DIC $\delta^{13}C$ values average -1.2 ± 1.3 ‰ (n = 13) and water $\delta^{18}O$ values average -11.1 ± 0.2 ‰ (n = 10). Pavilion Lake has a mean pH of 8.3 that remains stable on a yearly basis and throughout the water column and results in bicarbonate (HCO_3^-) being the dominant DIC species (Lim et al. 2009). Based upon the fractionation factor of Mook and colleagues (1974) (Mook et al. 1974) and the measured $\delta^{13}C$ of atmospheric CO_2 at Pavilion Lake ($\delta^{13}C$ = -9.6 ± 0.2 ‰), a temperature range of 0 to 20°C would result in equilibrium DIC $\delta^{13}C$ values ranging from +1.1 ‰ to -1.3 ‰. DIC $\delta^{13}C$ values average -1.2 ± 1.3 ‰ (n = 13) and generally fall within this predicted seasonal range confirming that the lake DIC is consistently at or near isotopic equilibrium with atmospheric CO_2. These findings are in agreement with a previous assessment using ^{14}C analysis (Chapter 2) (Brady et al. 2009).

Calcite is enriched in ^{13}C by +1.0 ± 0.2 ‰ above the bicarbonate from which it precipitates (Romanek et al. 1992) and, thus abiotic precipitation of microbialites would result in carbonate $\delta^{13}C$ values generally within 1 ‰ of the bulk measured DIC $\delta^{13}C$

values. The predicted carbonate equilibrium $\delta^{13}C$ value from the mean measured Pavilion Lake DIC was -0.2 ± 1.3 ‰.

Microbialite carbonate $\delta^{13}C$ and $\delta^{18}O$ values are listed in Table 4.2. The measured carbonate $\delta^{13}C$ values of all microbialite samples ranged from -1.4 to +2.2 ‰ (mean +0.7 ± 0.9 ‰, n = 27). Samples ranging from 20 to 52 m depth were typically within the range of predicted equilibrium values based on the mean Pavilion DIC while samples from 11 m were higher than the predicted mean abiotic $\delta^{13}C$ by up to 2.4 ‰, with one exception from LS2007 that was within the range of equilibrium precipitation. $\delta^{18}O$ values ranged from -11.7 to -9.0 ‰ (mean -10.2 ± 0.9 ‰, n = 27). The carbonate $\delta^{18}O$ values were used to determine the temperature of formation as they are a function of the $\delta^{18}O$ value in the water and the temperature of precipitation (Epstein and Mayeda 1953). Using the measured $\delta^{18}O$ values of the water and established temperature-dependent fractionation factors and equations (Kim and O'neil 1997), the carbonate $\delta^{18}O$ values corresponded to a predicted formation temperature range of 7.9 to 18.7°C.

4.3.2. Organic matter carbon isotope composition of microbial communities

Bulk organic $\delta^{13}C$ values of the microbialites are listed in Table 4.2. Microbialite bulk organic values were generally similar within analytical error with a mean of -25.5 ± 3.4 ‰ (n = 26). The 52 m microbialite $\delta^{13}C$ value of -37.6 ± 0.5 ‰ was distinct compared to other samples from above 33 m. In samples above 33 m, the $\delta^{13}C$ values ranged from -29.9 ± 2.1 ‰ in the brown region of the sample from 32 m to -20.5 ± 1.8 ‰ in fall 2008. The average offset between mean inorganic (DIC) and organic $\delta^{13}C$ values

($\Delta\delta^{13}C_{DIC\text{-}org}$) for all microbialites was -25.1 ± 3.5 ‰ (n = 24). The largest offset was observed in the sample recovered from 52 m with bulk organic matter depleted relative to the DIC $\delta^{13}C$ value by 37.8 ‰.

4.3.3. Microbial phospholipid fatty acid analysis

The microbial PLFA composition of microbialite samples are listed in Tables 4.3 to 4.8. The PLFA profiles from all microbialites showed similar distributions and for simplicity, only significant exceptions to the general distribution will be discussed with respect to light, depth and seasonal distributions. The general PLFA distribution from surface microbial mats in association with Pavilion Lake microbialites included saturated (typically under < 20:0), branched (*iso-, anteiso-* and mid-branched), monoenoic, polyenoic and cyclopropyl associated with a predominantly prokaryotic community (Findlay et al. 1990; Green and Scow 2000; White et al. 1996). Saturated straight chain PLFA from 14:0 to 20:0 ranged in proportion from ~ 20 to 25 %, dominated by 16:0 with an observed low of 13.7 % and a high of 31.6 %. Low percentages of odd-numbered saturated PLFA were present in the majority of samples (< 2 %). High percentages of monoenoic PLFA, chiefly 16:1 and 18:1 were present in all samples (typically > 35 %) suggestive of a Gram-negative bacteria (and cyanobacteria) dominated community (Kenyon et al. 1972; White et al. 1996). Branched PLFA were dominated by *iso-* and *anteiso* 15:0 and 17:0 and 10me16:0. Cyclopropyl PLFA cy17:0 and cy19:0 were identified in all samples in proportions ranging from ~ 3 – 11 %. Low proportions (< 1

%) of branched monoenoic PLFA that have been linked to sulfate reducing bacteria (Boon et al. 1977; Macalady et al. 2000) were identified in some samples.

Intra-microbialite variation

Samples of surface microbial mats representative of distinct colour regions within individual microbialites showed differences in PLFA profiles and biomass estimates (Table 4.3). Higher biomass and a greater variety of PLFA were associated with green regions of the microbialites covering the majority of the microbialite surface exposed to light (see Figure 4.3). The brown regions were near the sediment water interface (SWI) and/or were on shaded regions of the structure in comparison to the exposed green zones. Grey coloured zones were below the SWI. Biomass concentrations from surface mat samples sampled from green regions ranged from 9.7 to 29.2 μg total PLFA per gram of extracted sample (μg PLFA/g) corresponding to 8.5×10^2 to 2.6×10^3 cells per gram (cells/g) based on the conversion factor of 2.6×10^4 cells/pmol PLFA (Franzmann et al. 1996). In contrast, biomass from brown/grey zones from the same microbialites ranged from 0.8 to 1.0 μg PLFA/g or 7.1×10^1 to 9.8×10^2 cells/g. All sub-sections had saturated straight chain PLFA ranging from 14:0 to 18:0 with 20:0 only noted in the green zones. Typically, 16:0 was the dominant saturated PLFA with the exception of the grey section of the 33 m microbialite in which 18:0 dominated. Both green and brown/grey regions showed high proportions of monoenoic PLFA, with all samples dominated by 16:1 and 18:1 PLFA, specifically by 16:1Δ9 and 18:1Δ11. Branched PLFA were also present in all samples in proportions of 3 – 4 %, in particular *iso*- and

anteiso-15:0. The 10me16:0 PLFA was detected in all sections with the exception of one but was most abundant in the brown region (~ 6 to 7 %) indicative of sulfate reducing bacteria within these regions of the microbialites (Dowling et al. 1986; Londry et al. 2004; Parkes et al. 1993). The green sections had higher amounts of polyenoic PLFA, including 16:2, 18:2 and 20:4 which could indicate the presence of eukaryotes (Bobbie and White 1980) but have been detected in a few bacterial species (Delong and Yayanos 1986) and are also consistent with cyanobacteria (Findlay et al. 1990; Kenyon et al. 1972). Fewer PLFA types were observed in brown or grey regions below the SWI and typically no or very low proportions of polyenoic PLFA were observed. The grey, below SWI section of the 33 m microbialite showed the lowest biomass estimate of 0.8 μg PLFA/g and the least number of PLFA as compared to green and brown regions of the same sample and was dominated by saturated, branched and monoenoic PLFA.

Inter-microbialite variability

Sampling of other microbialites for assessment of light, depth and seasonal variability focused on upper green zones covering the majority of the exposed surface that demonstrated the highest biomass and greatest variety in PLFA. The PLFA profiles of the light limited microbialites were not significantly different from the non-light limited sample from the same depth and location despite light limitation for a period of either two weeks or four weeks (Table 4.4). The proportions of *iso*- and *anteiso*-branched, saturated (other than 16:0) and polyenoic PLFA were the least variable between samples (± < 0.8 %) while monoenoic and 16:0 PLFA varied by ± ~ 2 %.

Biomass estimates ranged from 4.1 to 6.8 μg PLFA/g, equivalent to 3.8×10^2 to 6.2×10^2 cells/g. These observations indicated that light limitation did not significantly influence the microbial community PLFA profile. The reproducibility of the three samples from 24 m was consistent with the variability observed in extractions of the surface microbial mat of three individual samples collected at the same time from 11 m in late summer 2007 that showed that PLFA mol % of individual microbialites were within ± 3 % (Table 4.5).

Depth and seasonal profile

Variation in PLFA distribution and biomass estimates separated the microbialites into three different groups: 11 to 20 m samples, samples between 20 and 33 m, and samples below 46 m. Seasonal PLFA profiles of 11 m and 26 m samples followed the general microbialite distribution described above and did not show extensive variability between samples collected during different seasons (Table 4.6, 4.7 and Figure 4.4). Over the course of a season, saturated and monounsaturated PLFA dominated the profiles, representing on average 19.9 – 23.8 % and 44.5 – 45.1 % respectively of total PLFA in both years. Terminal branched PLFA, predominantly *iso*- and *anteiso*- 15:0 and 17:0 accounted for 9 – 10 % of total PLFA while mid-branched fatty acids including 10me16:0 comprised 7 – 9 % of total PLFA.

PLFA profiles were similar between samples from 11 to 33 m and with PLFA distributions as described for all microbialites samples (Table 4.8, Figure 4.4c). Biomass estimates for samples recovered from 11 to 33 m samples ranged from 2.5 to 30.1 μg PLFA /g (Figure 4.5a). However, samples from above 20 m generally had higher

estimates of microbial biomass as reflected in total PLFA concentrations. The sample recovered from early summer 11 m in 2007 had the highest overall biomass estimate of 30.1 μg PLFA/g. Seasonal changes in biomass were noted as 11 m depth microbialites that were sampled in different seasons had high biomass estimates during the summer months, whereas biomass estimates in deeper 26 m samples were highest in the winter (Figure 4.5b). Biomass estimated in 11 m samples ranged from a maximum of 30.1 μg PLFA/g (2.7×10^3 cells/g) observed in ES2007 to a minimum biomass measure of 2.5 μg PLFA/g (1.7×10^2 cells/g) in W2008.

In contrast, samples below 46 m had distinct PLFA profiles and low estimates of biomass compared to samples recovered from above 33 m. Odd-numbered saturated PLFA present in samples above 33 m were not detected in samples below 46 m. Polyenoic PLFA were not detected in the sample from 46 m and only 18:2 was detected in the sample from 52 m at 5 % of total PLFA. Branched PLFA were present in higher proportions in samples below 46 m, dominated by *iso*- and *anteiso* 15:0 and 17:0 and 10me16:0. Cyclopropyl PLFA showed a general decrease with depth to 33 m from 11 – 12 % at 20 – 23 m to a minimum of 3.0 % at 33 m, but were present in higher proportions in samples recovered from depths greater than 46 m. Microbialites recovered from depths below 46 m had significantly lower biomass values than samples above 33 m, with a biomass estimate from the 52 m of 0.3 μg PLFA/g, corresponding to an estimates of 3.0×10^1 cells/g (Figure 4.5a).

4.3.4. Microbial phospholipid fatty acid carbon isotope composition

PLFA $\delta^{13}C$ values from microbialite samples are listed in Tables 4.3, 4.4, 4.6 and 4.8. The $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values of saturated and monoenoic PLFA generally ranged from 7 to 14 ‰, similar to that observed for cyanobacteria synthesis (Jahnke et al. 2004; Sakata et al. 1997) while the *iso*- and *anteiso*-15:0 and 10me16:0 had much smaller offsets that typically ranged from ~ 0.5 to 3 ‰ consistent with synthesis by heterotrophic organisms (Abraham et al. 1998; Blair et al. 1985). Within an individual microbialite showing distinct colour zones, green region PLFA generally showed greater depletions relative to the bulk cell ($\Delta\delta^{13}C_{PLFA\text{-}biomass}$) (~ 6 – 14 ‰) compared to PLFA from the brown sections that had smaller offsets (~ 1 – 4 ‰) (Figure 4.6). PLFA concentrations in the grey region were too low to obtain $\delta^{13}C$ values. These offsets generally fall within the ranges of predicted $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values linked to autotrophic and heterotrophic synthesis as illustrated in Figure 4.6.

Microbialites subjected to light limitation had consistent $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values that ranged from ~ 8 – 14 ‰ in saturated and monoenoics but were smaller (~ 4 – 8 ‰) in branched PLFA associated with heterotrophic bacteria (Figure 4.7). No trend in $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values was observed between samples. These finding were concurrent with no significant changes in PLFA distribution between the light limitation experiment samples.

Samples from above 33 m showed consistent $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values and no trends with depth or seasonal change were observed. Saturated and monoenoic $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values showed the largest offsets with values up to ~ 14 ‰. Other PLFA associated with heterotrophic bacteria such as *iso*- and *anteiso*-15:0 had smaller offsets of ~ 3 – 4 ‰.

The $\delta^{13}C$ value of 10me16:0 was only measured in the sample from 32 m and showed the smallest offset of 0.5 ‰ (within analytical error) relative to the bulk cell. The deep sample from 52 m was different from other samples as PLFA $\delta^{13}C$ values were generally similar within error of the bulk cell with exception of 14:0 and 16:0 that were depleted by values ranging from 2.5 to 4 ‰.

4.4. DISCUSSION

4.4.1. Surface microbial mat community

The microbial mat communities covering the majority of the exposed microbialite surface were dominated by PLFA associated with a predominantly prokaryotic community with high proportions of monoenoic PLFA linked to Gram-negative, including cyanobacteria, bacteria (Guckert et al. 1985; Kenyon 1972; Kenyon et al. 1972; Nichols et al. 1986). Polyenoic PLFA were identified that could suggest eukaryotic organisms such as diatoms, however these PLFA have also been identified in association with cyanobacteria (Findlay et al. 1990; Jahnke et al. 2004; Kenyon 1972) and sporadically in bacteria (Delong and Yayanos 1986). Long chain PLFA characteristic of eukaryotic organisms (Volkman et al. 1980) were not detected or were detected in minor proportions (< 1 %). Photosynthetic primary production was supported by microbialite bulk organic values with a mean of -25.5 ± 3.4‰ (n = 26) and a $\Delta\delta^{13}C_{DIC\text{-}org}$ value for all microbialites of -25.1 ± 3.5 ‰ (n = 24), consistent with primary production via C_3 photosynthesis (O'leary 1988). Saturated 14:0 and 16:0, the most abundant of ubiquitous saturated PLFA typically showed the largest $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values with offsets as high

as ~ 14 ‰ indicating that the photosynthetic bacteria make up a significant proportion of the microbial mat community. However, as saturated PLFA are common to many microbial groups (White et al. 1996), minor variations in the magnitude of offsets could also reflect contributions of heterotrophic bacteria. Monoenoic PLFA generally showed $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values in the range of 7 – 10 ‰, consistent with observed offsets observed in cyanobacteria (Jahnke et al. 2004; Sakata et al. 1997) supporting the main production of these PLFA by cyanobacteria in the microbialite samples. Cyclopropyl PLFA found in the microbialite surface communities may represent synthesis from various microbial groups as these PLFA have been linked to aerobic conditions (Parkes and Taylor 1983), anaerobic conditions (Guckert et al. 1985) and physiological changes within the same community (Guckert et al. 1986). The presence of cyclopropyl PLFA in the upper green zone and the previous identification of cyclopropyl PLFA within surface nodules composed predominantly of filamentous cyanobacteria (Chapter 3) in combination with PLFA $\delta^{13}C$ values that are offset relative to the bulk cell by 7 – 9 ‰ indicate synthesis by cyanobacteria under aerobic conditions within the microbialite surface communities.

Heterotrophic microbes were also present in the surface communities as suggested by the presence of branched saturated PLFA, in particular *iso*- and *anteiso*-15:0 characteristic for Gram-negative and Gram-positive bacteria but less common in cyanobacteria (Jahnke et al. 2004) and 10me16:0 and branched monoenoic PLFA linked to SRB (Boon et al. 1977; Macalady et al. 2000; Parkes et al. 1993). 10me16:0 in particular has been identified as a biomarker of sulfate reducing bacteria of the genus

Desulfobacter (Dowling et al. 1986). PLFA indicative of heterotrophic microbes such as *iso*- and *anteiso*-15:0 and 10me16:0 had smaller offsets (~ 1 – 4 ‰) characteristic of heterotrophic metabolisms (Abraham et al. 1998; Blair et al. 1985; Teece et al. 1999). These findings are consistent with previous microscopic characterizations of the Pavilion Lake microbialites that found surface communities composed of diatoms and cyanobacteria including *Synechococcus* sp., *Pseudoanabaena* sp. and purple pigmented *Oscillatoria* sp. in addition to heterotrophic organisms (Laval et al. 2000).

4.4.2. Autotrophic and heterotrophic dominance

Intra-microbialite variation

Distinct regions of photosynthetic dominated and heterotrophic dominated communities were present on individual microbialites as seen through variability in colour, biomass estimates, PLFA profiles and PLFA $\delta^{13}C$ values. Photosynthesis as the source of primary production in all regions was supported by a mean bulk organic of -25.5 ± 3.4 ‰ (n = 26) and an average $\Delta\delta^{13}C_{DIC\text{-}org}$ value of 25.1 ± 3.5 ‰ (n = 24) consistent with photosynthetic production of organic material that was subsequently consumed by heterotrophic organisms (O'leary 1988). Regions of the microbialites that were above the SWI and were green in colour had higher biomass as compared to regions near (brown) or below the SWI (grey) (see Table 4.3), suggesting high productivity in the surface microbial mat community on the upper light exposed surface. Green regions within an individual microbialite had high proportions of saturated, monoenoic and polyenoic PLFA with $\delta^{13}C$ values depleted relative to the bulk cell by $\Delta\delta^{13}C_{PLFA\text{-}biomass}$

values of ~ 7 to 14 characteristic of cyanobacteria (Sakata et al. 1997) and low relative contributions of branched and specific markers for SRB. In contrast, brown and grey regions of the microbialite showed a more heterotrophic dominant community with higher proportions of *iso*- and *anteiso*- PLFA and 10me16:0 with smaller $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values in the brown region (~ 1 – 4 ‰) characteristic of PLFA biosynthesis by heterotrophic bacteria, heterotrophic processes including sulfate reduction increased in importance in more light limited regions (Figure 4.6). The grey section of the microbialite was the only sample in which 18:0 was present in higher proportion than 16:0, a condition that has been observed in methylotrophic bacteria (Nichols et al. 1985) which may be expected closer to anaerobic zones located below the SWI. The same PLFA were present in both green and brown regions, but showed significantly different $\delta^{13}C$ values and offsets relative to the bulk cell in each region indicative of principal synthesis by either autotrophic cyanobacteria or heterotrophic bacteria. As an example, 16:0 showed large offsets (~ 8 – 9 ‰) within the green sub-samples consistent with autotrophic bacteria but much smaller offsets in the brown sections (~ 3 ‰) indicative of heterotrophic synthesis (Figure 4.6). As many individual PLFA are synthesized by more than one group of microbes including both heterotrophic and autotrophic bacteria, these results demonstrate that PLFA $\delta^{13}C$ values are useful tools to track changes in microbial metabolisms when PLFA distributions are similar.

Although evidence was found for distinct regions of autotrophy and heterotrophy within an individual microbialite, the carbonate $\delta^{13}C$ values were not significantly different between the coloured regions (Table 4.2). Within the microbialite samples from

32 and 33 m depth the green, brown and grey coloured regions carbonate $\delta^{13}C$ values ranged from -0.4 to +0.7 ‰, within the range of predicted abiotic equilibrium with the ambient DIC. No indication of autotrophic ^{13}C-enrichment in the green region was observed, in fact, the green region carbonate $\delta^{13}C$ value of the sample from 32 m $\delta^{13}C$ was slightly depleted relative to the brown region. These results indicate that within the structures showing distinct coloured regions, neither autotrophic nor heterotrophic microbial communities were influencing the isotopic composition of the DIC at a detectable level.

Light limitation experiment

Microbialite samples that were subjected to light limitation to assess the effects of decreased photosynthetic activity for a period of either two or four weeks did not show significant variability in biomass estimates, PLFA profiles or isotopic composition (Table 4.4 and Figure 4.4). Previous studies reported that cyanobacteria were capable of surviving burial for periods of up to 2 weeks (Perkins et al. 2007). The observation of no significant change in PLFA profiles or $\delta^{13}C$ values after two and four week periods of light limitation suggest the community was resistant to short-term environmental fluctuations and that more prolonged periods of change such as on a seasonal or spatial scale were required to significantly alter the microbial community PLFA profile.

4.4.3. Seasonal and spatial variability

Seasonal and spatial investigations of microbial PLFA distribution and isotopic biosignatures revealed three distinct groupings of microbialites: above 20 m, 20 to 33 m and below 46 m. The groupings identified in this study are consistent with depth related morphological categories identified in Laval et al. (2000) and support the distinct nature of microbialites within these depth ranges. The differences identified between each category and implications for the identification of isotopic biosignatures will form the basis of discussion in the following sections.

Seasonal variation in biomass estimates from 11 m samples

Although some variability in PLFA distributions existed between individual microbialites, no significant trend was observed between samples recovered from 11 to 33 m. However, samples recovered from 11 m did show trends in biomass and carbonate $\delta^{13}C$ values that were not observed in deeper samples. PLFA profiles of the seasonal samples were generally as described for the majority of Pavilion Lake microbialites and contained biomarkers common to many microbial groups as described for other microbialites including *iso*-15:0, *anteiso*-15:0, monoenoics and straight chain saturates ranging from 14:0 to 18:0 (Vestal and White, 1989) that did not change significantly with season. Seasonal investigations did reveal changes in microbial biomass that appeared to correspond to annual variations in temperature. Higher levels of biomass, in particular from shallow depths, were observed in samples collected during warm periods as compared to cooler seasons (Figure 4.5b), consistent with expected periods of increased photosynthetic activity and growth rate (Fritsen and Priscu 1998; Thompson et al. 1997).

Higher temperatures at shallow depths during summer periods were supported by annual lake water temperature profiles from 10 m and 18 m showing that temperatures at 10 m are elevated in the summer relative to 18 m depth (Figure 4.8). Similar or even greater differences in temperatures would be expected between the depths of 11 m and 26 m that correspond to the samples examined in this study. Higher biomass values present in the 11 m samples compared to 26 m samples during these periods may be related to increased growth rate and could explain the more porous and friable structures observed at the shallower depth if precipitation was microbially influenced and occurred during periods of extensive photosynthetic activity. Photosynthetic activity within the 11 m samples was supported by bulk organic $\delta^{13}C$ values that ranged from -26.5 to -20.5 ‰ with a mean $\Delta\delta^{13}C_{DIC\text{-}org}$ value of 23.4 ± 2.2 ‰ (n = 7). Carbonate $\delta^{13}C$ values in the shallow 11 m samples were significantly different (Student's t-test, $p = 0.013$) than those collected in the same season from 26 m and were enriched relative to the predicted equilibrium range with one exception. Enrichments relative to the predicted abiotic mean of up to 2.4 ‰ represented a potential biosignature of photosynthetic activity within these shallow samples. The proposed relationship between increased biomass and the elevated $\delta^{13}C$ values is explored below.

Microbial community between 20 and 33 m

The surface microbial mat community and biomass estimates did not vary significantly with depth between 20 m and 33 m. Seasonal investigations of microbialite samples at 26 m showed variability in biomass but did not reveal any significant trends in

PLFA distribution. PLFA profiles showed a dominance of saturated, monoenoic PLFA with some contributions of branched and cyclopropyl PLFA characteristic of a prokaryotic community (White et al. 1996). Bulk organic $\delta^{13}C$ values from microbialites above 33 m depth averaged -25.4 ± 2.6 ‰ (n = 10) and were depleted relative to the associated DIC by 24.6 ‰ on average supporting C_3 photosynthesis under non-CO_2 limited conditions (O'leary 1988). Despite evidence for a predominantly photosynthetic community, the carbonate $\delta^{13}C$ values from microbialites between 20 and 33 m depth ranged from -0.6 to 1.2 ‰, within the range of abiotic precipitation. These results suggest that carbonate precipitation at these depths was either not influenced by microbial activity, or that the level of microbial activity was not sufficient to result in a detectable biosignature. The lack of variation in the microbial communities with depth suggests that morphological differences in the microbialites down to 33 m were not caused by significant differences in the microbial community composition as reflected in PLFA profiles.

Microbialites below 46 m

The two samples recovered from below 46 m were different in appearance from shallow microbialites, having a black outer coating as opposed to the green and/or brown colouring observed on microbialites from shallower depths (see Figure 4.1c and d). The low biomass estimates of 0.3 and 0.5 μg PLFA/g in the two deepest samples and PLFA profiles consisting of low or below detection proportions of polyenoics associated with cyanobacteria (Kenyon 1972; Kenyon et al. 1972), a greater dominance of 10me16:0

linked to sulphate reduction (Parkes et al. 1993) and increased proportions of *i*17:0/*a*17:0, terminally branched PLFA typically associated with Gram-positive bacteria suggest a decrease in photosynthetic microbial growth and production at depths below 46 m. The unique nature of these deep samples was also supported by the study outlined in Chapter 2 where samples below 46 m were found to be highly depleted in ^{14}C as compared to shallower samples (Brady et al. 2009). As light limitation has been demonstrated to decrease photosynthetic activity in microbial mats (Bebout and Garcia-Pichel 1995; Garcia-Pichel et al. 1994), low biomass estimates observed in this current study are consistent with expected decreased photosynthetic activity and growth due to light limitation at depths greater than 33 m where PAR values are less than 1 % (Figure 4.9) (Chapter 2) (Brady et al. 2009; Lim et al. 2009). The carbonate $\delta^{13}C$ value of -1.4 ‰ from the 52 m sample was consistent with predicted abiotic precipitation.

The bulk organic $\delta^{13}C$ value of the sample collected from 52 m was also the lowest observed in any microbialite, -37.6 ± 0.5 ‰, depleted relative to the corresponding DIC $\delta^{13}C$ value of +0.3 ‰ by 37.8 ‰. This value is larger than fractionations typically associated with C_3 photosynthesis (O'leary 1988) and suggests the presence of different metabolisms such as methanogenesis or methanotrophy that could contribute to lower bulk organic $\delta^{13}C$ values or alternatively, use of a different carbon source by primary producers other than the ambient lake DIC at this depth. DIC representative of groundwater flowing through the soil surrounding the lake had a $\delta^{13}C$ value of -9.6 ‰ (Chapter 2) (Brady et al. 2009) and groundwater inputs could be a source of more ^{13}C-depleted DIC to the microbes growing at these depths. Increased groundwater inputs at

this depth was proposed as one explanation of the ^{14}C-depleted carbonate values identified in deep microbialite samples (Brady et al. 2009). If this groundwater DIC was the carbon source used, the discrimination between inorganic and organic carbon pools would be 28 ‰, approaching typical photosynthetic values. Alternatively, the bulk organic value may not reflect the active microbial community. The actual PLFA $\delta^{13}C$ values in most cases were similar to those recovered from other samples suggesting that the viable communities may be similar. As bulk $\delta^{13}C$ values were a measure of total organic material, the depleted values could reflect a greater incorporation of detrital material which may include biomass derived from more ^{13}C-depleted groundwater DIC.

4.4.4. Implications for isotopic biosignatures

Despite visual and PLFA support for distinct zones of autotrophy and heterotrophy within individual microbialites, autotrophic and heterotrophic dominance was not reflected in carbonate $\delta^{13}C$ values from these distinct coloured regions. Regions within individual microbialites with photosynthetic or heterotrophic dominated communities based on PLFA profiles and $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ offsets had carbonate $\delta^{13}C$ values within equilibrium predictions. These results imply that either precipitation within the surface microbial mats from these zones was not influenced by biological activity or that the level of activity in either photosynthetic or heterotrophic zones was not sufficient to alter the isotopic composition of the local DIC and thus, did not result in a detectable isotope biosignature.

Carbonate $\delta^{13}C$ values of samples collected from below 20 m including the deep samples from below 46 m were also generally within the range predicted for equilibrium precipitation indicating that precipitation at these depths was not occurring with any detectable biological influence. In contrast, carbonate $\delta^{13}C$ values in the shallow 11 m samples were enriched relative to the predicted equilibrium mean by up to 2.4 ‰, consistent with shifts in carbonate isotopic composition typically on the order of 2 – 5 ‰ induced by microbial photosynthetic activity (Ferris et al. 1997; Guo et al. 1996; Merz 1992; Thompson et al. 1997). Although carbonate $\delta^{13}C$ values from 26 m samples were slightly enriched relative to the predicted equilibrium mean the majority were within the range of error and one sample was depleted relative to the predicted mean value.

Seasonal PLFA distributions did not change significantly however increased photosynthetic activity during the summer within the surface microbial mat associated with 11 m microbialite samples was supported by higher biomass estimates during the summer sampling periods (Figure 4.5b). High levels of photosynthetic activity would be expected during this period (Fritsen and Priscu 1998; Thompson et al. 1997) and increased activity and growth in the shallow samples was consistent with elevated summer temperatures at this depth relative to 18 m (Figure 4.8) and increased % PAR relative to deeper samples (Figure 4.9). Precipitation during the warmer summer months at 11 m was also supported by predicated temperature of carbonate formation that were consistent with the range of water temperatures observed in late summer (Figure 4.8). Predicted temperature for formation for 26 m samples was also consistent with summer precipitation, however carbonate $\delta^{13}C$ values were within the predicted equilibrium range

indicating that a biosignature was either not induced, or that the signal was not preserved. Less extensive photosynthetic activity and growth within samples 26 m and deeper would be expected due to overall lower temperatures and light levels. As well, microbial growth rates at deeper temperatures may be overall more consistent throughout the year as the water temperature profile from 18 m demonstrates that deeper samples experienced less temperature fluctuation than shallow samples. Lower levels of microbial activity at deeper depths would also be consistent with the observation of no significant difference in carbonate $\delta^{13}C$ values between autotrophic and heterotrophic dominated regions in the comparatively deep 32 and 33 m samples. These results support a link between microbial activity level and biosignature formation as seen in elevated carbonate $\delta^{13}C$ values from 11 m surface microbial mats due to summer periods of increased photosynthetic activity associated with comparatively high temperatures and light levels.

4.5. CONCLUSIONS

Elevated carbonate $\delta^{13}C$ values consistent with photosynthetic influence on carbonate precipitation were observed in microbialite samples from 11 m. These shallow samples did not show distinctly different PLFA distributions from other microbialites or trends in PLFA with season but rather had higher summer biomass values and were exposed to higher water temperatures and % surface PAR in the summer than deeper samples, consistent with high levels of photosynthetic activity. Increased microbial growth and precipitation within the 11 m samples may be linked to the porous and friable nature of these samples as compared to deeper samples exhibiting a different morphology.

Surface microbial mat communities covering the majority of exposed microbialite surfaces were largely composed of prokaryotic organisms including both photosynthetic and heterotrophic ones as identified through PLFA distributions and $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values. The PLFA distribution of this surface microbial mat did not change significantly with depth or season between 11 m and 33 m depth. However in contrast to the 11 m microbialite samples, carbonate $\delta^{13}C$ values of samples collected below 20 m were within the range predicted for equilibrium precipitation indicating that precipitation at these depths was not occurring with any detectable biological influence.

Within an individual microbialite, variability in the relative dominance of photosynthetic versus heterotrophic was supported by PLFA profiles and PLFA $\delta^{13}C$ values that were depleted relative to the bulk cell by amounts characteristic of cyanobacteria in the green region and heterotrophic synthesis in the brown region. Despite identification of zones of relative autotrophic versus heterotrophic dominance, dominance of either microbial metabolic activity was not reflected in different carbonate $\delta^{13}C$ values from these regions implying that either precipitation was abiotic or that microbial metabolic activity was not influencing DIC isotopic compositions at a detectable level.

Samples from below 46 m were distinct from samples recovered above 33 m as seen in lower total PLFA biomass and a more heterotrophic dominated community reflected by increased proportions of branched PLFA and smaller $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values including some that were enriched relative the bulk biomass. Lower biomass was likely due to low light levels limiting growth as samples below 46 m are at PAR values lower

than 1 % of surface values. Variation in the microbial community or activity level could have some influence on the distinct morphology and colouring of samples from these depths.

These results suggest that although regions of autotrophy and heterotrophy were identifiable within individual microbialites, the overall surface microbial mat community as reflected by PLFA distributions does not change significantly with seasonal or depth, up to 33 m, light and temperature related changes. Rather changes in microbial activity levels may have more important implications for both morphology and the formation of isotope biosignatures in Pavilion Lake microbialites.

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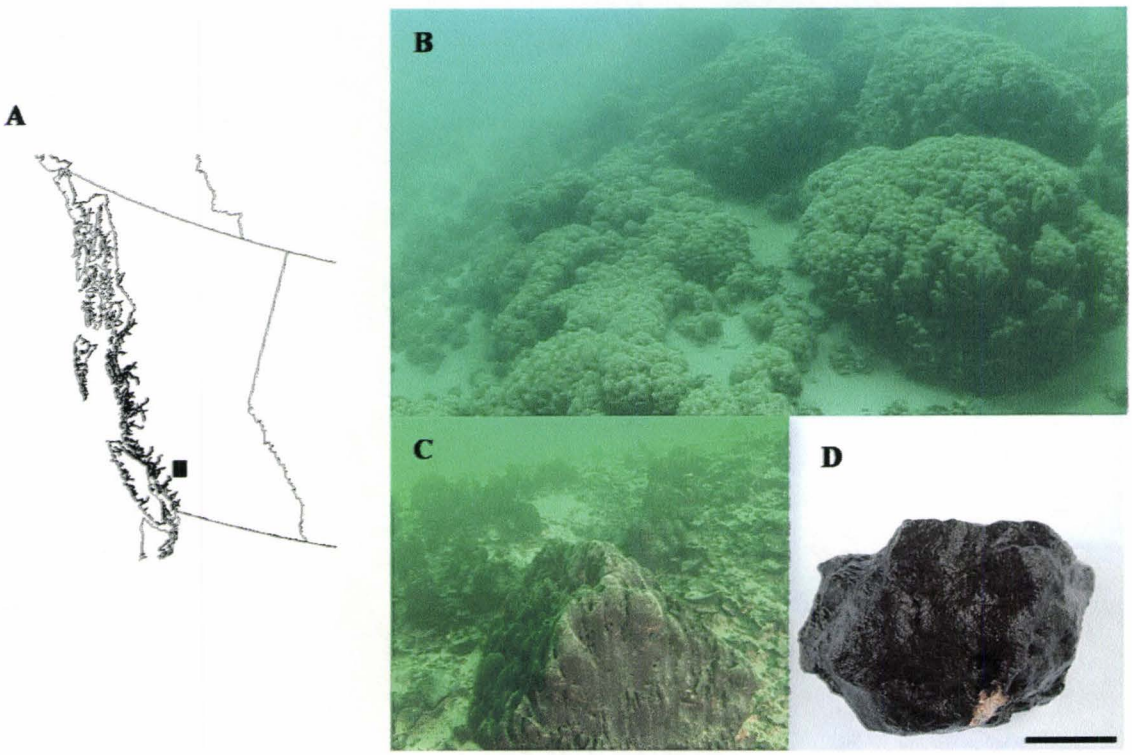

Figure 4.1: a) Map showing location of Pavilion Lake and b) examples of microbialite morphologies from above 45 m c) from below 45 m and d) collected sample from below 45 m illustrating distinct dark colouring (scale bar is 2 cm).

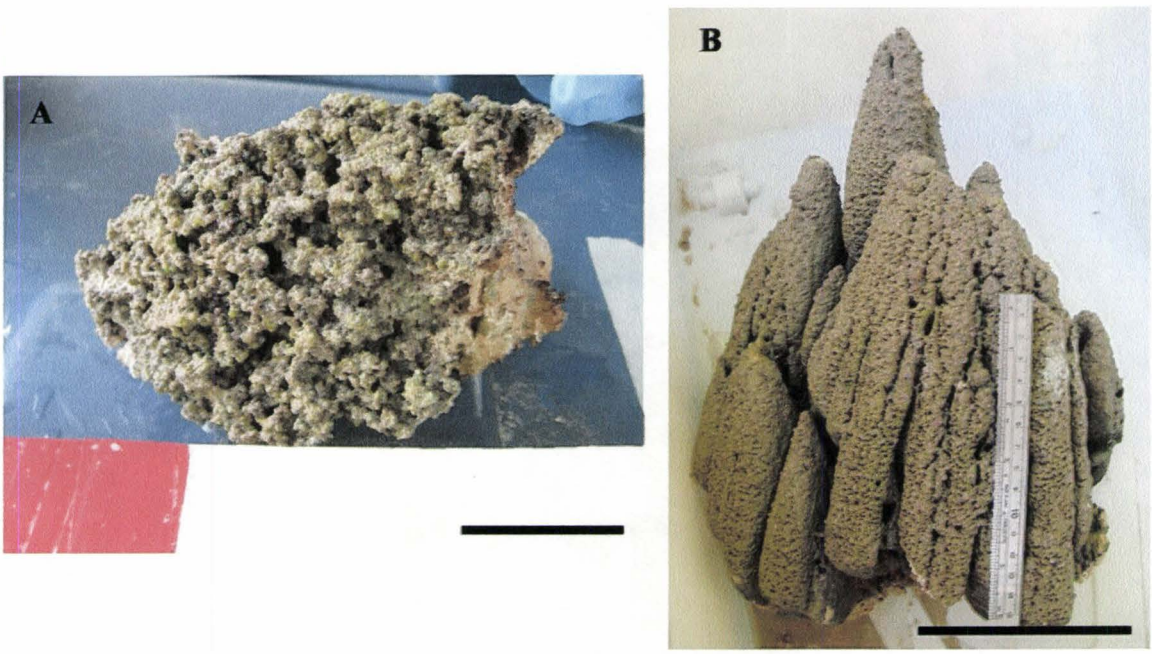

Figure 4.2: Examples of microbialites collected for assessment of seasonal variation showing differences in morphology a) porous, friable sample from 11 m and b) less friable, more dense structure from 26 m. Scale bar represents 10 cm.

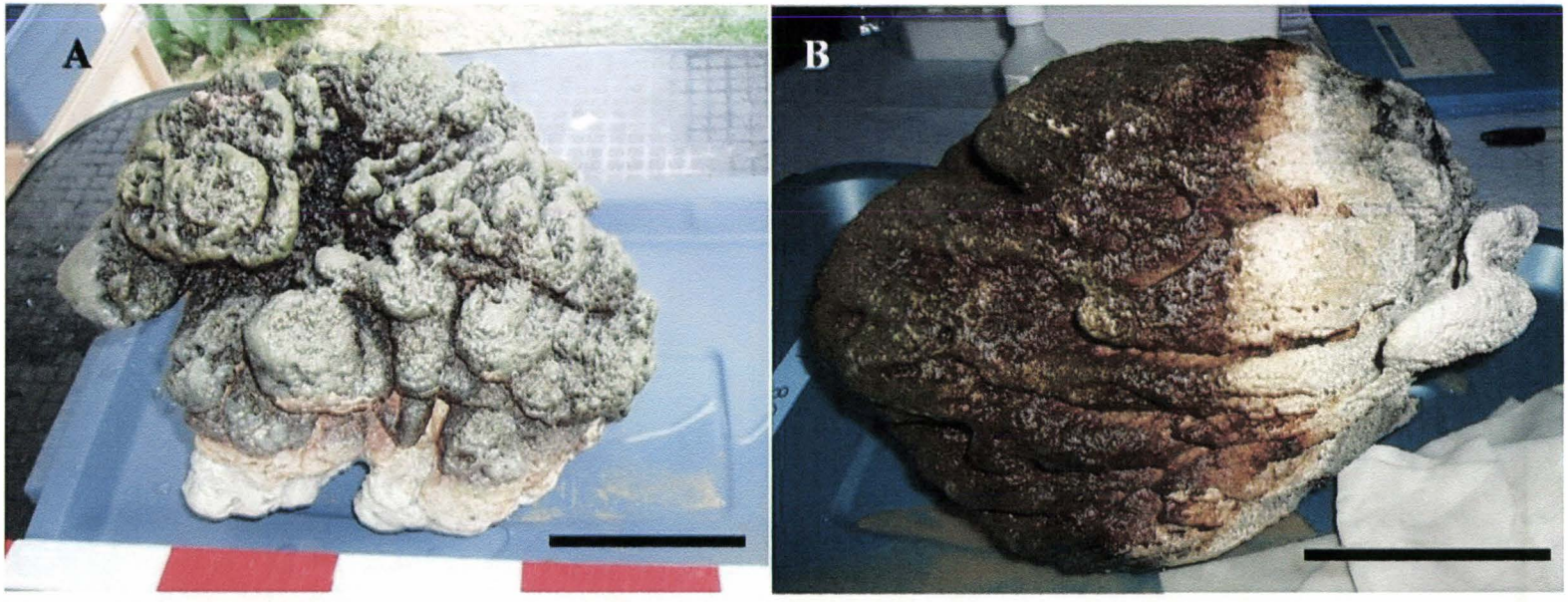

Figure 4.3: a) Sample from 32 m recovered in 2006 and b) sample from 33 m recovered in 2008. Note the variation in colouring in both samples and the transition from green to brown to grey below the sediment water interface. Scale bar represents 10 cm.

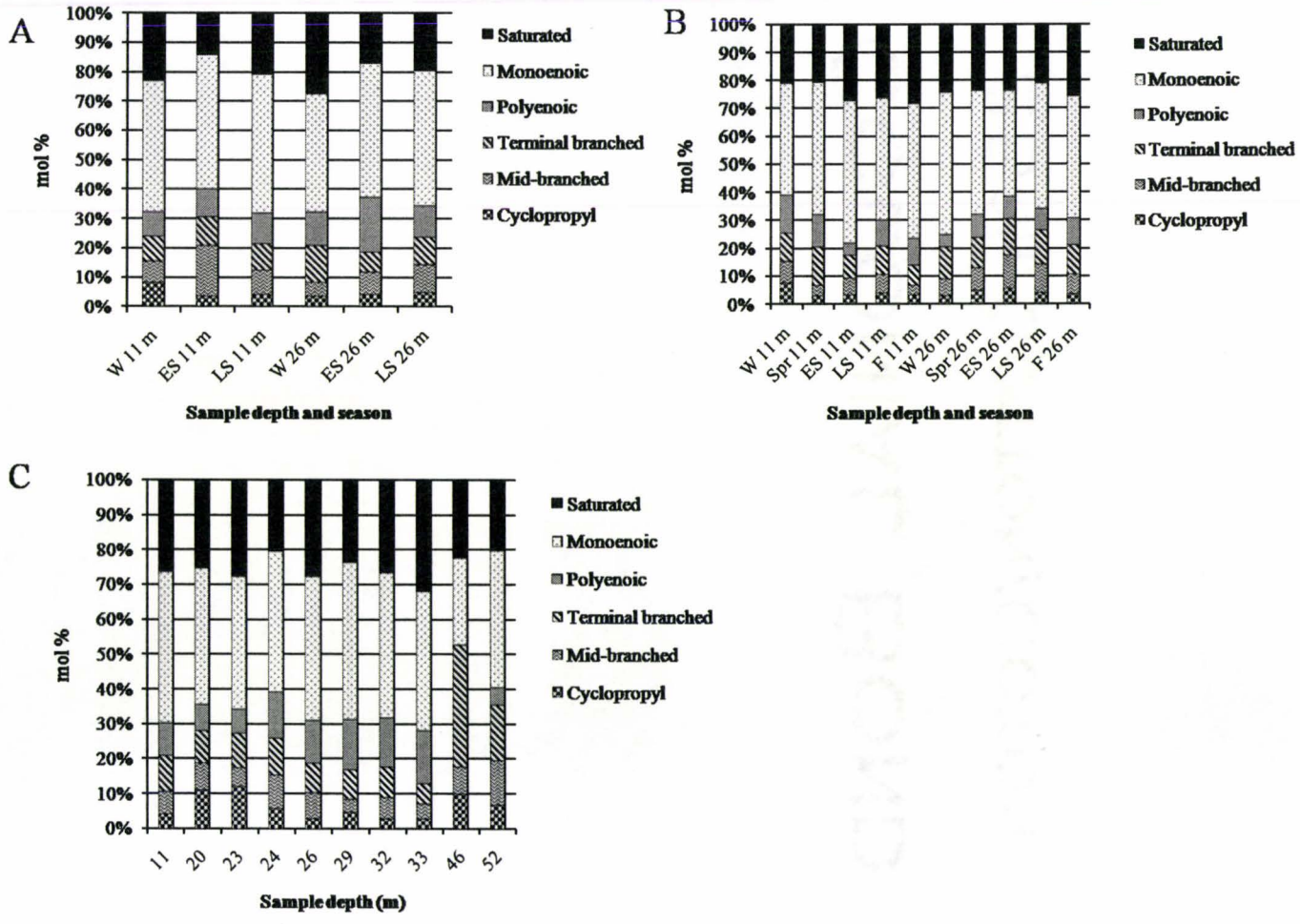

Figure 4.4: Variation in PLFA proportions for seasonal samples from 11 and 26 m depth from 2007 b) seasonal samples from 2008 and c) samples from 11 to 52 m. Sample time periods as described in text.

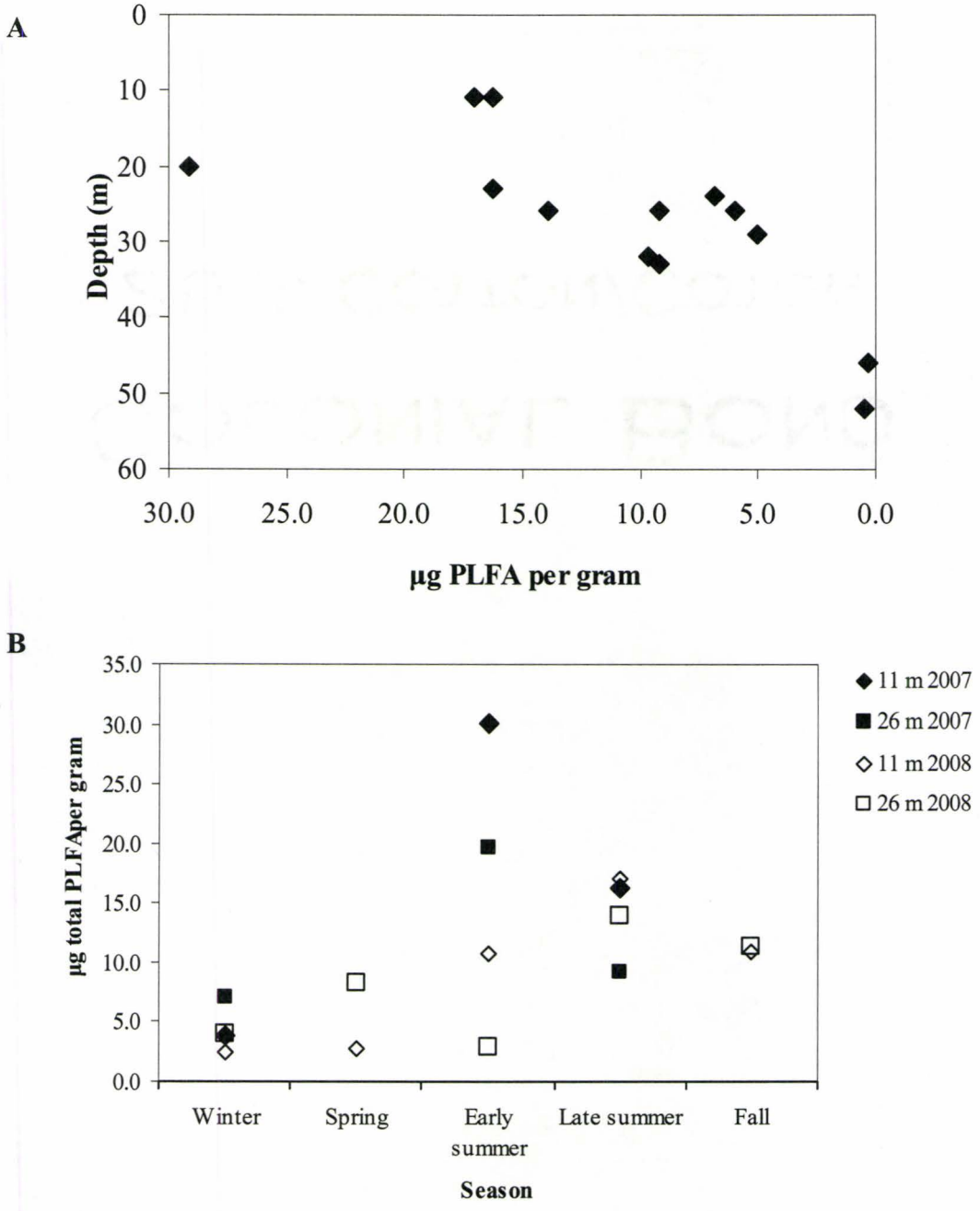

Figure 4.5: a) Variation in biomass as µg total PLFA per gram of extracted samples from microbialites recovered from 11 to 52 m depth showing lower biomass in samples recovered from depths below of 46 m. b) Variation in biomass as µg g total PLFA per gram of extracted sample at depths of 11 m and 26 m from different seasons demonstrating that biomass was higher in samples from 11 m during the summer and 26 m in the cooler winter and spring.

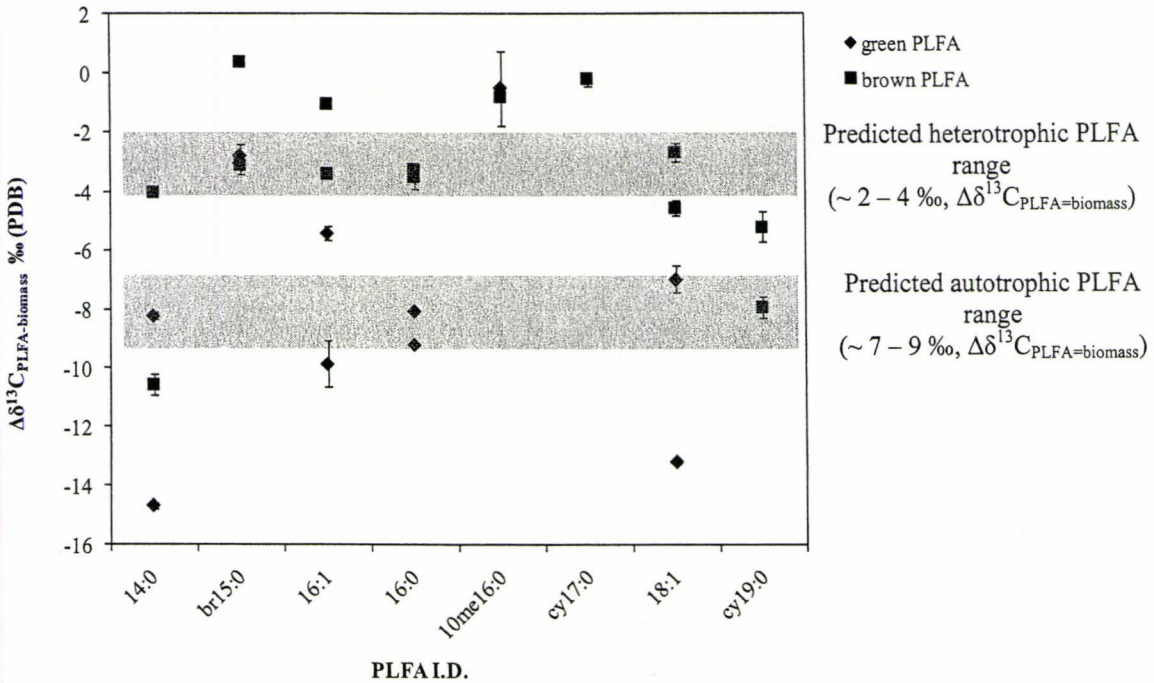

Figure 4.6: Graph illustrating measured $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values for PLFA extracted from green (♦) and brown (■) regions of microbialites showing distinct colour zones. Predicted ranges of autotrophic and heterotrophic offsets are included to illustrate that green region PLFA tend to show larger offsets consistent with autotrophic synthesis while heterotrophic PLFA have smaller offsets.

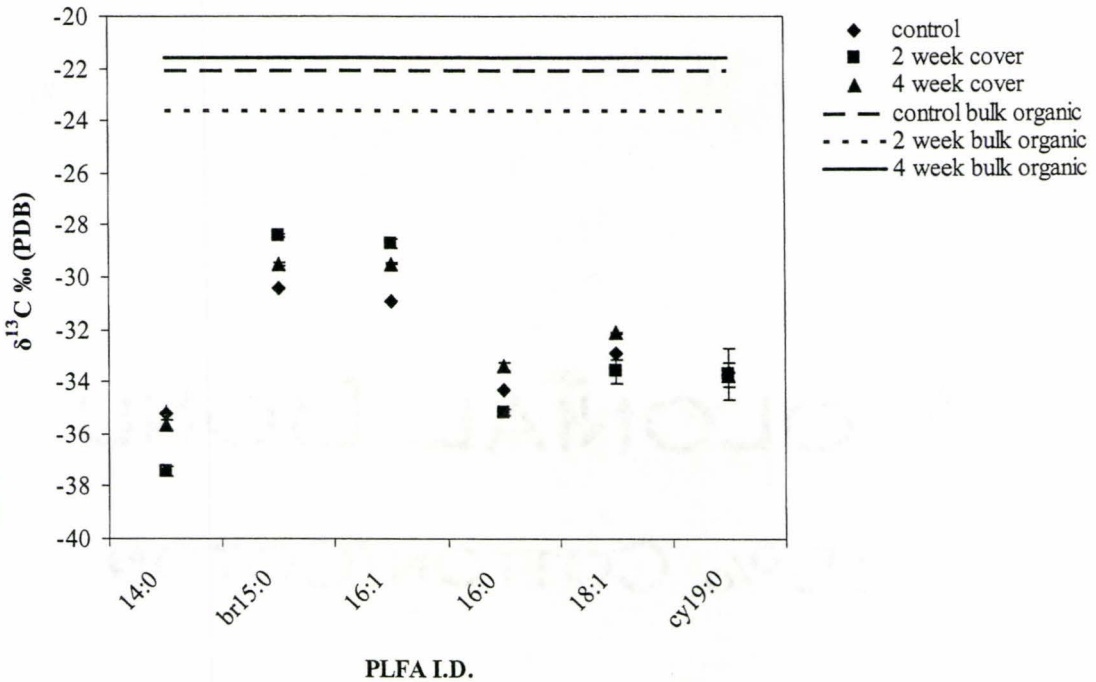

Figure 4.7: Results of light inhibition experiment illustrating that despite coverage for periods of either 2 or 4 weeks, no significant changes in PLFA $\delta^{13}C$ values consistent with a decrease in photosynthetic growth or a shift towards a more heterotrophic dominated community was observed.

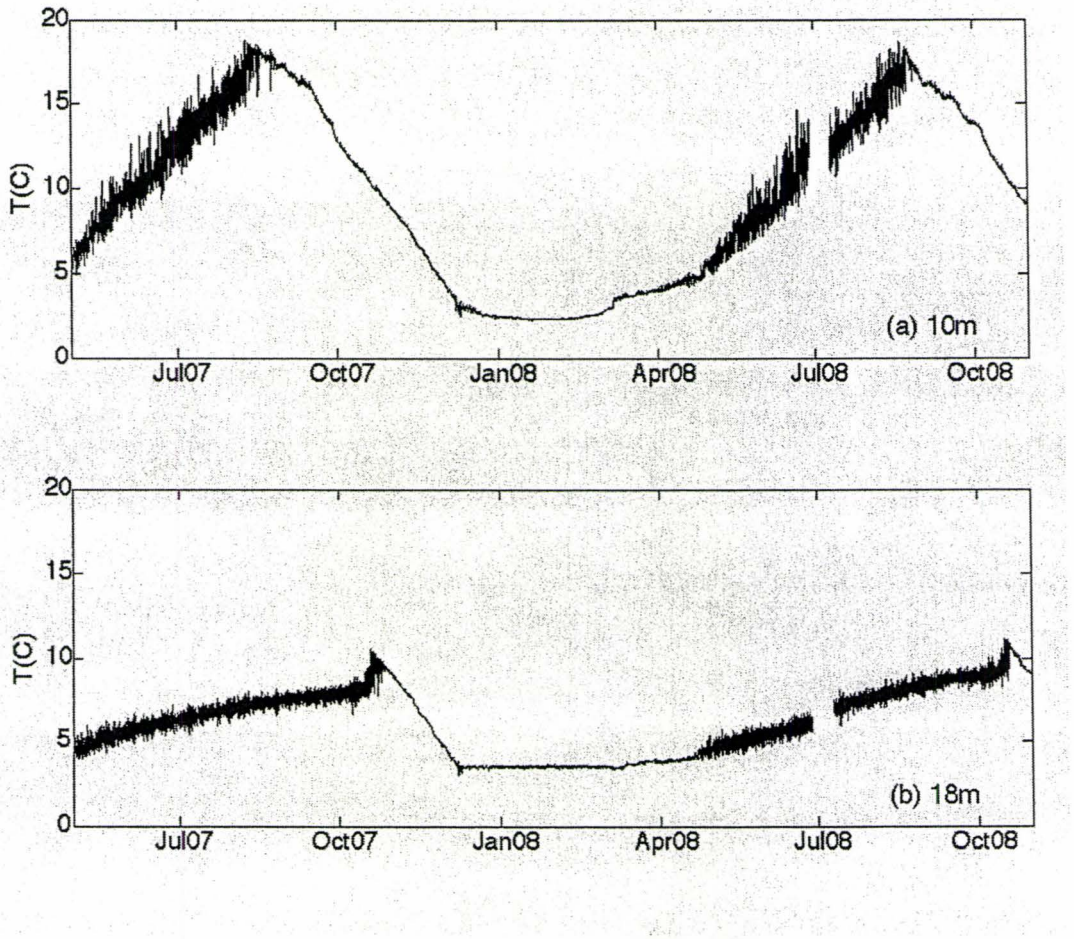

Figure 4.8: Annual temperature variation in Pavilion Lake at depths of a) 10 m and b) 18 m. Summer temperatures are higher at 10 m depth as compared to 18 m and the magnitude of annual temperature variation is less at 18 m.

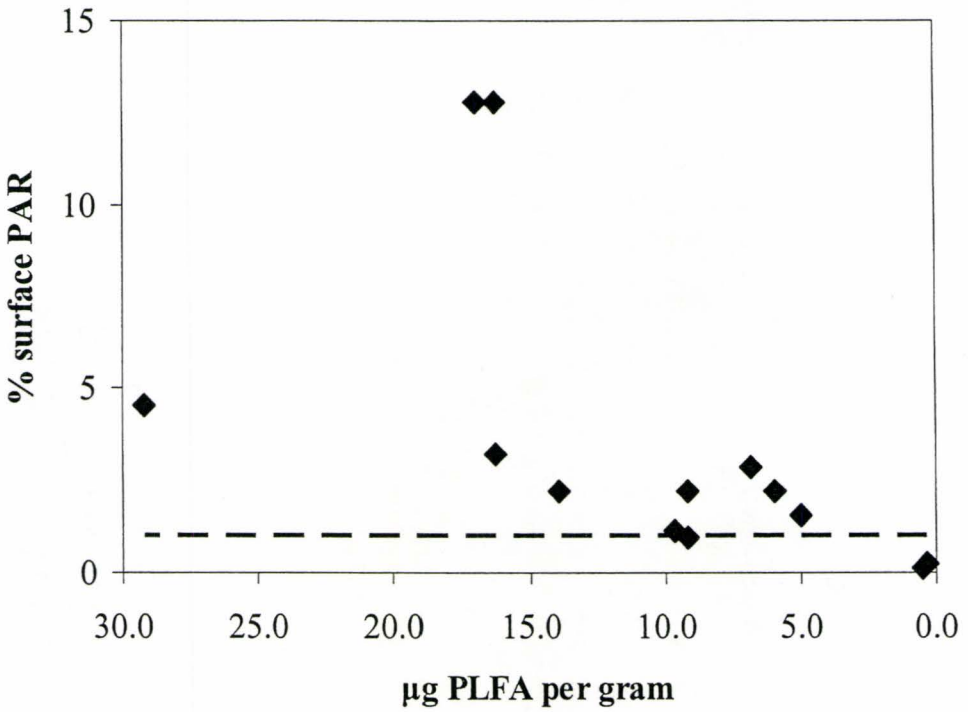

Figure 4.9: Relationship between % of surface photosynthetically active radiation (PAR) and biomass demonstrating that samples with the lowest biomass estimates were at PAR levels well below 1 % (dashed line).

Table 4.1: Isotopic composition of DIC and water samples collected from Pavilion Lake.

Sample	$\delta^{13}C_{DIC}$ ‰ (PDB)	$\delta^{18}O$ ‰ (VSMOW)
02/2005	-2.5	-
02/2005	-2.4	-
08/2005	-1.6	-
06/2006	-3.7	-11.1
08/2006	-1.9	-10.7
06/2007	-1.0	-11.1
08/2007	-1.1	-10.9
10/2007	-1.7	-11.0
02/2008	-0.3	-11.4
04/2008	0.6	-11.2
06/2008	0.4	-11.2
07/2008	0.3	-11.1
10/2008	-0.2	-10.9
mean	-1.2	-11.1
s.d.	1.3	0.2

-, not determined

Table 4.2: Depth, seasonal and isotopic composition of microbialite samples collected for this study. Mean and standard deviation presented for triplicate analysis.

Sample	Collection Date	$\delta^{13}C_{carb}$ ‰ PDB	$\delta^{18}O_{carb}$ ‰ PDB	Predicted Formation Temperature (°C)	$\delta^{13}C_{org}$ ‰ PDB
20 m	08/2005	1.2	-10.6	n.d.	-23.2 ± 2.2
23 m	08/2005	-0.6	-10.7	n.d.	-29.2
24 m	08/2008	1.0 ± 0.2	-9.4 ± 0.1	9.3	-22.1 ± 1.0
26 m	08/2004	-0.6	-10.3	n.d.	n.d.
29 m	08/2006	0.1 ± 0.0	-9.4 ± 0.2	10.6	-27.0 ± 0.3
32 m green	08/2008	-0.4	-9.5	9.9	-27.3
32 m brown	08/2008	-0.2	-10.8	15.0	-29.9 ± 2.1
33 m green	08/2006	0.2	-11.3	18.4	-27.8 ± 0.5
33 m brown	08/2006	-0.2	-10.9	16.8	-26.1 ± 1.7
33 m grey	08/2006	0.7	-10.0	13.0	-26.7
52 m	08/2008	-1.4	-10.8	14.9	-37.6 ± 0.5
W2007 11 m	02/2007	2.1 ± 0.1	-9.7 ± 0.1	-	-23.9 ± 0.5
W2007 26 m	02/2007	0.3 ± 0.0	-9.2 ± 0.1	-	-24.8 ± 2.3
ES2007 11 m	06/2007	1.7	-11.7	18.7	-26.2
ES2007 26 m	06/2007	1.3	-9.2	8.7	-23.7 ± 0.3
LS2007 11 m	08/2007	0.9	-11.1	16.9	-26.5
LS2007 26 m	08/2007	0.7 ± 0.1	-9.2 ± 0.1	9.5	-25.9 ± 1.4
W2008 11 m	02/2008	1.3	-11.7	17.8	-21.1
W2008 26 m	02/2008	0.8	-9.3	8.5	-26.7
Spr2008 11 m	04/2008	1.3	-11.5	17.1	-23.6
Spr2008 26 m	04/2008	0.9	-9.2	8.1	-25.3
ES2008 11 m	06/2008	1.4	-11.4	16.7	-24.9
ES2008 26 m	06/2008	0.8	-9.1	7.9	-23.4 ± 2.5
LS2008 11 m	07/2008	1.5	-9.0	8.0	-22.0
LS2008 26 m	07/2008	0.3 ± 0.1	-9.2 ± 0.2	8.6	-23.3
F2008 11 m	10/2008	2.2 ± 0.2	-10.5 ± 0.2	14.3	-20.5 ± 1.8
F2008 26 m	10/2008	-0.3	-10.3	13.5	-24.1

Table 4.3: Biomass estimates, PLFA distribution and $\delta^{13}C$ values of PLFA from microbialite samples showing distinct colour zones. PLFA distributions are reported in mol % and $\delta^{13}C$ values are mean and standard deviation for triplicate analysis unless otherwise noted.

	20 m Green	20 m Brown/grey	23 m Green	23 m Brown	32 m Green		32 m Brown		33 m Green		33 m Brown		33 m Grey
µg PLFA/g	29.2	1.0	16.3	7.9	9.2		1.1		9.7		3.6		0.8
PLFA I.D.	mol %	mol %	mol %	mol %	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %
br14:0	0.9	0.0	0.7	1.2	0.6		0.0		0.0		0.0		0.0
14:0	2.8	11.6	3.5	2.5	5.5	-35.5 ± 0.1	2.2	-40.5 ± 0.4	5.7	-42.0 ± 0.1	1.5	-30.1 ± 0.1	5.0
i15:0*	2.3	10.0	3.0	4.5	2.9	-30.9 ± 0.4	2.6	-29.5 ± 0.2	2.1	-30.3 ± 0.1	3.6	-29.2 ± 0.3	4.2
a15:0*	3.8	10.2	3.9	4.6	2.7		3.1		2.0		4.5		5.9
15:1	2.4	0.0	0.9	0.0	1.8		0.0		0.0		0.0		0.0
15:0	0.8	0.0	0.7	1.1	0.5		0.0		0.5		0.0		0.0
br16:0	0.0	0.0	0.0	0.0	0.4		0.0		0.0		0.0		0.0
i16:0	1.2	9.1	1.0	1.5	1.4		4.6		0.7		1.2		0.0
16:3	0.0	0.0	0.0	0.0	0.5		0.0		0.8		0.0		0.0
16:2	0.8	0.0	0.9	0.0	0.7		0.0		1.2		0.0		0.0
16:1	14.7	9.8	15.7	16.7	13.6	-32.3 ± 0.2	17.4	-30.9 ± 0.2	13.1	-37.2 ± 0.8	16.1	-29.5 ± 0.2	7.8
16:0	18.2	15.0	20.6	22.3	17.9	-35.4 ± 0.1	14.6	-33.4 ± 0.4	22.1	-36.5 ± 0.1	9.2	-29.3 ± 0.2	9.7
br17:1	0.0	0.0	0.0	0.0	0.4		0.0		0.0		0.0		0.0
10me16:0	1.2	0.0	1.5	3.3	3.0	-27.8 ± 1.3	6.1		2.4		7.2	-26.9 (n = 1)	3.9
br17:0	1.6	0.0	0.8	1.7	1.2		4.4		0.6		2.1		3.2
i17:0	0.9	8.6	0.8	1.5	0.7		4.2		0.5		1.1		0.0
a17:0	1.1	0.0	1.1	1.6	1.0		4.4		0.6		1.3		3.2
17:1	1.6	0.0	0.8	1.4	0.5		0.0		0.6		0.0		0.0
cy17:0	8.7	0.0	9.6	9.9	1.3		9.6	-30.1 ± 0.2	1.4		3.7		3.5
17:0	0.8	0.0	0.6	0.0	0.5		0.0		0.4		0.0		0.0
br18:0	1.1	0.0	0.0	0.0	0.0		0.0		0.0		0.0		0.0
18:3	0.0	0.0	0.0	0.0	2.4		0.0		1.4		0.0		0.0
18:2	4.4	0.0	5.2	1.7	8.0		3.1		8.3		4.3		0.0
18:1	19.2	17.3	20.8	18.5	23.9	-34.3 ± 0.5	14.4	-32.6 ± 0.3	25.5	-40.5 ± 0.1	23.4	-30.6 ± 0.3	35.8
18:0	1.9	8.4	1.7	1.9	1.5		2.4		2.0		5.2		17.7

Table 4.3 continued

	20 m	20 m	23 m	23 m	32 m		32 m		33 m		33 m		33 m
	Green	Brown/grey	Green	Brown	Green		Brown		Green		Brown		Grey
µg PLFA/g	29.2	1.0	16.3	7.9	9.2		1.1		9.7		3.6		0.8
PLFA I.D.	mol %	mol %	mol %	mol %	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %
br19:0	3.0	0.0	2.3	1.5	0.8		2.0		1.2		4.8		0.0
19:1	0.0	0.0	0.0	0.0	0.6		2.0		0.0		4.5		0.0
cy19:0	2.4	0.0	2.7	2.6	1.8		2.9	-35.1 ± 0.6	1.6		6.3	-34.0 ± 0.4	0.0
20:5	0.8	0.0	0.0	0.0	1.0		0.0		1.7		0.0		0.0
20:4	0.8	0.0	0.8	0.0	1.3		0.0		1.6		0.0		0.0
20:3	0.6	0.0	0.0	0.0	0.0		0.0		0.0		0.0		0.0
20:1	1.3	0.0	0.0	0.0	1.3		0.0		1.1		0.0		0.0
20:0	0.6	0.0	0.4	0.0	0.4		0.0		0.8		0.0		0.0

*$\delta^{13}C$ value reported as single value as peaks were not completely resolved

Table 4.4: Total PLFA total biomass, distribution and $\delta^{13}C$ values of microbialite samples from 24 m subjected to light limitation for a period of either two weeks or four weeks and an adjacent microbialite from the same depth that was not subjected to light limitation. Results are for triplicate analysis unless otherwise noted.

	Control		2 week		4 week	
µg PLFA/g	6.8		4.1		6.6	
PLFA I.D.	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**
br14:0	0.6		0.4		0.5	
14:0	2.6	-35.2 ± 0.3	2.9	-34.7 ± 0.2	2.3	-35.7 ± 0.2
br15:0	0.3		0.4		0.3	
i15:0*	3.4	-30.5 ± 0.1	2.8	-28.4 ± 0.1	3.4	-29.5 ± 0.1
a15:0*	3.9		3.3		3.6	
15:1	1.0		0.5		1.1	
15:0	0.2		0.4		0.2	
br16:0	0.2		0.0		0.3	
i16:0	1.2		1.0		1.0	
16:2	0.4		0.6		0.0	
16:1	15.7	-30.9 ± 0.1	12.5	-28.7 ± 0.2	16.2	-29.5 ± 0.0
16:0	15.0	-34.3 ± 0.1	17.8	-35.1 ± 0.1	14.1	-33.4 ± 0.1
br17:1	0.5		0.0		0.0	
10me16:0	5.0		3.1		4.7	
br17:0	2.1		1.8		2.0	
i17:0	0.9		0.7		0.8	
a17:0	1.1		1.0		1.1	
17:1	0.7		1.9		3.2	
cy17:0	2.9		0.9		1.3	
17:0	0.4		0.4		0.4	
br18:0	0.0		1.1		0.5	
18:3	2.1		1.1		0.8	
18:2	4.6		8.3		6.9	
18:1	21.1	-32.9 ± 0.3	23.6	-33.6 ± 0.5	22.7	-32.1 ± 0.0
18:0	1.9		2.3		2.0	
br19:0	1.3		1.1		2.1	
19:1	0.8		1.0		1.2	
cy19:0	3.0	-33.6 ± 0.7	2.7	-33.7 ± 1.0	3.4	-33.7 ± 0.5
20:5	2.6		2.1		1.3	
20:4	3.5		3.0		1.8	
20:1	0.9		1.3		0.9	

*$\delta^{13}C$ value reported as single value as peaks were not completely resolved

Table 4.5: Total PLFA biomass and distribution in mol % of three adjacent microbialite samples collected from 11 m during the same sampling period to assess inter-sample variability.

	LS2007 #1	LS2007 #2	LS2007 #3	
µg PLFA/g	16.3	16.8	8.7	
PLFA I.D.	**mol %**	**mol %**	**mol %**	**Mean ± s.d.**
br14:0	0.4	0.6	0.8	0.6 ± 0.2
14:0	2.0	2.0	1.6	1.9 ± 0.2
br15:0	0.4	0.0	0.0	0.1 ± 0.2
i15:0	3.3	3.0	3.0	3.1 ± 0.2
a15:0	2.9	2.7	2.7	2.8 ± 0.1
15:1	1.1	0.5	0.0	0.5 ± 0.6
15:0	0.3	0.6	0.0	0.3 ± 0.3
br16:0	0.0	0.6	1.3	0.6 ± 0.7
i16:0	0.9	1.1	1.3	1.1 ± 0.2
16:2	0.5	0.7	0.0	0.4 ± 0.4
16:1	15.1	12.4	10.5	12.7 ± 2.3
16:0	14.8	10.9	8.5	11.4 ± 3.2
br17:1	1.2	0.5	0.8	0.8 ± 0.3
10me16:0	4.9	5.5	9.5	6.6 ± 2.5
br17:0	0.4	0.7	1.1	0.7 ± 0.4
i17:0	0.9	1.1	1.5	1.2 ± 0.3
a17:0	1.0	1.3	1.5	1.3 ± 0.3
17:1	0.6	0.5	3.0	1.4 ± 1.4
cy17:0	2.0	1.9	1.4	1.8 ± 0.3
17:0	0.3	0.6	1.1	0.7 ± 0.4
br18:0	0.5	2.0	3.2	1.9 ± 1.3
18:3	0.4	0.0	0.0	0.1 ± 0.2
18:2	7.6	5.5	4.3	5.8 ± 1.7
18:1	28.5	29.4	23.5	27.1 ± 3.2
18:0	2.8	4.2	4.1	3.7 ± 0.8
br19:0	1.5	1.8	2.6	2.0 ± 0.6
19:1	0.8	1.7	2.0	1.5 ± 0.6
cy19:0	2.1	3.8	5.0	3.6 ± 1.4
20:5	1.0	1.2	1.5	1.2 ± 0.3
20:4	0.8	1.0	1.4	1.1 ± 0.3
20:3	0.1	0.0	0.0	0.0 ± 0.1
20:1	0.5	1.2	1.5	1.1 ± 0.5
20:0	0.2	0.8	1.3	0.8 ± 0.6

Table 4.6: Total PLFA total biomass, distribution and $\delta^{13}C$ values of microbialite samples from 11 m and 26 m during winter (W), early summer (ES) and late summer (LS) in 2007. PLFA $\delta^{13}C$ values reported for triplicate analyses unless otherwise noted and all values are reported relative to PDB.

	W2007 11 m		W2007 26 m		ES2007 11 m		ES2007 26 m		LS2007 11 m		LS2007 26 m	
µg PLFA/g	3.8		7.1		30.1		19.7		16.3		9.2	
PLFA I.D.	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰	mol %	$\delta^{13}C$ ‰
br14:0	0.0		1.1		0.4		0.4		0.4		0.6	
14:0	1.6	-36.5 ± 0.1	2.6	-36.5 ± 0.0	1.4	-31.8 ± 0.2	1.9	-33.1 ± 0.2	2.0	-33.7 ± 0.1	2.6	
i15:0*	2.3	-28.6 ± 0.0	4.2	-27.7 ± 0.1	3.4	-27.4 ± 0.1	2.6	-28.1 ± 0.1	3.3	-27.9 ± 0.2	3.2	-29.4 ± 0.1
a15:0*	2.6		5.2		2.8		2.5		2.9		3.5	
15:1	0.0		0.8		1.1		1.4		1.1		1.5	
i16:0	1.2		1.2		1.2		0.9		0.9		1.0	
16:2	0.0		0.0		0.4		1.2		0.5		0.5	
16:1	11.2	-29.7 ± 0.2	13.4	-29.7 ± 0.4	15.9	-28.8 ± 0.3	15.1	-29.9 ± 0.3	15.1	-31.0 ± 0.0	14.7	-31.4 ± 0.1
16:0	14.1	-34.7 ± 0.8	19.3	-34.9 ± 0.1	9.3	-31.0 ± 0.2	12.0	-33.4 ± 0.1	14.8	-33.4 ± 0.3	14.0	-34.9 ± 0.1
br17:1	0.0		0.0		0.5		0.0		1.2		1.4	
10me16:0	5.1	-27.0 ± 0.7	1.8		11.5	-25.6 ± 0.3	3.7	-25.7 ± 0.2	4.9		3.3	-25.6 ± 0.1
br17:0	0.0		0.0		0.8		0.4		1.4		3.2	
i17:0	1.1		0.8		1.1		0.6		0.9		0.7	
a17:0	1.2		1.2		1.1		0.7		0.0		1.0	
17:1	0.0		0.7		5.5		1.4		0.6		0.4	
cy17:0	3.7	-29.5 ± 0.5	1.7		0.2		1.5		2.0		1.9	
br18:0	0.0		0.0		1.5		0.6		0.5		0.2	
18:3	0.0		0.0		0.0		1.4		0.4		0.8	
18:2	6.2		9.6		5.4		8.9		7.6		6.6	
18:1	31.6	-28.7 ± 0.4	23.1	-30.0 ± 0.6	20.3	-30.3 ± 0.0	26.4	-30.8 ± 0.5	28.5	-32.3 ± 0.0	28.4	-32.6 ± 0.4
18:0	2.5	-30.6 ± 0.7	2.0		1.9		1.4		2.8		1.6	
br19:0	2.1		0.9		1.8		1.4		1.5		1.2	
19:1	0.0		1.2		0.8		0.0		0.8		0.0	
cy19:0	4.5	-32.6 ± 0.5	2.2		3.5	-32.9 ± 0.3	2.7	-31.6 (n = 1)	2.1		2.9	
20:5	0.0		0.9		1.4		0.0		1.0		1.1	

Table 4.6 continued

	W2007 11 m		W2007 26 m		ES2007 11 m		ES2007 26 m		LS2007 11 m		LS2007 26 m	
µg PLFA/g	3.8		7.1		30.1		19.7		16.3		9.2	
PLFA I.D.	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**
20:4	1.9		0.9		1.0		2.9		0.8		1.6	
20:1	1.8		0.9		1.1		1.3		0.5		0.4	
20:0	1.2		0.7		0.3		0.3		0.2		0.1	
21:0	1.1		0.0		0.0		0.0		0.0		0.1	
22:6	0.0		0.0		0.0		1.6		0.0		0.0	
22:4	0.0		0.0		0.8		1.5		0.0		0.0	
22:0	1.1		0.7		0.2		0.3		0.1		0.0	

Table 4.7: Total PLFA biomass and distribution reported in mol % of microbialite samples from 11 m and 26 m depth during winter (W), spring (Spr), early summer (ES), late summer (LS) and fall (F) in 2008.

	W2008 11 m	**W2008 26 m**	**Spr2008 11 m**	**Spr2008 26 m**	**ES2008 11 m**	**ES2008 26 m**	**LS2008 11 m**	**LS2008 26 m**	**F2008 11 m**	**F2008 26 m**
µg PLFA/g	2.5	4.0	2.8	8.3	10.7	2.8	17.0	13.9	10.9	11.4
PLFA I.D.	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**
br14:0	0.0	0.6	0.8	0.6	0.5	1.1	0.7	0.4	0.0	0.6
14:0	1.4	2.1	1.4	2.1	1.4	2.9	2.8	2.8	4.3	2.8
br15:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
i15:0	2.6	3.3	6.8	2.9	2.2	3.7	3.2	4.0	2.2	3.5
a15:0	2.6	3.9	2.9	4.4	3.1	4.2	3.6	4.3	1.9	3.7
15:1	0.0	0.0	1.3	0.4	0.0	0.0	0.0	0.7	0.0	1.5
15:0	1.0	0.0	1.0	0.4	0.4	0.0	0.5	0.6	0.0	0.4
i16:0	1.4	1.3	1.4	1.1	1.0	1.7	1.1	1.3	1.1	1.2
16:2	0.0	0.0	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.4
16:1	12.1	14.7	22.3	17.1	22.8	12.5	16.0	17.2	15.7	18.2
16:0	12.4	17.7	15.0	16.7	22.0	16.3	19.5	14.9	20.0	18.7
br17:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10me16:0	5.7	3.6	3.0	3.6	2.8	4.4	4.0	6.8	2.7	3.3
br17:0	2.1	1.6	0.0	2.2	1.4	3.6	0.6	2.6	0.0	1.8
i17:0	1.6	1.2	1.1	1.0	0.9	1.5	1.0	1.1	1.0	0.9
a17:0	1.9	1.7	1.4	1.4	1.1	1.9	1.3	1.4	1.2	1.1
17:1	2.8	1.4	0.0	0.8	0.0	2.0	0.7	0.8	0.0	1.1
cy17:0	2.8	1.8	1.3	2.6	1.1	1.7	2.0	1.8	1.4	1.9
17:0	2.1	0.8	1.0	0.9	0.6	1.2	0.7	0.8	0.6	0.5
br18:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:3	0.0	0.0	0.0	0.9	0.0	0.0	1.8	1.1	0.9	0.9
18:2	13.6	4.3	3.0	5.4	3.0	5.4	5.8	4.7	6.2	5.3
18:1	25.2	29.0	22.1	23.4	27.0	21.9	24.9	24.2	30.4	21.0
18:0	3.6	2.9	2.0	2.3	2.4	3.0	2.6	1.8	3.0	1.9
br19:0	0.0	1.7	0.0	1.5	1.3	2.8	1.2	0.5	0.6	1.1
19:1	0.0	1.5	1.5	1.1	0.8	0.0	1.1	1.0	1.3	0.7

Table 4.7 continued

	W2008 11 m	**W2008 26 m**	**Spr2008 11 m**	**Spr2008 26 m**	**ES2008 11 m**	**ES2008 26 m**	**LS2008 11 m**	**LS2008 26 m**	**F2008 11 m**	**F2008 26 m**
µg PLFA/g	2.5	4.0	2.8	8.3	10.7	2.8	17.0	13.9	10.9	11.4
PLFA I.D.	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**
cy19:0	5.0	3.4	1.8	2.6	2.4	4.0	2.3	2.6	2.3	1.9
20:5	0.0	0.0	0.0	1.2	0.6	1.3	0.9	0.9	1.5	1.5
20:4	0.0	0.0	0.0	0.0	0.6	1.4	0.8	1.0	1.0	1.2
20:3	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.5
20:1	0.0	1.7	0.0	1.1	0.6	1.4	0.8	0.9	0.9	1.2
20:0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.3
dimethyl21:0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
24:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3

Table 4.8: a) Total PLFA biomass and distribution reported in mol % for microbialite samples collected from depths between 11 m and 52 m. A representative 11 m sample is included from late summer 2007, the same seasonal period in which the other samples were collected.

	11 m (LS07)	**20 m**	**23 m**	**24 m**	**26 m**	**29 m**	**32 m**	**33 m**	**46 m**	**52 m**
µg PLFA/g	16.3	29.2	16.3	6.8	6.0	5.0	9.7	9.2	0.3	0.5
PLFA I.D.	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**
br14:0	0.4	0.9	0.7	0.6	1.6	0.0	0.6	0.0	0.0	0.0
14:0	2.0	2.8	3.5	2.6	5.2	3.6	5.5	5.7	9.0	2.4
br15:0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
i15:0	3.3	2.3	3.0	3.4	2.2	2.8	2.9	2.1	8.8	2.9
a15:0	2.9	3.8	3.9	3.9	2.2	2.6	2.6	2.0	9.8	2.8
15:1	1.1	2.4	0.9	1.0	3.1	0.0	1.8	0.0	0.0	0.0
15:0	0.0	0.8	0.7	0.2	1.5	0.0	0.5	0.5	0.0	0.0
br16:0	0.0	0.0	0.0	0.2	1.4	0.0	0.4	0.0	0.0	1.7
i16:0	0.9	1.2	1.0	1.2	1.4	1.1	1.4	0.7	8.1	0.0
16:3	0.0	0.0	0.0	0.0	1.6	0.0	0.5	0.8	0.0	0.0
16:2	0.5	0.8	0.9	0.4	1.9	0.0	0.7	1.2	0.0	0.0
16:1	15.1	14.7	15.7	15.7	16.5	12.6	13.6	13.1	17.0	26.3
16:0	14.8	18.2	20.6	15.0	17.9	16.5	17.9	22.1	13.2	11.5
br17:1	1.2	0.0	0.0	0.5	0.0	0.0	0.4	0.0	0.0	0.0
10me16:0	4.9	1.2	1.5	5.0	1.3	2.8	3.0	2.4	7.7	9.1
br17:0	1.4	1.6	0.8	2.1	3.2	0.9	1.2	0.6	0.0	0.0
i17:0	0.9	0.9	0.8	0.9	1.3	0.9	0.7	0.5	8.3	5.2
a17:0	0.0	1.1	1.1	1.1	1.4	1.1	1.0	0.6	0.0	5.1
17:1	0.6	1.6	0.8	0.7	1.4	0.0	0.5	0.6	0.0	2.5
cy17:0	2.0	8.7	9.6	2.9	1.5	1.7	1.3	1.4	10.1	2.5
17:0	0.0	0.8	0.6	0.4	1.3	0.6	0.5	0.4	0.0	0.0
br18:0	0.5	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:3	0.4	0.0	0.0	2.1	0.0	2.2	2.4	1.4	0.0	0.0
18:2	7.6	4.4	5.2	4.6	7.5	7.3	8.0	8.3	0.0	5.0
18:1	28.5	19.2	20.8	21.1	17.6	29.1	23.9	25.5	8.1	10.5
18:0	2.8	1.9	1.7	1.9	1.5	2.9	1.5	2.0	0.0	2.7

Table 4.8 continued

	11 m (LS07)	20 m	23 m	24 m	26 m	29 m	32 m	33 m	46 m	52 m
µg PLFA/g	16.3	29.2	16.3	6.8	6.0	5.0	9.7	9.2	0.3	0.5
PLFA I.D.	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**	**mol %**
br19:0	1.5	3.0	2.3	1.3	0.0	0.0	0.8	1.2	0.0	2.0
19:1	0.8	0.0	0.0	0.8	1.3	1.8	0.6	0.0	0.0	0.0
cy19:0	2.1	2.4	2.7	3.0	1.5	3.1	1.8	1.6	0.0	4.4
20:5	1.0	0.0	0.0	2.6	0.0	2.0	1.0	1.7	0.0	0.0
20:4	0.8	1.6	0.8	3.5	1.2	2.8	1.3	1.6	0.0	0.0
20:3	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1	0.5	1.3	0.0	0.9	1.4	1.6	1.3	1.1	0.0	0.0
20:0	0.2	0.6	0.4	0.0	0.0	0.0	0.4	0.8	0.0	3.5

Table 4.8: b) PLFA $\delta^{13}C$ values for samples collected from 11 m to 52 m. Representative 11 m sample was collected during late summer 2007, the same seasonal period in which the other samples were collected. PLFA $\delta^{13}C$ values reported for triplicate analyses unless otherwise noted and all values are reported relative to PDB.

	11 m (LS07)	20 m	23 m	24 m	26 m	29 m	32 m	33 m	52 m
PLFA I.D.	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**	**$\delta^{13}C$ ‰**
14:0	-33.7 ± 0.1	-37.4 ± 0.3	-40.4 ± 0.1	-35.2 ± 0.3	-42.1 ± 0.1	-35.1 ± 0.1	-35.5 ± 0.1	-42.0 ± 0.1	-42.0 ± 0.1
i15:0* a15:0*	-27.9 ± 0.2	-29.1 ± 0.2	-30.1 ± 0.1	-30.5 ± 0.1	-30.1 ± 0.2	-30.9 ± 0.1	-30.9 ± 0.4	-30.3 ± 0.1	-29.0 ± 0.2
16:1	-31.0 ± 0.0	-29.2 ± 0.5	-31.8 ± 0.1	-30.9 ± 0.1	-31.1 ± 0.3	-31.3 ± 0.4	-32.3 ± 0.2	-37.2 ± 0.8	-33.8 ± 0.2
16:0	-33.4 ± 0.3	-36.0 ± 0.2	-33.5 ± 0.3	-34.3 ± 0.1	-34.3 ± 0.3	-36.2 ± 0.2	-35.4 ± 0.1	-36.5 ± 0.1	-39.6 ± 0.0
10me16:0					-28.9 (n = 1)		-27.8 ± 1.3		
cy17:0		-28.0 ± 0.7	-32.6 ± 0.2		-32.4 ± 0.2				
18:1	-32.3 ± 0.0	-32.0 ± 0.4	-33.0 ± 0.3	-32.9 ± 0.3	-33.7 ± 0.3	-34.1 ± 0.1	-34.3 ± 0.5	-40.5 ± 0.1	-36.9 ± 0.2
18:0									-32.3± 0.3
cy19:0		-33.9 ± 0.1	-34.7 ± 0.4	-33.3 ± 0.4	-33.5 ± 1.9	-36.4 ± 0.1			-34.3 ± 0.0

*$\delta^{13}C$ value reported as single value as peaks were not completely resolved

CHAPTER 5

CARBON CYCLING IN CARBONATE RICH, CYANOBACTERIA DOMINATED MICROBIAL MATS OF THE CARIBOO PLATEAU, B.C.

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ABSTRACT

Carbonate rich microbial mats located in saline, alkaline lakes on the Cariboo Plateau, B.C. showed extensive photosynthetic activity resulting in pCO_2 below equilibrium during the summer and DIC $\delta^{13}C$ values enriched up to +6.0 ‰ above values predicted for isotopic equilibrium with atmospheric CO_2. Carbonate $\delta^{13}C$ values exhibited enrichments of up to 3.8 ‰ and depletion of up to 11.6 ‰ relative to the DIC. Within an individual lake, carbonate $\delta^{13}C$ values had an overall range of ~ 4 to 8 ‰ over the course of this study. Microbial phospholipid fatty acid (PLFA) profiles supported the presence of a prokaryotic community consisting of both photosynthetic and heterotrophic microbes. Variations in the relative contributions of photosynthetic and heterotrophic carbon inputs and outputs led to seasonal and annual variability in DIC $\delta^{13}C$ and carbonate $\delta^{13}C$ values.

Microelectrode profiling within microbial mats identified oxygenated zones and zones of oxygen depletion and sulfide production. The $\delta^{13}C$ value of carbonate within the photosynthetic zone was within 0.2 ‰ of the surface DIC, consistent with a biosignature of photosynthetic activity on the lake DIC. In contrast, carbonate $\delta^{13}C$ associated with the heterotrophic zones were depleted relative to the DIC by 5 ‰ indicating that heterotrophic inputs via sulfate reduction were controlling the carbonate $\delta^{13}C$ values within this zone. Sulfate reduction within the mats was supported by observation of sulfide production within the anoxic zones of the mats and PLFA profiles with high proportions of monoenoic 16:1 and 18:1 and sulfate reducing bacteria

biomarkers that were depleted relative to the bulk cell by ~ 4 ‰, consistent with heterotrophic synthesis.

Despite extensive photosynthetic drawdown of CO_2 in the lakes, bulk organic values in the Cariboo lakes ranged from a mean of -18.7 ± 0.1 to -25.3 ± 1.0 ‰ with mean $\Delta^{13}C_{inorg\text{-}org}$ values that ranged from 21.1 to 24.2 ‰, consistent with non-CO_2 limited photosynthesis. These observations are in contrast to the saline, organic rich mat and hot spring microbial mats that have been extensively studied. The observation of such large photosynthetic isotopic discriminations in these microbial mat systems suggests that the observation of Precambrian $\delta^{13}C$ values around -26 ‰ does not necessitate atmospheric CO_2 higher than today. Rather, low bulk organic $\delta^{13}C$ values and large isotopic discriminations can be observed in microbial mat systems where high dissolved inorganic carbon (DIC) concentrations and carbonate content provide a non limiting carbon source to replenish photosynthetic drawdown.

5.1. INTRODUCTION

Modern microbial mats form complex, typically layered communities in which major biochemical metabolic pathways exist in close proximity and over short vertical distances (Canfield and Des Marais, 1993; van Germerden, 1993; Paerl and Pinckney, 1996). Cyanobacteria are typically the major microbial group in most microbial mat systems and are the primary producers, supporting a host of other organisms employing different metabolisms. Other major groups include purple sulfur bacteria, colourless sulfur bacteria, sulfate reducing bacteria and methanogens (Stal et al., 1985; Paerl et al.,

2000; Bebout et al., 2004). Microbial mats are capable of growing under extreme environmental conditions including hypersaline (e.g. Bauld, 1981; Des Marais et al., 1989; Pinckney and Paerl, 1997), high temperature (e.g. van der Meer et al., 2000) and polar regions (e.g. Jungblut et al., 2009).

Modern microbial mats are considered analogues of early ecosystems and stromatolite forming communities, although many do not lead to stromatolite formation (Schidlowski et al., 1992; Riding, 2000). Stromatolites are lithified layered organo-sedimentary structures that are found throughout the geologic record as far as ~ 3.4 billion years ago and are believed to some of the earliest evidence for life on Earth (Schopf et al., 1971; Walter et al., 1980; Awramik, 1992; Riding, 2000). The restriction of modern stromatolites and microbial mats to extreme environments where grazing activity is limited (Logan, 1961; Rasmussen et al., 1993) has lead to the suggestion that the decline in stromatolites abundance coincides with the rise of metazoan grazers and the corresponding predation of microbial mats (Walter and Heys, 1985). Further, the observation of ^{13}C-enriched organic matter in modern microbial mats in contrast to the relatively ^{13}C-depleted organic material observed in ancient stromatolitic systems has lead to the suggestion that this is a biosignature of atmospheric CO_2 levels that were higher in the Precambrian than they are today (Mizutani and Wada, 1982; Schidlowski, 1985; Des Marais et al., 1989). However, previous studies have focused on non-lithifying organic rich microbial mats such as those growing in salt ponds of Guererro Negro and Solar Lake (e.g. Des Marais et al., 1989; Schidlowski et al., 1992) and hot spring mats in Yellowstone National Park (e.g. Estep, 1984; van der Meer et al., 2000).

In contrast to these studies, the carbonate rich microbial mats found in the saline, alkaline lakes of the Cariboo Plateau, British Columbia present an opportunity to investigate a distinct system to contribute to our understanding of biosignatures within microbial mats and their implication to the geologic record. Carbonate rich microbial mats have been proposed as important potential stromatolite analogue systems due to expected differences in photosynthetic activity and the potential for non-CO_2 photosynthesis (Des Marais, 1992). Beyond understanding earth history, understanding microbial biosignatures associated with carbonates is also important as carbonates have recently been identified on Mars that are believed to have formed in an aqueous environment (Bandfield et al., 2003; Ehlmann et al., 2008). The Cariboo Plateau mats thus provide a unique comparative system to the organic rich and hot spring mats and an opportunity to examine potential differences in microbial community structure, variation in isotopic composition of dissolved inorganic carbon (DIC) and associated carbonate precipitates and related isotopic discriminations.

5.1.1. Microbial mat carbon cycling

The development of biosignatures associated with microbial mat systems is primarily controlled by the nature and extent of microbial metabolic activities. Carbon dioxide uptake by photosynthesis and release via respiration are major processes by which microbial mat communities may influence the DIC concentrations and isotopic composition in lake systems. Microbial heterotrophic and autotrophic metabolic activity has been shown to induce changes in the local geochemical environment leading to the

promotion of calcium carbonate ($CaCO_3$) precipitation (Revsbech et al., 1983; Merz, 1992; Thompson et al., 1997; Merz-Preiß and Riding, 1999; Merz-Preiß, 2000). These metabolic processes may also influence the isotopic composition of the dissolved inorganic carbon and the $\delta^{13}C$ value of carbonate precipitated from this carbon pool will reflect those microbial effects on the isotopic composition (Merz, 1992; Sumner, 2001). The direction of deviations in measured $\delta^{13}C$ carbonate values from the predicted equilibrium values provides a biosignature of autotrophic or heterotrophic microbial influence on precipitation (McConnaughey, 1989b; Merz, 1992; Ferris et al., 1997; McConnaughey et al., 1997; Thompson et al., 1997; Hodell et al., 1998). High levels of photosynthetic activity have been shown to result in CO_2 undersaturation with respect to atmospheric levels of freshwater lakes (McConnaughey et al., 1994) and experimental ponds (Portielje and Lijklema, 1995) due to uptake rates that are faster than replenishment from the atmosphere or organic matter mineralization. At the same time, biological preference for ^{12}C during photosynthesis leads to incorporation of the lighter isotope into cell biomass and a corresponding enrichment in ^{13}C of the residual DIC and precipitated carbonate (O'Leary, 1988; Hollander and McKenzie, 1991). Carbonates enriched in ^{13}C have been reported from freshwater lakes with high levels of photosynthetic activity (Hollander and McKenzie, 1991; Thompson et al., 1997). Conversely, heterotrophic decomposition of organic matter results in little fractionation, leading to the formation of CO_2 similar in isotopic composition to the source organic matter (Blair et al., 1985; Abraham et al., 1998). Thus, heterotrophic degradation of ^{13}C-

depleted organic matter inputs correspondingly low $\delta^{13}C$ CO_2 leading to a ^{13}C-depletion in the residual DIC and precipitated carbonate (Andres et al., 2006; Breitbart et al., 2009).

Analysis of the distribution and $\delta^{13}C$ values of microbial phospholipid fatty acids (PLFA) provides a tool to examine *in situ* carbon cycling and identification of active autotrophic and heterotrophic metabolic activity (Vestal and White, 1989; Abraham et al., 1998). Phospholipids are membrane components that are known to degrade rapidly upon death (White et al., 1979) and specific fatty acids have been shown to be linked to certain microbial groups (Vestal and White, 1989; Zelles, 1999; Boschker and Middelburg, 2002). PLFA analysis has been used to assess microbial diversity within modern environmental systems such as eutrophic bays (e.g. Rajendran et al., 1992); sediments (e.g. Rajendran et al., 1995; Fang et al., 2006); and modern microbial mats (e.g. Navarrete et al., 2000; Zhang et al., 2005; Scherf and Rullkötter, 2009). In contrast to bulk cell isotopic composition, PLFA may be used to link carbon sources to specific microbial groups and metabolic pathways (Summons et al., 1994; Abraham et al., 1998; Boschker et al., 1998; Boschker and Middelburg, 2002; Londry and Des Marais, 2003; Gu et al., 2004; Boschker et al., 2005; Zhang et al., 2005). PLFA in photosynthetic cyanobacteria are typically depleted by ~ 7 – 9 ‰ compared to bulk cell $\delta^{13}C$ values (Sakata et al., 1997; Jahnke et al., 2004) while heterotrophic microbes have PLFA depleted relative to the bulk cell value by ~ 2 – 4 ‰ (Monson and Hayes, 1982; Blair et al., 1985; Abraham et al., 1998; Teece et al., 1999).

The isotope discrimination between DIC (inorganic) and bulk organic carbon pools ($\Delta^{13}C_{inorg\text{-}org} = \delta^{13}C_{inorg} - \delta^{13}C_{org}$) provides additional information regarding

microbial carbon utilization pathways and/or growth conditions including CO_2 limitation (O'Leary, 1988; Hollander and McKenzie, 1991; van der Meer et al., 2000; Schouten et al., 2001). Microbial mats in non-lithifying, hypersaline and hot spring environments have been noted to produce organic matter with highly enriched $\delta^{13}C$ values typically ranging from -10 to -18 ‰, but up to -5 ‰ in some cases (Schidlowski et al., 1984; Des Marais et al., 1989; Schouten et al., 2001; Scherf and Rullkötter, 2009). ^{13}C-enriched organic matter has been attributed to CO_2 limitation during photosynthesis or in some cases, to microbial metabolisms that do not use the Calvin cycle for CO_2 fixation (Preuß et al., 1989; van der Meer et al., 1998; van der Meer et al., 2000). As CO_2 concentrations become limited, the observed discrimination between the inorganic and organic carbon pools decreases due to increasingly quantitative conversion resulting in organic matter depleted relative to DIC by less than 25 ‰ (O'Leary, 1988; Hollander and McKenzie, 1991; Hodell and Schelske, 1998). Environmental factors such as salinity and reductions in CO_2 solubility have been linked to CO_2-limited environments in modern microbial mats in order to explain the observation of ^{13}C-enriched organic matter (Schidlowski et al., 1984; Des Marais et al., 1989; Schouten et al., 2001). However further research has demonstrated that other factors must also play a role as no consistent trends have been identified in microbial mats at varying salinities (Des Marais et al., 1989; Schidlowski et al., 1994; Scherf and Rullkötter, 2009). Microbial utilization of different carbon fixation pathways, such as the 3-hydroxypropionoate pathway and reversed tricarboxylic acid (TCA) cycle have been proposed to account for elevated bulk organic $\delta^{13}C$ values in hot spring microbial mats from Yellowstone National Park that are composed of

cyanobacteria and anoxygenic photosynthetic bacteria (Ward et al., 1998; van der Meer et al., 2000). Both of these autotrophic pathways have small fractionations between inorganic carbon and bulk organic biomass relative to the Calvin cycle (Quandt et al., 1977; Preuß et al., 1989) leading to ^{13}C-enriched biomass (van der Meer et al., 1998; van der Meer et al., 2000). In contrast to values often observed in organic rich modern microbial mats, organic matter $\delta^{13}C$ values from the geologic record are closer to -26 ‰ (Eichmann and Schidlowski, 1975; Schidlowski, 1988; Schidlowski et al., 1994) typical of photosynthesis. These observations have been used to propose higher atmospheric CO_2 concentrations during the Precambrian allowing for non-CO_2 limited photosynthesis (Mizutani and Wada, 1982; Schidlowski, 1985; Des Marais, 1992).

Carbonate rich microbial mats provide an important comparative system to organic rich and hot spring microbial mats in which to gain an understanding of carbon cycling and associated biosignatures. Variation in the microbial community or level of metabolic activity in these systems could lead to the identification of distinct isotopic signatures that further contribute to our understanding of the genesis of carbonate and organic isotope values recovered from the geologic record. Interpretation of the isotopic biosignatures associated with ancient microbial mats also requires an understanding of seasonal and annual variability in modern microbial ecosystems and associated biosignatures because comparable variability would be expected to have influenced similar signals preserved in the geologic record. Investigation of modern, evaporative microbial mats provides insight into the range of inorganic and organic carbon $\delta^{13}C$ values that might be expected in evaporative systems on Mars, thereby contributing to

our ability to interpret any biosignatures of past life on Mars. To further our ability to understand carbon isotope biosignatures, this study focuses on annual and seasonal variability in inorganic and organic biosignatures of the carbonate rich Cariboo Plateau microbial mats and implications for interpreting the geologic record.

5.2. SAMPLING AND LABORATORY ANALYSIS

5.2.1. Study Site

The Cariboo Plateau is an arid region with elevations ranging from 1050 to 1250 m located in the interior of British Columbia (Figure 5.1) (Renaut and Long, 1989). Most of the Plateau is underlain by Miocene and Pliocene basalt flows and the region was last glaciated between 10,000 and 9,500 years ago leaving glacial till 1 to 5 m thick. Several hundred saline lakes exist in this area ranging from large freshwater lakes to small hypersaline lakes with total dissolved solids (TDS) greater than 350 g/L. Most are small, shallow groundwater and precipitation fed lakes, occupying depressions within glacial deposits, typically poor in Ca and Mg but dominated by Na-CO_3-(SO_4) (Renaut and Long, 1989; Renaut, 1990). Carbonate deposits have been identified within the microbial mats in the lakes and in surrounding mudflats (Renaut and Long, 1989). The depth of the water varies with the season and is usually greatest in spring and early summer and lowest in late August and fall with most of the lakes frozen between November and April. Freezing of the lakes, spring precipitation and summer evaporation leads to variability in the salinity of the water (Renaut and Long, 1989; Renaut, 1990). Within these evaporative lakes, filamentous and coccoid cyanobacteria dominate and there is extensive

formation of carbonate rich microbial mats but no persistent stromatolitic structures are present (Schultze-Lam et al., 1996). Based on previous data, three of these lakes were chosen for this study that provided varying geochemistry particularly with respect to sulfate and methane concentrations: Probe Lake (PL-M), Deer Lake (DL-M) and Goodenough Lake (GEL-M) (Slater, 1997).

5.2.2. Field Sampling

5.2.2.1. Microbial mat and water chemistry collection and characterization

Microbial mat samples were collected maximally 4 times a year: Spring (Spr: April), Early Summer (ES: early June), Late Summer (LS: late July or early August) and Fall (F: October) over the course of 3 years. Mats were collected in combusted, organic-free glass jars and frozen prior to transport on dry ice to McMaster University. Mats were lyophilized at McMaster.

Parameters including temperature, salinity and pH were determined in the field using a hand-held YSI instrument (YSI Incorporated, Yellowsprings, OH). Water samples were collected for chemical analyses using protocols provided by the Environment Canada, Pacific Environmental Science Centre (PESC) in Vancouver, Canada. Samples were kept cool and dark prior to shipment to the PESC labs for analysis. Saturation indices (S.I.) were determined according to the relationship:

$$S.I. = \log (IAP/K_{sp}) \quad (1)$$

where IAP is the ion activity product and K_{sp} is the solubility product of the mineral of interest using measured pH, temperature, DIC and ionic concentrations and the program

PHREEQC (Parkhurst and Appelo, 1999). Spring and Goodenough Lake late summer 2007 Ca and Mg concentrations were not determined and were estimated based on the ratio of these ions to Cl as a conservative tracer during other sampling periods Table 5.1. Flux of CO_2 between the lakes and atmosphere was calculated using the following relationship:

$$\text{Flux (J)} = D \times \partial C/\partial z \times f \quad (2)$$

where D is the dissolution coefficient (cm^{-2} s^{-1}) (Jähne et al., 1987), ∂C is the difference in concentration between $[CO_2]$ in equilibrium with atmospheric pCO_2 and measured dissolved aqueous CO_2 as calculated using PHREEQC, z is the thickness of the boundary layer (estimated at 200 μm as per (Kling et al., 1992) and f represents an enhancement factor that reflects increased diffusion with increased pH and DIC concentration and was assumed to be 3 for the purposes of this study (Emerson, 1975; Portielje and Lijklema, 1995). Equilibrium concentrations of dissolved CO_2 were determined according to Henry's law:

$$[CO_2] = K_h * pCO_2 \quad (3)$$

where K_h represents a dissolution constant of CO_2 that varies with temperature and salinity and pCO_2 represents atmospheric CO_2 concentrations (atm) (Langmuir, 1997).

Microelectrode profiling of oxygen and sulfide concentrations within the microbial mats used preformed glass Au-Hg amalgam electrodes (with tips drawn to ~500 micron diameter) constructed in the lab according to methods published in (Brendel and Luther, 1995) and lowered vertically in 1 – 5 mm increments using a manual micromanipulator. A sequence of ten cyclic voltammograms (-0.1 to -1.8 V vs. Ag/AgCl

at 1V/sec with 2 second deposition at -0.1 V) was obtained from each electrode at each depth using DLK-60 (AIS Instruments) software. The current response for signals of the last 5 scans of each sequence were measured and averaged (AIS Instruments DLK-60 Analysis program). The instrumental variability between measurements is extremely small (typically less than 1 %). The electrodes were calibrated using 2-point O_2 calibrations (air-saturated and N_2 purged), standard additions of freshly prepared Na_2S*9H_2O, and standard additions of MnCl to N_2 purged water, with calibration for other ions, relative to Mn^{2+}, accomplished using the pilot ion method (Brendel and Luther, 1995).

Porewater chemistry was collected using dialysis chambers or 'peepers' installed in the upper 30 cm of the sediment. Each set consisted of two peepers, with chambers 20 cm long by 1 cm wide by 1 cm deep machined into polypropylene boards fitted with nylon machine screws. Prior to placement in each lake, chambers were filled in the field with He-sparged water and covered with a Suprapor membrane (3 mil Teflon) and a second polypropylene board with small holes machined in that corresponded to the location of the 1x 1 cm chambers. Upon retrieval of the peepers, approximately 20 ml of porewater was removed from each chamber using a 22 gauge disposable needle fitted to 60 ml plastic syringe. For methane sampling from surface water and porewater, a syringe gas phase equilibration technique was used following (Rudd et al., 1974). A volume of He gas equivalent to the water removed from the port was added to the syringe. The sample and helium mixture was then vigorously shaken for 3 to 5 minutes in order to completely partition the volatile CH_4 into the gas phase. The gas phase was then

transferred into a previously prepared crimp sealed 60 mL serum vial filled with He-sparged distilled water. A surface water sample was collected in the same manner from each lake. For methane isotope sampling, water from 4 – 5 ports were combined due to low concentrations and sample size requirements. Volumes of water were removed using a syringe as described above and injected into an evacuated 125 ml serum bottle crimp sealed with a blue butyl stoppers that were boiled in a 0.1 N NaOH solution for 45 min and soaked in distilled water overnight were used in order to eliminate any potential contamination. Porewater chemistry was determined using a peeper with a 0.45 μm PVDF membrane, water from individual chambers was collected using the above described 60 ml syringe and was analyzed in the same manner as surface water samples by PESC.

Methane concentration was determined using an SRI GC (SRI Instruments, Torrance, CA) equipped with a 3 m packed silica column and flame ionization detector (FID). Column conditions were held isothermal at a temperature of 30°C with He carrier gas with concentrations determined using calibration curves created from injections of known volumes of methane reference gas. Reproducibility was better than 5 % for triplicate samples.

5.2.2.2. Isotope sampling

Water samples for ^{13}C analysis were collected in crimp sealed glass serum bottles with no headspace and fixed with mercuric chloride to prevent further microbial activity.

Porewater samples for ^{13}C analysis were collected in 15 ml acid rinsed Nalgene bottles, sealed with no headspace and fixed with mercuric chloride.

5.2.3. Laboratory Analysis

5.2.3.1. Microbial mat characterization

Loss on ignition (LOI) of microbial mats was conducted to measure the percentage of organic and inorganic content in the samples. Organic loss was determined by the mass difference from the original dry weight after combustion at 550°C for one hour (Dean, 1974; Howard and Howard, 1990). Triplicate analysis reproducibility was < 1 %.

Microbial PLFA were extracted from freeze-dried microbial mat samples according to a modified Bligh and Dyer method (Bligh and Dyer, 1959) and purified using silica gel chromatography to separate lipids into non-polar, neutral and polar fractions. Phospholipids recovered from the polar fraction were subjected to a mild alkaline methanolysis and converted to fatty acid methyl esters (FAMEs) (Guckert et al., 1985). 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). Identification of PLFA were made based on the retention time and mass spectra of known reference standards (Bacterial Acid Methyl Esters Mix, Matreya Inc., Pleasant Gap, Pennsylvania, USA) and characteristic ion fragments (e.g. Fellenberg et al., 1987). Monoenoic double-bond

position and geometry of PLFA were determined by GC-MS analysis of dimethyl disulfide adducts (Nichols et al., 1986). In addition to ester-linked PLFA, the presence of methanogen associated ether-linked lipids was determined by derivatization of polar lipids using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Teixidor and Grimalt, 1992).

Fatty acids are named as follows; total number of carbon atoms:number of double bonds, followed by the position (Δ) of the double bond from the carboxyl end of the molecule. *Iso-* and *anteiso-* branching are denoted by the prefixes i or a respectively. "br" indicates a branch in an unknown location followed by the total number of carbon atoms. Other methyl branching is indicated as the position of the additional methyl carbon from the carboxylic end. Cyclopropyl PLFA are denoted by "cy".

5.2.3.2. Isotope analysis

DIC isotopic composition was determined by acidification and conversion to CO_2 analyzed by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa (St-Jean, 2003). All $\delta^{13}C_{DIC}$ values are reported in standard delta notation in reference to PeeDee Belemnite (PDB). Water oxygen values were determined by CO_2-water equilibration at 25°C prior to analysis on a Gasbench and Finnigan MAT Deta Delta^{Plus} XP. $\delta^{18}O$ values are reported in standard delta notation in reference to Vienna Standard Mean Ocean Water (VSMOW).

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 less ± 0.4 ‰ (1 σ) for $\delta^{13}C_{carb}$ and ± 0.2 ‰ (1 σ) for $\delta^{18}O_{carb}$ analyses. Samples for bulk organic carbon analysis were dried and treated with 1 M HCl to remove carbonate. Bulk organic isotopic analyses were conducted on an EA-Delta XL at McMaster University. Triplicate analyses gave a precision of less ± 0.3 ‰ (1 σ) for $\delta^{13}C_{org}$ values of the microbial mats. All carbonate and organic $\delta^{13}C$ and $\delta^{18}O_{carb}$ values are reported in standard delta notation relative to PeeDee Belemnite (PDB).

Methane carbon isotope compositions were determined via manual injections via a split/splitless injector into an Agilent GC-MS with a GS-Q capillary column (30 m x 0.32 mm I.D.) with the temperature held isothermal at 30°C. Methane was combusted to CO_2 as it eluted from the column via a combustion oven set at 960°C. Evolved CO_2 was analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS). Precision based on triplicate analysis was ± 0.4 ‰. $\delta^{13}C_{CH4}$ values are reported relative to PDB. Methane $\delta^{13}C$ values were determined for all 3 lakes in 2006 but were only determined for Goodenough Lake in 2007.

Aliquots of microbial FAMEs were injected into a split/splitless injector set to splitless mode at 300°C prior to separation using gas chromatography mass spectrometry (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 combusted to CO_2 as they eluted from the column via a combustion oven set at 960°C. Evolved CO_2 was analyzed using a Delta^{Plus} XP continuous flow isotope ratio mass spectrometer (IRMS).

The methanol used during methanolysis was characterized for ^{13}C and FAME $\delta^{13}C$ values were corrected for the added methyl carbon via the relationship:

$$\delta^{13}C_{FAME} = [(N+1)* \delta^{13}C_{meeasured} - \delta^{13}C_{MeOH}]/N \quad (4)$$

where N is the number of carbon atoms. All PLFA $\delta^{13}C$ values are reported in standard delta notation relative to PDB. Individual samples were analyzed in triplicate and precision is reported as one standard deviation (s.d.).

5.3. RESULTS

5.3.1. Visual appearance of the mats and inorganic carbon content

Probe Lake was the deepest of the three lakes sampled with maximum depths ranging from 2 – 2.5 m depending upon the time of the year. Evaporation leads to the development of extensive mudflats and formation of salt crust around the edge of the lake that is particularly pronounced in late summer (Figure 5.2). The Probe Lake microbial mat was relatively thin (0.5 – 1 cm), with a leathery texture and dark green colouring. The % LOI of mats collected in early and late summer 2007 were 39.2 ± 0.9 % and 35.4 % of the total dry weight respectively indicating that approximately 60 – 65 % of the mass of the mat was inorganic.

Deer Lake water depths ranged from a maximum of 0.5 m to 0.25 m with extensive microbial mat development. The flocculent mat was approximately 2 cm thick with visible laminations in the upper 0.5 – 1 cm underlain by about 50 cm of non-cohesive microbial debris and appeared uniform throughout the lake. The upper layer was brown/orange in colour, covering a distinct green layer, followed by a pink layer

with a darker brown/grey layer at depths below 1 cm. LOI results for ES and LS2007 were 26.2 % and 23.4 ± 0.7 % of the total dry weight, indicating an inorganic content of 73.8 and 76.6 % respectively.

Goodenough Lake water depths ranged from 1 m – 0.5 m. In late summer and fall, periods of lowest water depth, extensive salt precipitate was observed on the shoreline (Figure 5.2). The mat was dark green in colour and approximately 1 – 2 cm thick. Goodenough Lake LOI was 31.0 % for ES2007 and 25.2 % for LS2007, representing an inorganic content of these mats of 69 and 74.8 %.

5.3.2. Cariboo Plateau lake water samples

Results of in-field and water chemistry analysis for all Cariboo Lakes and sampling time periods are listed in Table 5.1 and Table 5.2. The pH of all lakes varies little over the season and between years, maintaining mean values of 10.1 to 10.2 ± 0.1 that were consistent with previous data (Slater, 1997). Specific conductance, salinity and DIC concentrations of surface waters from Cariboo lake samples typically increased from spring to late summer or fall (Table 5.1). The lakes are all dominated by Na, Ca, and Mg with concentrations of Na reaching a maximum observed concentration of 32600 mg/L in Goodenough Lake in Fall2008. These concentrations are comparable to previous measurements of 30050 mg/L Na in Goodenough Lake during August taken ~ 20 years prior (Renaut, 1990). All three lakes had high concentrations of DIC that showed a trend of increased concentrations over the course of the year. The lowest values in all lakes was 218 mg/L observed in Deer Lake in spring 2008 and a maximum of 9200 mg/L was

observed in Goodenough Lake in fall 2008. pCO_2 values indicated that the lakes were undersaturated with respect to atmospheric CO_2 during most of the sampling periods with the exception of one early summer period and fall in Probe Lake and fall in Goodenough Lake, consistent with previous findings that lakes with pH > 9 and high DIC concentrations were typically undersaturated and acted as CO_2 sinks (Duarte et al., 2008). Flux estimates of CO_2 into the lakes during periods of undersaturation ranged from 0.6 to 9.2 mol m^{-2} yr^{-1}. All of the lakes were saturated (S.I. > 0) with respect to dolomite, magnesite, calcite, and aragonite with the exception of predicted S.I. values for spring sampling periods (Table 5.1). These results were consistent with identification of extensive precipitation of carbonate minerals in previous studies of the Cariboo Plateau (Renaut and Long, 1989; Renaut, 1990). The most significant differences between the three lakes were in the sulfate and methane concentrations. Goodenough Lake showed elevated levels of sulfate in both the surface water and porewater (712 to 3460 mg/L SO_4^{2-}) as compared to Probe (maximum surface value of 115 mg/L) and Deer Lake (maximum surface value of 45 mg/L). Methane surface water concentrations in Goodenough were < 0.0 µmol/L to 0.3 µmol/L and porewater methane concentrations reached a maximum of 13.4 µmol/L (Table 5.2). Deer Lake had the highest observed surface water methane concentration of 4.1 µmol/L in 2007. Porewater methane concentrations were higher with an observed maximum of 791.8 µmol/L. Probe Lake surface methane concentrations were similar to those observed in Deer Lake, ranging from 1.7 µmol to 3.7 µmol with porewater concentrations that ranged from 356.1 to 934.1 µmol/L.

Microelectrode profiling of Deer Lake showed distinct regions within the microbial mat of oxygen concentrations extending to depths of ~ 5 to 8 mm before concentrations decline, concurrent with increased sulfide production indicative of underlying regions dominated by sulfate reducing bacteria (Figure 5.3). Subsamples of the microbial mat were taken in 2007 representative of communities from the oxygenated and anoxic portions of the mat. Similar profiles were obtained for Probe Lake demonstrating upper oxygenated zones with lower regions of oxygen depletion and sulfide production (Figure 5.3). Profiles were not obtained for Goodenough Lake.

5.3.3. Carbonate and organic carbon isotopic variability

At the pH and salinity values observed within the Cariboo Lakes, the DIC will be 80 – 95 % CO_3^{2-} (Mehrbach et al., 1973). Based upon the fractionation factor of Mook et al., (1974), the measured $\delta^{13}C$ of atmospheric CO_2 from a nearby site ($\delta^{13}C = -9.6 \pm 0.2$ ‰) (Chapter 2) (Brady et al., 2009), and a temperature range of 15 – 20°C (as typically measured during the summer sampling periods) equilibrium DIC $\delta^{13}C$ values were predicted to range from -1.8 to -1.2 ‰. Values as high as +0.1 ‰ would be expected with the lower measured salinity and surface water temperatures of ~ 5 °C during spring sampling periods.

DIC, carbonate and bulk organic $\delta^{13}C$ values are presented in Table 5.3 and seasonal and annual variation in $\delta^{13}C$ values is presented in Figure 5.4. DIC $\delta^{13}C$ values were typically elevated relative to the range predicted for atmospheric equilibrium and were highest in Deer Lake (Figure 5.4). The lowest DIC $\delta^{13}C$ values were typically

measured during the spring and in the majority of cases, there was an observed increase in $\delta^{13}C$ from the earliest sampling period to the later seasonal sampling period within a given year. However, not all sampling periods showed this trend. The general range of variation in surface DIC $\delta^{13}C$ values within an individual lake over the course of this study was approximately ~ 4 – 8 ‰, with the largest range observed in Deer Lake (Table 5.3). Porewater DIC $\delta^{13}C$ values in the upper sediments of Probe and Deer were lower than the corresponding surface DIC $\delta^{13}C$ values by 1.6 ‰ and 0.6 ‰ respectively (Table 5.2). The $\delta^{13}C$ values of Probe Lake and Deer Lake porewater DIC showed trends of increased values with increased depth, in Probe Lake the values increased to +0.5 ‰, slightly higher than the surface value of 0.0 ‰ while Deer increased to 3.8 ‰. In contrast, porewater DIC $\delta^{13}C$ values in Goodenough Lake had a mean value of -2.4 ± 0.1 ‰, depleted relative to the associated surface DIC $\delta^{13}C$ value of -1.5 ‰ and showed little variation with depth (Figure 5.5). Porewater methane $\delta^{13}C$ values in all lakes ranged from -95.9 ‰ to -56.4 ‰ (Table 5.2).

Carbonate $\delta^{13}C$ values were generally within 1 – 2 ‰ of the surface DIC $\delta^{13}C$ values and showed the same approximate 4 ‰ range of variation. However within each lake there was seasonal and annual variability with examples of both depletion and enrichment relative to the corresponding DIC (Figure 5.4). The lowest carbonate $\delta^{13}C$ value observed in any of the lakes was -3.8 ‰ in Probe Lake LS2007 while the highest $\delta^{13}C$ value was +8.0 ‰ in Deer Lake Spr2008. No correlation was observed between carbonate $\delta^{13}C$ and $\delta^{18}O$ values in any of the lakes (Figure 5.6). Carbonate sampled from the oxygenated upper layers of the Deer Lake mat had a $\delta^{13}C$ value of +3.4 ‰, within 0.2

‰ of the corresponding DIC $\delta^{13}C$ value of +3.2 ‰. In contrast, the carbonate sampled from the lower oxygen depleted zone had a $\delta^{13}C$ value of -1.8 ‰, 5 ‰ lower than the corresponding surface DIC $\delta^{13}C$ value.

The bulk organic $\delta^{13}C$ values are presented in Table 5.3. Probe Lake $\delta^{13}C_{org}$ values ranged from -24.6 to -22.3 ‰ with a mean of -24.0 ± 0.6 ‰ (n = 8). Deer Lake had a range of values from -19.0 to -18.6 ‰, mean $\delta^{13}C_{org}$ = -18.7 ± 0.1 ‰ (n = 8) while Goodenough Lake bulk organic $\delta^{13}C$ values had a mean of -25.3 ± 1.0 (n = 8) and ranged from -26.5 to -23.3 ‰. These bulk organic values were stable over all sampling periods and are consistent with previous investigations that reported $\delta^{13}C$ values of -18.5 ± 0.5 (Deer Lake), -23.0 ± 0.4 ‰ (Probe Lake), and -24.4 ± 0.2 ‰ (Goodenough Lake) 10 years earlier (Slater, 1997). Samples collected from Deer Lake in the upper oxygenated mat and lower anoxic mat had bulk organic $\delta^{13}C$ values similar to other Deer Lake values of -19.1 ‰ and -18.4 ‰ respectively. Isotopic discrimination between inorganic DIC and organic $\delta^{13}C$ values ($\Delta^{13}C_{inorg - org}$) are shown in Table 5.3. Goodenough Lake had the greatest mean discrimination of 24.0 ± 2.2. ‰, while the Probe Lake mean isotopic discrimination was 23.4 ± 1.9 ‰ and Deer Lake mean discrimination was 21.1 ± 2.7 ‰. Two sampling periods in Deer Lake showed small discriminations (< 20 ‰) due to the low DIC $\delta^{13}C$ values recorded at the time as the bulk organic $\delta^{13}C_{org}$ values from these samples were consistent with other time periods.

5.3.4. Phospholipid fatty acid analysis

The PLFA of all Cariboo mats from all sampling periods are listed in Table 5.4 to Table 5.6 and relative contributions of various PLFA groups during each time period are presented in Figure 5.7. PLFA distributions from the upper and lower sub-sampled portion of the Deer Lake mat corresponding to microelectrode profiles are listed in Table 5.7. Similar PLFA were identified in all of the lakes with varying proportions indicating some differences in the microbial communities between the lakes. No significant trends with season were noted. All of the lakes had significant proportions of saturated straight chain PLFA ranging from 14:0 to 20:0, with 16:0 generally the most abundant, however these PLFA are ubiquitous in prokaryote and eukaryote organisms and are not linked to specific microbial groups (Boschker and Middelburg, 2002). The presence of both monoenoic and branched PLFA suggest that the Cariboo mats contained both Gram-positive and Gram-negative bacteria (Parkes and Taylor, 1983; Findlay et al., 1990; Kaneda, 1991). In particular 18:1 on average accounted for 40 – 50 % of total PLFA with two PLFA (18:1Δ11 and 18:1Δ9) the most dominant indicating a significant presence of Gram-negative bacteria, including cyanobacteria (Nichols and Wood, 1968; Findlay et al., 1990; Grimalt et al., 1992). The PLFA *iso*- and *anteiso*-15:0 (*i*15:0/*a*15:0) and 17:0 (*i*17:0/*a*17:0) were present in proportions typically ranging from 10 – 20 % indicative of Gram-positive bacteria (Kaneda, 1991; White et al., 1996). Tetramethyl16:0 (phytanic acid) was detected in almost all of the samples in varying proportions ranging from less than 1 % to ~ 9 %. Phytanic acid has been noted to arise from the degradation of bacteriochlorophyll *a* found in purple sulfur bacteria indicating that these microbes are part of the Cariboo microbial mat communities (Marchand and Rontani, 2003), consistent

with previous identification of purple sulfur bacteria in Goodenough Lake (Schultze-Lam et al., 1996). Polyunsaturated PLFA (PUFA) were detected in Cariboo microbial mats (including 18:2 and 20:3, 20:4 and 20:5) and are often linked to the presence of eukaryotic organisms, however cyanobacteria have been shown to synthesize PUFA as well (Kenyon, 1972; Kenyon et al., 1972; Oren et al., 2009) and it is likely that cyanobacteria are contributing to the observed PUFA in these lakes. Derivatization with BSTFA identified ether-linked lipids indicative of the presence of methanogens (Teixidor and Grimalt, 1992) in Deer Lake only, consistent with the high methane concentrations (surface water values of 0.2 to 4.1 μmol).

PLFA $\delta^{13}C$ values are also reported in Table 5.8. The greatest depletion relative to the bulk organic $\delta^{13}C$ was typically observed for 16:0 (~ 5 – 7 ‰). The smallest depletions were generally observed for *i*15:0/*a*15:0 with offsets that ranged from 2 to 3 ‰. Monounsaturated 18:1 PLFA showed variability between lakes and seasons in the range of offsets with values as low as ~ 2 ‰ and as high as 10 ‰ likely reflecting synthesis by differing microbial groups in each lake. PLFA $\delta^{13}C$ values from the upper zone mat sampled in Deer Lake generally had greater offsets relative to the bulk cell (5 – 8 ‰) than the lower anoxic zone consistent with cyanobacteria synthesis (Sakata et al., 1997; Teece et al. 1999).

5.4. DISCUSSION

The Cariboo Plateau microbial mats are evaporative, carbonate rich (> 60 % inorganic content by weight) systems where extensive photosynthetic activity results in

undersaturation of the lakes with respect to CO_2 and typically to DIC $\delta^{13}C$ values that are higher than those predicted for equilibrium with atmospheric CO_2. At the same time, the discrimination between inorganic and organic carbon from the Cariboo systems show evidence for non-CO_2 limited photosynthesis. One of the primary goals of this research was to understand the interplay between carbon sources and cycling and the resulting biosignatures. A proposed schematic of the major carbon cycling pathways for the Cariboo Plateau lakes is presented in Figure 5.8 will form the basis of the following discussion.

5.4.1. Carbon cycling within a carbonate rich microbial mat system

The DIC $\delta^{13}C$ values from the Cariboo Plateau lakes showed both enrichment (values as high as 4.2 ‰) and depletion (low of -3.9 ‰) relative to the predicted range of -1.8 to -1.2 ‰ (Figure 5.4). In most cases the DIC $\delta^{13}C$ values were higher than predicted based on equilibrium with atmospheric CO_2, a situation that may be the result of physical and/or biological factors. As both biological and physical processes may be responsible for the observed enrichments, the ability to distinguish between these processes is important for interpretation of elevated carbonate $\delta^{13}C$ values as a biosignature in the geologic record. Preservation of these enrichments and depletions in precipitated carbonate represents a biosignature of influences on DIC at the time of formation. As outlined in Figure 5.8, the main processes that could contribute to ^{13}C-enriched DIC are evaporation, photosynthesis and methanogenesis.

5.4.1.1. Biological influences on DIC isotopic composition

Primary productivity has been proposed as the most significant biogeochemical control on DIC isotopic composition in eutrophic lacustrine systems (Hollander and McKenzie, 1991). PLFA profiles indicate that the Cariboo microbial mats were composed of a diverse, prokaryotic community that produced saturated (typically under < 20:0), branched (*iso-, anteiso-* and mid-branched), monoenoic, polyenoic and cyclopropyl PLFA (Figure 5.7) (Findlay et al., 1990; White et al., 1996; Green and Scow, 2000). Phytanic acid generated by degradation of bacteriochlorophyll *a* (Marchand and Rontani, 2003) was present in all lakes and indicated that purple sulfur bacteria were part of the Cariboo microbial mat communities. However, high proportions of monoenoic and PUFA that may be linked to cyanobacteria (Kenyon, 1972; Kenyon et al., 1972) and $\Delta\delta^{13}C_{PLFA\text{-}biomass}$ values on the order of 5 – 7 ‰ in 16:0 and monoenoic PLFA were consistent with cyanobacterial production (Sakata et al., 1997) indicating that cyanobacteria were dominant members of the mat communities. These results were consistent with previous identification of abundant cyanobacteria in the presence of other bacteria including purple sulfur bacteria within Goodenough Lake (Schultze-Lam et al., 1996).

The Cariboo lakes were undersaturated with respect to atmospheric CO_2 during the majority of the sampling periods consistent with high levels of photosynthetic uptake at a rate greater it can be replenished. Low pCO_2 values in freshwater lakes have been suggested to result in the invasion of ^{13}C-depleted CO_2 from the atmosphere due to faster kinetics for ^{12}C versus ^{13}C and resulting in DIC with low $\delta^{13}C$ values (Herczeg and

Fairbanks, 1987; Lazar and Erez, 1990). However, DIC $\delta^{13}C$ values in the Cariboo lakes were typically ^{13}C-enriched relative to the range predicted for equilibrium with atmospheric CO_2 indicating that this is not the case. Rather, the observed enrichment of DIC can be explained by preferential uptake of ^{13}C-depleted carbon during photosynthesis leading to ^{13}C-enrichment in the residual carbon pool (O'Leary, 1988). This observation is consistent with previous work linking undersaturated pCO_2 levels and high DIC $\delta^{13}C$ values to high levels of photosynthetic activity (Herczeg, 1987; McConnaughey et al., 1994; Thompson et al., 1997; Hollander and Smith, 2001).

Methanogenesis is another biological mechanism that could contribute to the observed ^{13}C-enrichment. Methanogensis results in some of the largest isotopic fractionations of carbon observed in natural systems due to a high preference for ^{12}C versus ^{13}C during methane production (Botz et al., 1996; Whiticar, 1999). This highly preferential uptake of ^{12}C results in the residual DIC pool being strongly enriched in ^{13}C. Methanogenesis has been linked to isotopically enriched DIC surface $\delta^{13}C$ values averaging +9 ‰ with porewater $\delta^{13}C$ values as high as +26.4 ‰ (Gu et al., 2004). Although there was evidence for methane production within the Cariboo lakes there are several factors that indicate that methanogenesis is not a primary driver of DIC $\delta^{13}C$ values. The strongest factor is the much lower concentrations of methane than DIC. Methane is only equivalent to less than 0.5 % of the DIC in the porewater where concentrations are highest. The second factor is the lack of significantly enriched DIC $\delta^{13}C$ values in the sediment porewater as compared to the overlying water column. Methanogenic influences on porewater DIC ^{13}C-content may have been expected in Deer

Lake as this lake had methane concentrations of up to 4.1 µmol in the surface water and derivatization with BSTFA indicated the presence of methanogenic archaea within the microbial mat. Mean Deer Lake methane $\delta^{13}C$ values were -70.6 ± 2.6 ‰ within the range of -60 to -110 ‰ predicted for methane production via CO_2 reduction (Whiticar, 1999), this process would be expected to result in elevated $\delta^{13}C$ values in the porewater where methanogenesis could occur under anaerobic conditions. However, porewater DIC $\delta^{13}C$ values in Deer Lake were within 0.6 ‰ of the corresponding surface DIC $\delta^{13}C$ value of +3.4 ‰ indicating that ^{13}C-enrichment associated with methanogenesis did not alter surface DIC (Figure 5.5). In the upper sediments of Deer Lake, evidence for methanotrophy was observed resulting in an increase of ~ 5 ‰ in methane $\delta^{13}C$ values due to preferential metabolism of $^{12}CH_4$. Inputs of ^{13}C-depleted carbon via methanotropy would aid in lessening any potential impacts on DIC resulting from methanogenesis. Probe Lake porewater DIC values were lower in the upper sediments by up to 1.6 ‰ as compared to corresponding surface DIC, indicating that any ^{13}C-enriched CO_2 produced via methanogenesis in the lower sediments was not contributing to surface DIC. Methanogenesis was unlikely to have been a dominant process in Goodenough Lake as methane concentrations were even lower than in the other two systems (less than 0.3 µmol in surface water). Sulfate concentrations in the surface water were in the range of 20 – 30 mM and at these concentrations methanogenic bacteria are generally outcompeted by sulfate reducing bacteria (Whiticar, 1999; Bebout et al., 2004).

5.4.1.2. Potential isotopic impacts of evaporation

Observed increases in salinity, specific conductance and concentrations of ions such as Na and Cl between sampling periods early in the season and late was consistent with evaporitic effects on the Cariboo lakes over the course of the year. DIC $\delta^{13}C$ values higher than the predicted range for isotopic equilibrium with atmospheric CO_2 have been suggested to be related to evaporation and outgassing of ^{13}C-depleted CO_2 leading to ^{13}C-enrichments in residual DIC (Valero-Garcés et al., 1999) with enrichments of up to +16.5 ‰ reported in evaporating brines (Stiller et al., 1985). However, the CO_2 undersaturation of the lakes (minimum of 93 µatm) during the majority of the sampling periods supports invasion of CO_2 rather than significant de-gassing suggesting that biological influences on isotopic composition dominate during the majority of sampling periods. Further the lack of correlation between carbonate $\delta^{13}C$ and $\delta^{18}O$ values (Figure 5.6) expected for evaportic control on isotopic compositions indicated that biological processes that only affect the carbon isotope system were more likely the cause of the observed enrichment (McConnaughey, 1989a; Léveillé et al., 2007; Kremer et al., 2008).

5.4.1.3. Heterotrophic inputs of ^{13}C-depleted carbon

As illustrated in Figure 5.8, microbial heterotrophic metabolism of ^{13}C-depleted organic matter results in inputs of ^{13}C-depleted carbon into the aqueous environment resulting in opposite influences on the isotopic composition of the lake DIC to those of photosynthesis. DIC $\delta^{13}C$ values showed overall variations of ~ 4 – 8 ‰ within an individual lake over the course of this study (Table 5.3 and Figure 5.4). The observed variability in pCO_2 and DIC $\delta^{13}C$ values reflected the dynamic nature of these lakes

including periods of relative ^{13}C-depletion that were likely caused by shifts in the balance of photosynthetic ^{13}C-enrichment versus heterotrophic inputs of ^{13}C-depleted carbon. Observed reversals in the direction of flux CO_2 in the fall are consistent with previous studies of freshwater lakes that found pCO_2 increased during cooler periods as photosynthetic activity decreased, allowing for both increased atmospheric invasion and heterotrophic inputs of CO_2 (McConnaughey et al., 1994). Increased heterotrophic activity could also support the observed low DIC $\delta^{13}C$ values in spring sampling periods in Probe and Deer Lake if photosynthesis had been limited during the winter due to ice cover on the lakes and reduced light levels. Reduced photosynthetic activity in the winter would be expected to result in relatively increased inputs of heterotrophy derived ^{13}C-depleted carbon to the lake. Both of these lakes still had partial ice coverage during the sampling period and photosynthetic activity levels may not have been high enough or sustained long enough to have yet caused the ^{13}C-enrichment in the DIC that developed later that year. In comparison, Goodenough Lake was ice free during this spring sampling period and DIC $\delta^{13}C$ values were comparable to values measured in the lake later that year. This observation suggested that if heterotrophic activity had been dominant under ice during the winter, increased spring photosynthetic activity and uptake of ^{12}C was already influencing the DIC isotopic composition. Other periods of increased heterotrophic activity relative to photosynthesis in the Cariboo lakes explains the variability in DIC $\delta^{13}C$ values observed annually.

No significant trends in seasonal PLFA distribution were observed, however, on-going heterotrophic activity in the Cariboo microbial mats was supported by the presence

of heterotrophic markers such as *iso-, anteiso-*, mid-branched and monoenoic PLFA that indicated that the Cariboo communities were not solely dominated by cyanobacteria and that other microbes were present in significant proportions (Dowling et al., 1986; Vestal and White, 1989; Parkes et al., 1993; Londry et al., 2004) (Vestal and White, 1989). PLFA $\delta^{13}C$ values for markers of heterotrophic bacteria including *iso-, anteiso-* and mid-branched were typically depleted relative the bulk cell by values ranging from 3 – 4 ‰ in all Cariboo microbial mats supportive of synthesis of these PLFA by heterotrophic bacteria (Blair et al., 1985; Abraham et al., 1998; Teece et al., 1999). Organic matter degradation via sulfate reduction is an important heterotrophic process in microbial mats (Teske et al., 1998; Paerl et al., 2000; Baumgartner et al., 2006). Markers 10me16:0 and br17:1 linked to sulfate reducing bacteria (SRB) (Boon et al., 1977; Taylor and Parkes, 1983; Wieland et al., 2003; Fang et al., 2006) were detected in all of the Cariboo lakes during at least one sampling period indicating that SRB were present within the community. The proportions of these PLFA markers were generally highest in Probe and Goodenough Lake and were either not observed in the majority of Deer Lake samples or were observed in minor quantities (< 2 %) (Table 5.4 to Table 5.6). Microelectrode sulfide profiles also indicate the presence of sulfate reduction zones within the microbial mats (Figure 5.3). Sulfate reduction in the upper sediments of Probe and Goodenough Lake was supported by porewater DIC $\delta^{13}C$ values depleted by 1 – 2 ‰ relative to the overlying surface water values due to inputs of ^{13}C-depleted carbon (Figure 5.5). Increased heterotrophic activity in the early summer periods within Probe and Goodenough microbial mats accounts for periods of relatively ^{13}C-depleted DIC observed

in the early summer sampling periods. In particular, PLFA profiles in Goodenough Lake had high proportions of monoenoic (16:1 and 18:1) and branched PLFA with $\delta^{13}C$ values that were typically depleted relative to the bulk organic values by less than 4 ‰, consistent with heterotrophic metabolisms (Blair et al., 1985; Abraham et al., 1998; Teece et al., 1999). At the same time, Goodenough Lake DIC $\delta^{13}C$ values in 2006 and 2007 ranged from -3.5 to -1.5 ‰, lower than expected for equilibrium with atmospheric CO_2 supportive of heterotrophic inputs of ^{13}C-depleted DIC.

5.4.2. Implications for identification of carbonate biosignatures

Biologic influences on the DIC composition may be reflected in precipitated carbonate representing a potential biosignature of the dominant microbial activity. Change in the relative influence of autotrophic versus heterotrophic metabolic activity on DIC is recorded in carbonate ^{13}C-enrichments and depletions relative to DIC. This variation in carbonate $\delta^{13}C$ values may account for some of the variability observed in carbonate recovered from the geologic record. The Cariboo microbial mat carbonate $\delta^{13}C$ values were not consistently enriched or depleted relative to the DIC with offsets ranging from an enrichment of 3.8 ‰ to a depletion of 11.6 ‰ (Figure 5.4). However, the range in variability of carbonate $\delta^{13}C$ values was generally similar to that observed for DIC $\delta^{13}C$ values (~ 4 ‰ in Probe and Goodenough and up to ~ 8 ‰ in Deer Lake) and is consistent with precipitation during periods of varying autotrophic versus heterotrophic dominance within the lakes. The variability in carbonate isotopic composition in the Cariboo lakes also likely reflects varying microbial metabolic activity

within localized zones of carbonate precipitation within the mats. The $\delta^{13}C$ value of mat associated carbonates reflects microbial influences on the isotopic composition of the DIC within the local environment (Sumner, 2001). Microenvironments of varying geochemistry within microbial mats are supported by studies employing microelectrode profiling to assess variations in oxygen, pH, sulfide within a microbial mat (Jørgensen et al., 1983; Revsbech et al., 1983; Bissett et al., 2008). In this study, microelectrode profiling within Deer Lake demonstrated the presence of an oxygenated zone above a zone of oxygen depletion and sulfide production (Figure 5.3). Carbonate $\delta^{13}C$ values associated with mat sampled from these distinct zones reflect the influences of the dominant microbial metabolic activity on localized DIC and the carbonate that precipitated within this zone. The carbonate $\delta^{13}C$ value of 3.4 ‰ from the upper oxygenated zone reflected the overall photosynthetic influence on DIC in Deer Lake. In contrast, carbonate sampled from the lower oxygen free zone was -1.8 ± 1.8 ‰, depleted relative to the surface DIC by 5 ‰ consistent with heterotrophic influences on the isotopic composition of the local DIC. Greater heterotrophic activity within the lower anoxic zone of the Deer Lake mat was supported by higher proportions of branched PLFA (~ 20 %) consistent with Gram-positive heterotrophic bacteria (Kaneda, 1991; White et al., 1996) and generally smaller offsets (~ 4 ‰) between bulk cell and PLFA $\delta^{13}C$ values (Monson and Hayes, 1982; Blair et al., 1985) as compared to the upper oxygenated zone. Carbonate precipitated within mat microenvironments or during time periods of varying photosynthetic versus heterotrophic activity reflect the range of DIC isotopic compositions that existed in the Cariboo lakes. The relatively constant state of

saturation and high carbonate content (> 60 %) of the mats indicated that the variability in carbonate $\delta^{13}C$ values within the mats was likely the result of several years or seasons of precipitation during varying photosynthetic versus heterotrophic activity levels.

5.4.3. Carbon cycling within the Cariboo lakes

Despite high levels of photosynthetic activity and drawdown of CO_2, all of the Cariboo lakes had isotopic discriminations between inorganic (DIC) and organic carbon pools ($\Delta^{13}C_{DIC\text{-}org}$) values consistent with non-CO_2 limited photosynthesis. The mean bulk organic $\delta^{13}C$ values for Probe Lake, Deer Lake and Goodenough Lake were -24.0 ± 0.6 ‰, -18.7 ± 0.1 ‰ and -25.2 ± 1.0 ‰ respectively, with $\Delta^{13}C_{DIC\text{-}org}$ values that averaged between 21.1 and 24.2 ‰. The mean bulk organic $\delta^{13}C$ and $\Delta^{13}C_{DIC\text{-}org}$ values for the Cariboo microbial mats were similar to those observed previously (Slater, 1997) and were consistent with C_3 photosynthesis (O'Leary, 1988). These results indicate that despite undergoing annual freeze-thaw cycles, the carbon isotope dynamics in the microbial ecosystems are stable over the last decade and conditions within the lakes allow for non-limited photosynthesis.

The high rates of photosynthesis in the Cariboo mats led to undersaturation of CO_2 yet photosynthesis was non-CO_2 limited suggesting that either atmospheric invasion was equal to the uptake rate of CO_2, or the cyanobacteria were able to draw on an additional supply of inorganic carbon. Re-supply of CO_2 via diffusion from the atmosphere to the photosynthetic uptake site could not account for non-CO_2 limited conditions if rates of consumption were higher than or equal diffusion rates. Estimates of

atmospheric replenishment varies greatly between systems, previous studies have found that atmospheric influx could account for up to 60 % of photosynthetic drawdown in a study of experimental ditches with 0.5 m water depth dominated by benthic algae during the summer (Portielje and Lijklema, 1995) and 90 % of photosynthetic uptake during summer months in a softwater lake (Herczeg, 1987). Although the photosynthetic uptake rates for the Cariboo microbial mats are currently unknown, proposed rates for other microbial mats are on the on the order of 1.56 g C m^{-2} d^{-1} (Revsbech et al., 1983) to 8 g C m^{-2} d^{-1} (Cohen et al., 1977). Estimates of CO_2 influx from the atmosphere in the Cariboo lakes ranged from 0.6 to 9.2 mol m^{-2} yr^{-1}, or 0.0 to 0.8 g C m^{-2} yr^{-1}, lower than proposed rates photosynthetic uptake for other microbial mats indicating that atmospheric invasion alone does not account for photosynthetic uptake in these systems. Heterotrophic input of CO_2 from degradation of organic matter within the microbial mats was another potential source of inorganic carbon that could re-supply photosynthetic uptake (Figure 5.8). Within the Solar Lake microbial mats, carbon recycling via sulfate reduction was estimated to account for 7 – 8 % of the photosynthetic carbon demand (Teske et al., 1998) and sulfate reduction could be contributing within the Cariboo microbial mats. However, as the DIC $\delta^{13}C$ values in the Cariboo lakes generally show photosynthetic influences, heterotrophic inputs of ^{13}C-depleted CO_2 were likely not the most significant carbon source.

Alternatively, the high concentration of DIC in the lakes provides a large supply of inorganic carbon for photosynthetic uptake. Carbonate equilibrium (as illustrated in Figure 5.8) can respond quickly to CO_2 uptake and the high amounts of DIC, carbonate

content within the mats (> 60 %) and generally constant carbonate saturation levels suggest that this may be an important source of inorganic supply allowing for high levels of photosynthesis (Hollander and Smith, 2001). Cyanobacteria have the ability to uptake HCO_3^- under conditions of low [CO_2] (Miller and Colman, 1980; Hollander and McKenzie, 1991) and CO_2 concentrating mechanisms allows for up to 1000-fold increases in cellular DIC concentrations as compared to the ambient water (Kaplan et al., 1991). If the Cariboo microbial mat photosynthetic bacteria were using HCO_3^- in addition to CO_2, the high DIC concentrations in the lakes would provide a significant source of inorganic carbon leading to non-limited photosynthesis. Shifts in the carbonate equilibrium to replace CO_2 and HCO_3^- lost during photosynthesis at a salinity of 35 and 25°C is on the order of 15.9 s with the slowest step being the conversion between CO_2 and HCO_3^- at ~ 10 s while equilibrium between HCO_3^- and CO_3^{2-} is ~ 10^{-2} s (Zeebe et al., 1999). High DIC concentrations and carbonate content within the Cariboo lakes supporting high levels of photosynthetic activity are consistent with previous studies that have identified carbon stores as important source of CO_2 in lakes exhibiting undersaturation of pCO_2 during summer photosynthesis (McConnaughey et al., 1994; Hollander and Smith, 2001).

5.4.3.1. Implications for organic biosignatures within Cariboo Plateau microbial mats

Enriched bulk organic matter $\delta^{13}C$ values and decreased $\Delta^{13}C_{inorg\text{-}org}$ values in saline organic rich microbial mats have been linked to low concentrations of CO_2 resulting from high levels of productivity and increased demand (Des Marais, 1992;

Schidlowski et al., 1992) or non-Calvin cycle metabolisms with smaller fractionations as in the case of hot spring microbial mats (Preuß et al., 1989; van der Meer et al., 2000). Bulk organic values ranging from -10 to -18 ‰ and up to -5 ‰ have been recovered from other non-lithifying, saline and hot spring microbial mats (Schidlowski et al., 1984; van der Meer et al., 2000; Schouten et al., 2001; Scherf and Rullkötter, 2009). In contrast, both Probe and Goodenough Lake had $\delta^{13}C$ values that were not as enriched in ^{13}C as organic matter recovered from these other microbial mat environments. Deer Lake had a more ^{13}C-enriched mean bulk organic $\delta^{13}C$ value of -18.7 ‰, near the lower end of the range of values recovered from proposed CO_2-limited systems (Des Marais et al., 1989; Scherf and Rullkötter, 2009). Viewed independently, observation of this value suggests that Deer Lake cyanobacteria experienced CO_2 limited photosynthesis or that the photosynthetic community was composed of autotrophs employing non-Calvin cycle metabolisms. Non-Calvin cycle metabolisms utilized by anoxygenic photosynthetic bacteria in hot spring microbial mats have ^{13}C-enriched organic values (van der Meer et al., 2000). Anoxygenic photosynthetic bacteria were present within the Cariboo microbial mats as indicated by the observation of phytanic acid, indicative of purple sulfur bacteria (Marchand and Rontani, 2003) and a previous microscopic investigation of Goodenough Lake (Schultze-Lam et al., 1996). However, in hot spring microbial mats with ^{13}C-enriched bulk organic compositions, anoxygenic photosynthetic bacteria make up significant portions of the microbial community and in some cases, dominate as the primary producers (Ward et al., 1998; van der Meer et al., 2000). Enriched bulk organic $\delta^{13}C$ values in these systems are the result of autotrophic primary production employing

the 3-hydroxypropionoate pathway (van der Meer et al., 2003) or the reverse tricarboxylic acid cycle (van der Meer et al., 1998; van der Meer et al., 2000). The small fractionations associated with these pathways leads to $\Delta^{13}C_{inorg\text{-}org}$ values that range from ~ 12 to 14 ‰ (Quandt et al., 1977; Preuß et al., 1989; Strauss and Fuchs, 1993). The mean Deer Lake $\Delta^{13}C_{inorg\text{-}org}$ value was 21.1 ± 2.7 ‰, too large to be associated with anoxygenic photosynthesis metabolisms but within the range of non-CO_2 limited oxygenic photosynthesis (O'Leary, 1988). Furthermore, PLFA from Deer Lake were depleted relative to the bulk cell by values of ~ 9 ‰, consistent with cyanobacteria synthesis (Sakata et al., 1997) whereas lipids recovered from hot spring bacteria employing the 3-hydropropionoate pathway are depleted relative to the bulk cells by ~ 4 ‰ (van der Meer et al., 2007), similar to values expected for heterotrophic synthesis.

The Cariboo lakes have a high level of photosynthetic activity as supported by undersaturated pCO_2 due to carbon uptake yet do not show highly enriched $\delta^{13}C$ organic values nor a small $\Delta^{13}C_{inorg\text{-}org}$. These bulk organic values, in particular those of Probe and Goodenough Lake, are consistent with organic $\delta^{13}C$ values of ~ - 26 ‰ recovered from stromatolitic deposits in the geologic record (Eichmann and Schidlowski, 1975; Schidlowski, 1988). Increased levels of atmospheric CO_2 in the past have been proposed to explain how values consistent with non-CO_2 limited photosynthesis could have been produced (Mizutani and Wada, 1982; Schidlowski, 1985; Des Marais et al., 1992). In contrast, results from the Cariboo microbial mats indicate that similar values can be obtained from saline, microbial mats supporting high levels of productivity under modern atmospheric conditions without becoming CO_2 limited. The high surface water DIC

concentrations and carbonate content within the mats provided a large supply of inorganic carbon that enabled non-limiting photosynthesis and the production of ^{13}C-depleted organic matter. The observation of non-CO_2 limited photosynthesis within modern carbonate rich, microbial mats suggests that proposed changes in atmospheric CO_2 concentrations are not necessary to produce the C_3 photosynthesis derived organic matter $\delta^{13}C$ values observed in Precambrian stromatolites.

5.5. CONCLUSIONS

High levels of photosynthetic activity resulted in undersaturation of the Cariboo lakes with respect to atmospheric CO_2 and ^{13}C-enriched water-column DIC relative to predicted atmospheric values. DIC $\delta^{13}C$ values within individual lakes were seen to vary by ~ 4 – 8 ‰ over the course of this study. Periods of variable DIC $\delta^{13}C$ values including ^{13}C-depleted values were the result of deviations in the relative balance of photosynthetic removal versus heterotrophic input of ^{13}C-depleted CO_2. Photosynthetic activity within the mats with contributions from heterotrophic organisms, in particular sulfate reducing bacteria was supported by PLFA profiles with high proportions of saturated and monoenoic fatty acids that were depleted relative to bulk organic $\delta^{13}C$ values by amounts characteristic of either cyanobacteria or heterotrophic synthesis. These results indicated that DIC $\delta^{13}C$ values can vary seasonally and annually as a result of changes in photosynthetic versus heterotrophic activity levels.

Carbonate $\delta^{13}C$ values within microbial mats reflect the isotopic composition of DIC within the mat including localized influences of microbial activity that varied

spatially and temporally. The carbonate $\delta^{13}C$ values were generally within 1 – 2 ‰ of the measured surface DIC and exhibited the same overall variation of approximately 4 – 8 ‰ but did not vary consistently with the DIC $\delta^{13}C$ values, showing both enrichment and depletion. Variation in the carbonate $\delta^{13}C$ values reflected shifts in the balance of photosynthetic ^{13}C-enrichment versus heterotrophic inputs of ^{13}C-depleted carbon. These influences resulted in varying surface DIC $\delta^{13}C$ values within the lakes, but were also observed within microenvironments within the microbial mat. Microelectrode profiles within Deer Lake demonstrated zones of oxygen depletion and sulfide production consistent with carbonate depleted in ^{13}C relative to the overlying DIC by 5 ‰. The high carbonate content of the microbial mats and near constant saturation with respect to carbonate minerals indicated that the carbonate $\delta^{13}C$ values reflected multiple seasons of both photosynthetically and heterotropically influenced carbonate precipitation. These findings highlight the dynamic nature of microbial mat systems and suggest that carbonate $\delta^{13}C$ values in the geologic record can vary by ~ 4 – 8 ‰ as a result of variation in the relative balance of autotrophic versus heterotrophic influences on DIC occurring on a system wide scale or within localized microenvironments within the mat.

Isotopically depleted bulk organic matter $\delta^{13}C$ values and $\Delta^{13}C_{inorg\text{-}org}$ values of ~ 20 – 25 ‰ in the Cariboo microbial mats reflected non-CO_2 limited photosynthesis in contrast to other saline, organic rich or hot spring microbial mats. The high levels of photosynthetic activity within the Cariboo microbial mats were supported by high concentrations of DIC and carbonate contents (> 60 %) that provided a non-limiting supply of inorganic carbon. These findings suggest that early stromatolite-forming

communities with organic $\delta^{13}C$ values of ~ -26 ‰ may have existed under non-CO_2 limited conditions resulting from continual renewal linked to high DIC concentrations and carbonate content, and that atmospheric CO_2 concentrations were not necessarily significantly different than current levels.

These results support periods of varying autotrophic versus heterotrophic activity levels that can lead to wide ranges in DIC and carbonate $\delta^{13}C$ values. Understanding variation in dominant microbial activities and associated isotopic biosignatures in modern microbial mats leads to a greater understanding and interpretation of microbial ecosystems and evaporative environments found on early Earth or on Mars.

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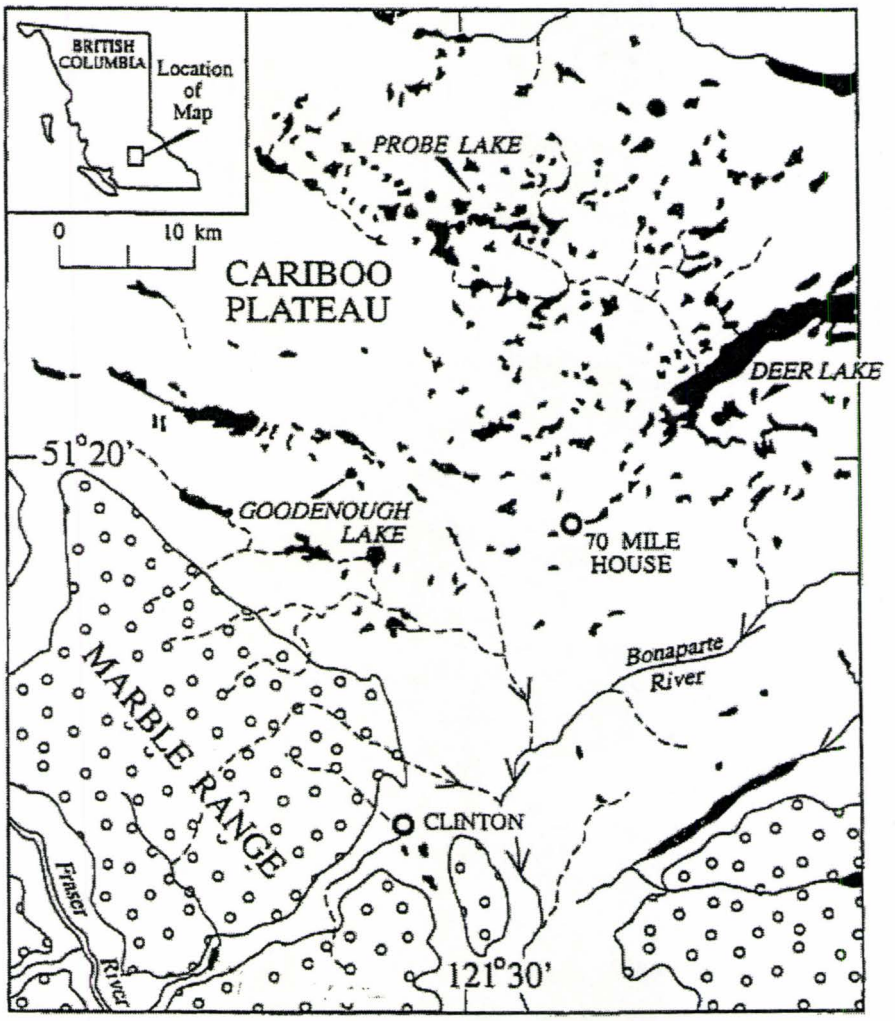

Figure 5.1: Location of the three Cariboo Plateau lakes with carbonate rich microbial mats that were the focus of this study

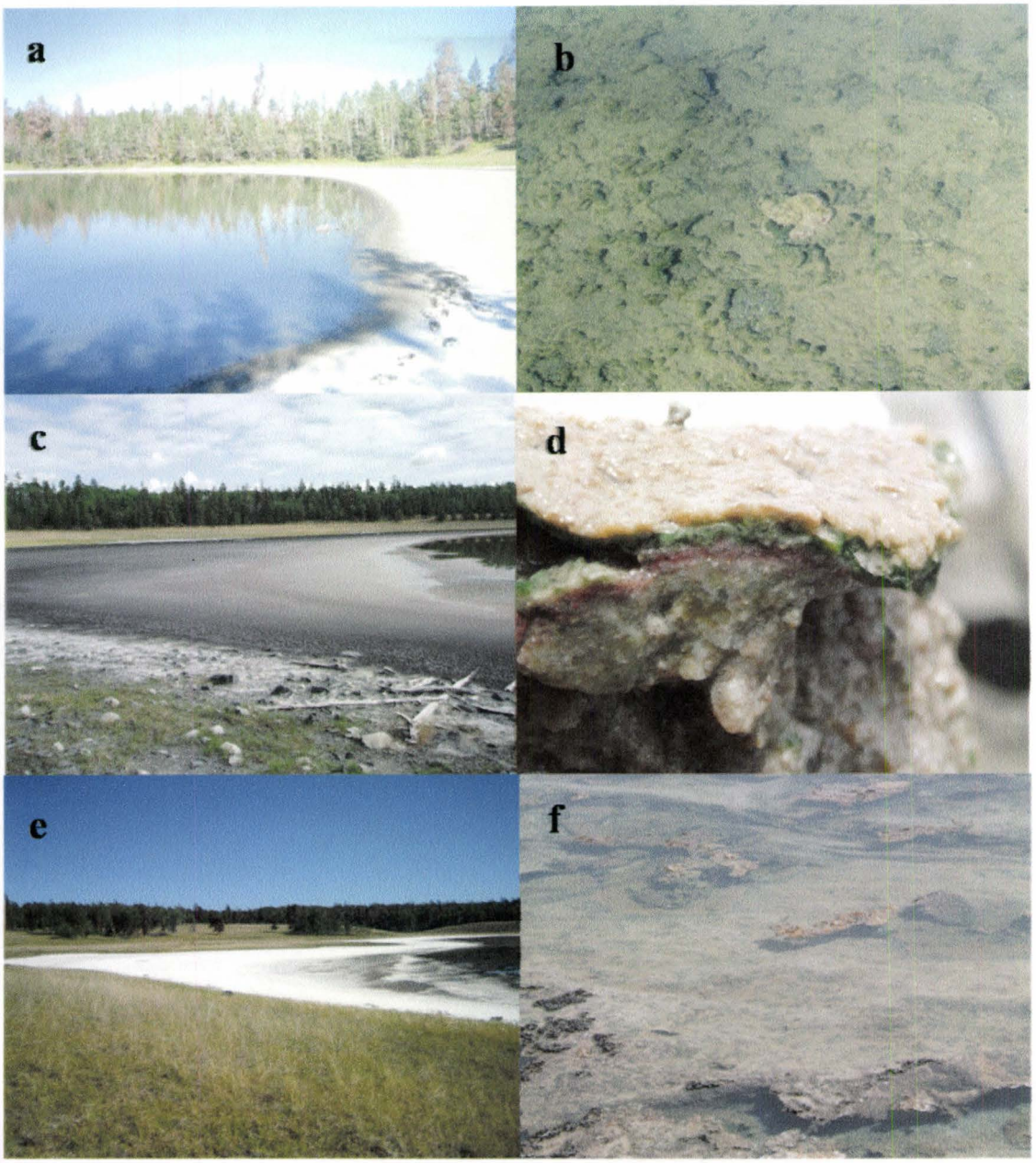

Figure 5.2: Images of Cariboo lakes showing desiccated mud, microbial and salt precipitates around edge and close-up of microbial mats showing texture and layering a) Probe Lake b) benthic mat in Probe Lake c) Deer Lake showing extent of dried microbial mat and mud d) layering present in Deer Lake microbial mat e) Goodenough Lake showing extensive salt encrustation f) microbial mat lifting off sediment surface

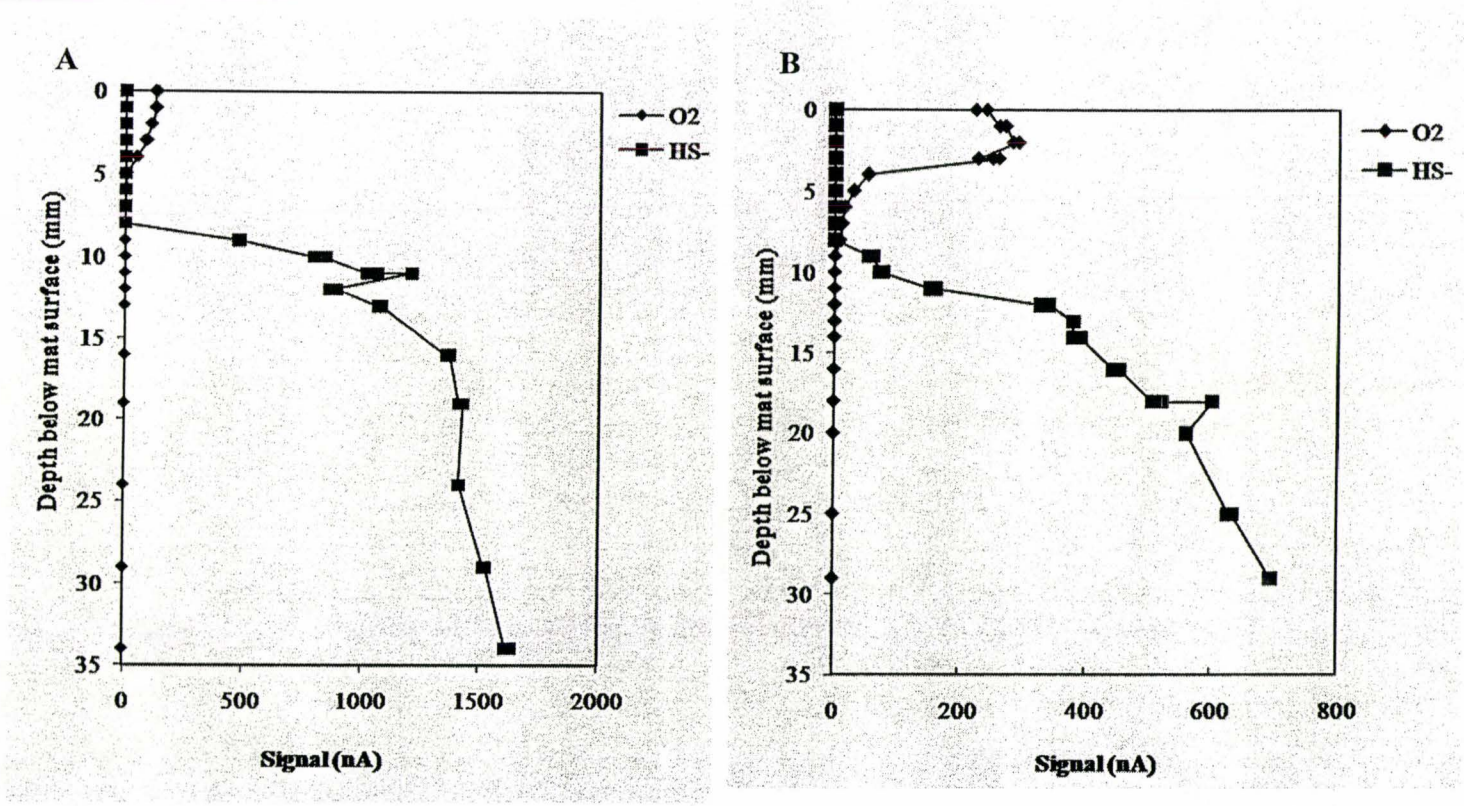

Figure 5.3: a) Vertical profiles through Probe Lake microbial mat showing depletion of oxygen beginning at a depth of 4 mm and sulfide production beginning at 8 mm b) Vertical profiles through Deer Lake microbial mat showing depletion of oxygen beginning at a depth of 5 mm before becoming fully depleted at ~ 9 mm concurrent with sulfide production occurring at a similar depth of 9 mm. These zones of oxygen production and depletion correspond to Deer Lake microbial mat sub-samples collected during this sampling period.

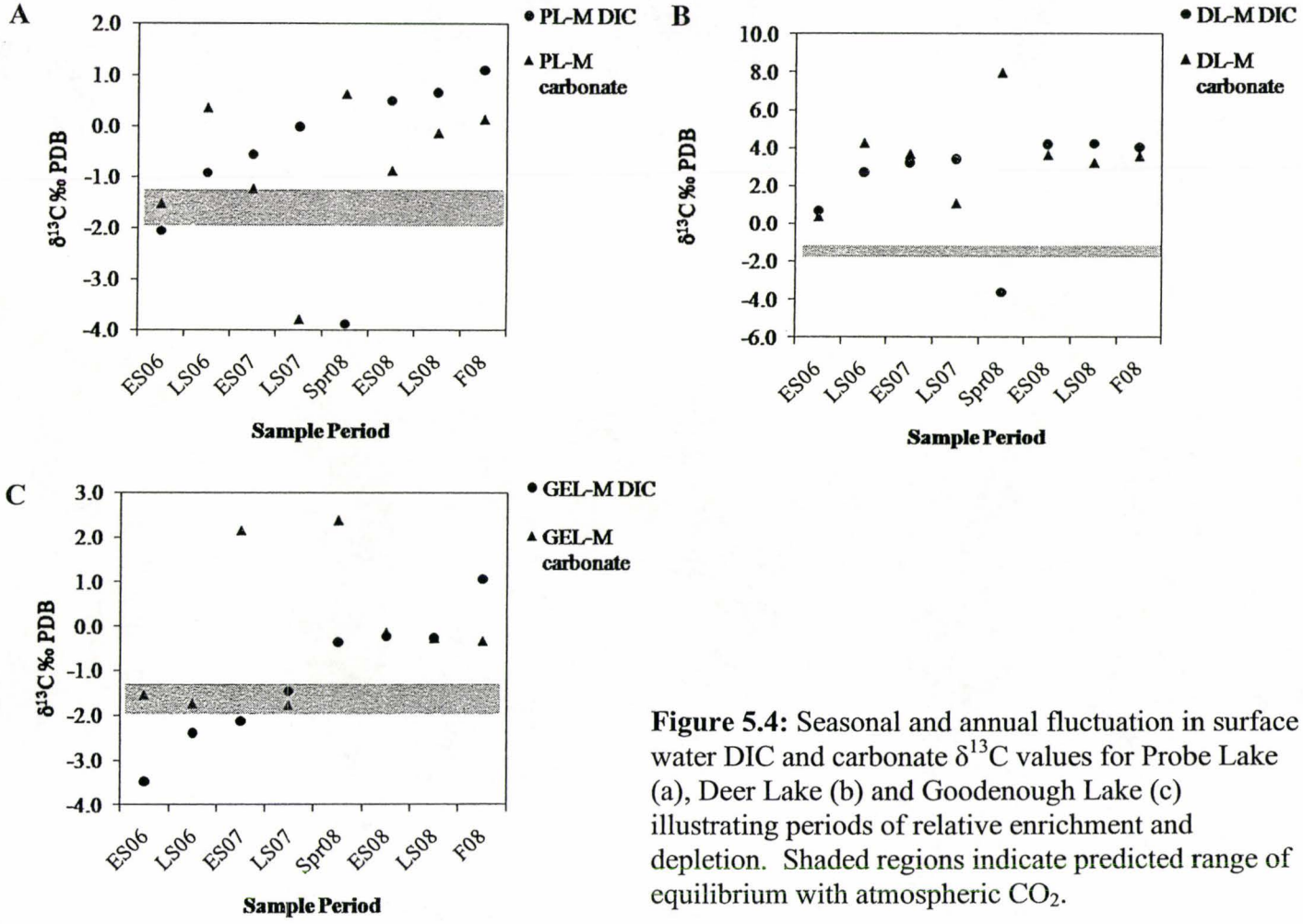

Figure 5.4: Seasonal and annual fluctuation in surface water DIC and carbonate $\delta^{13}C$ values for Probe Lake (a), Deer Lake (b) and Goodenough Lake (c) illustrating periods of relative enrichment and depletion. Shaded regions indicate predicted range of equilibrium with atmospheric CO_2.

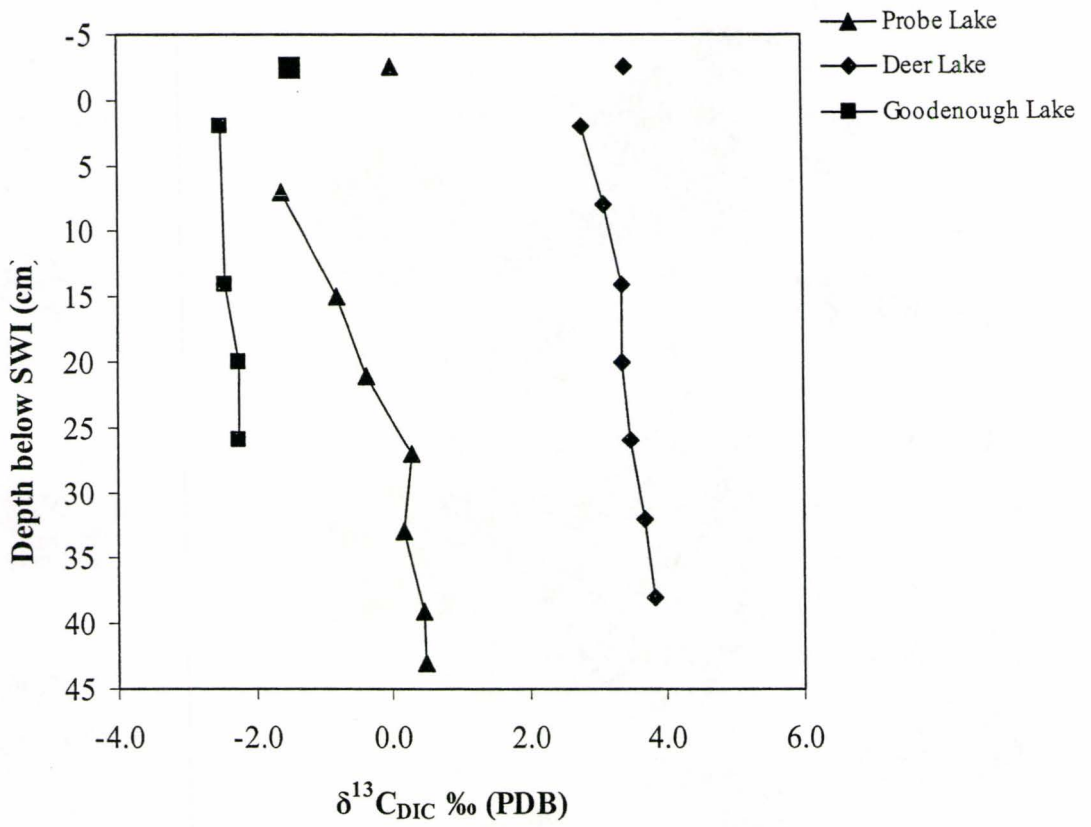

Figure 5.5: Porewater DIC $\delta^{13}C$ values for Cariboo lakes showing trends of more ^{13}C enriched values with depth. Corresponding surface water DIC values are represented as values above the sediment water interface.

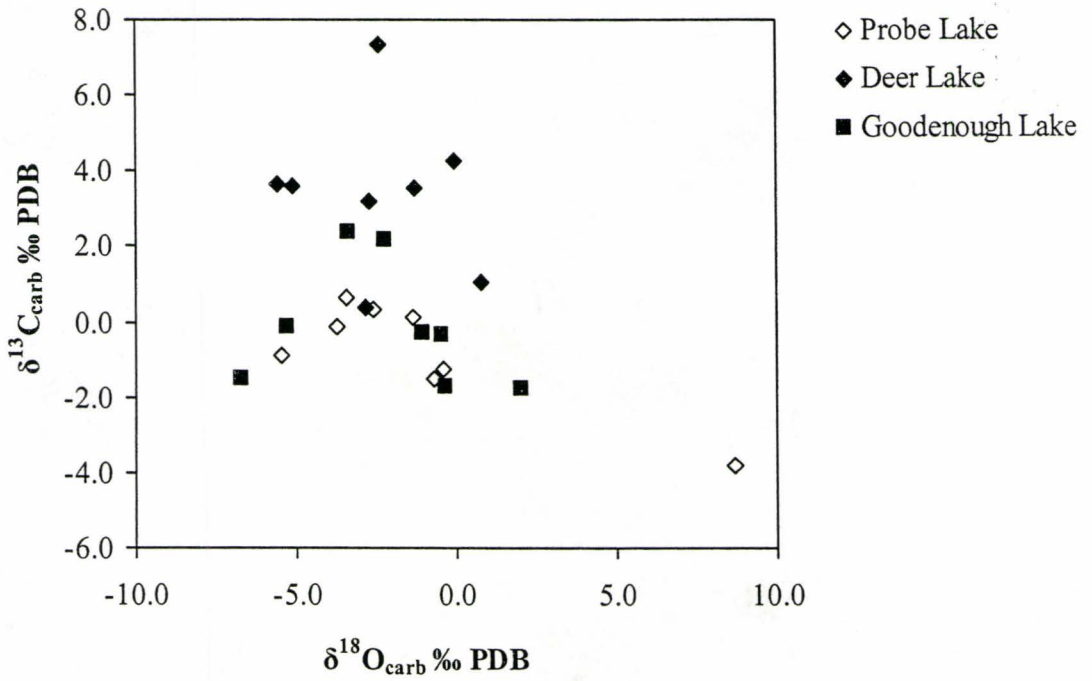

Figure 5.6: Carbonate $\delta^{13}C$ and $\delta^{18}O$ value for Cariboo Plateau microbial mats illustrating wide scatter and lack of correlation between values.

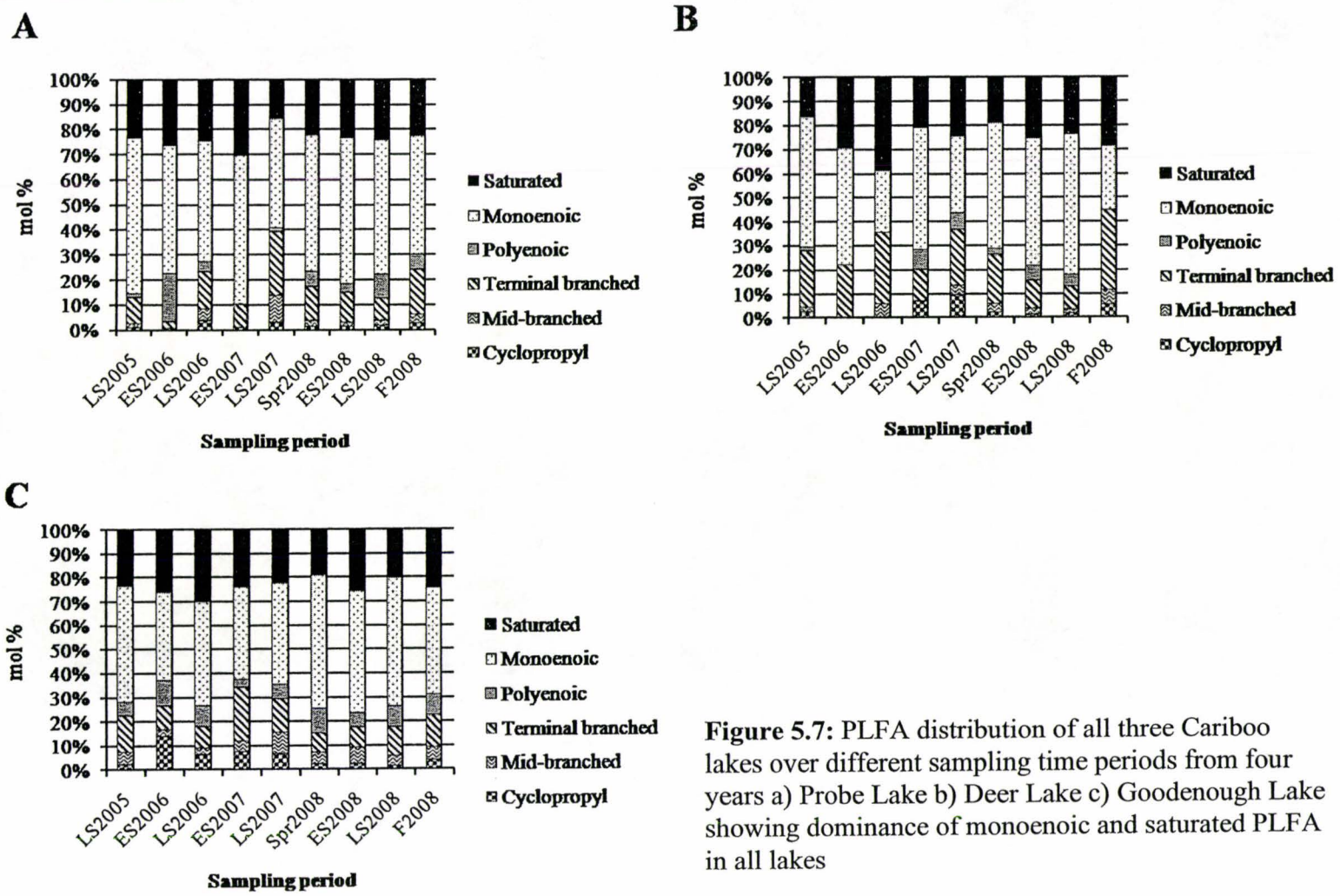

Figure 5.7: PLFA distribution of all three Cariboo lakes over different sampling time periods from four years a) Probe Lake b) Deer Lake c) Goodenough Lake showing dominance of monoenoic and saturated PLFA in all lakes

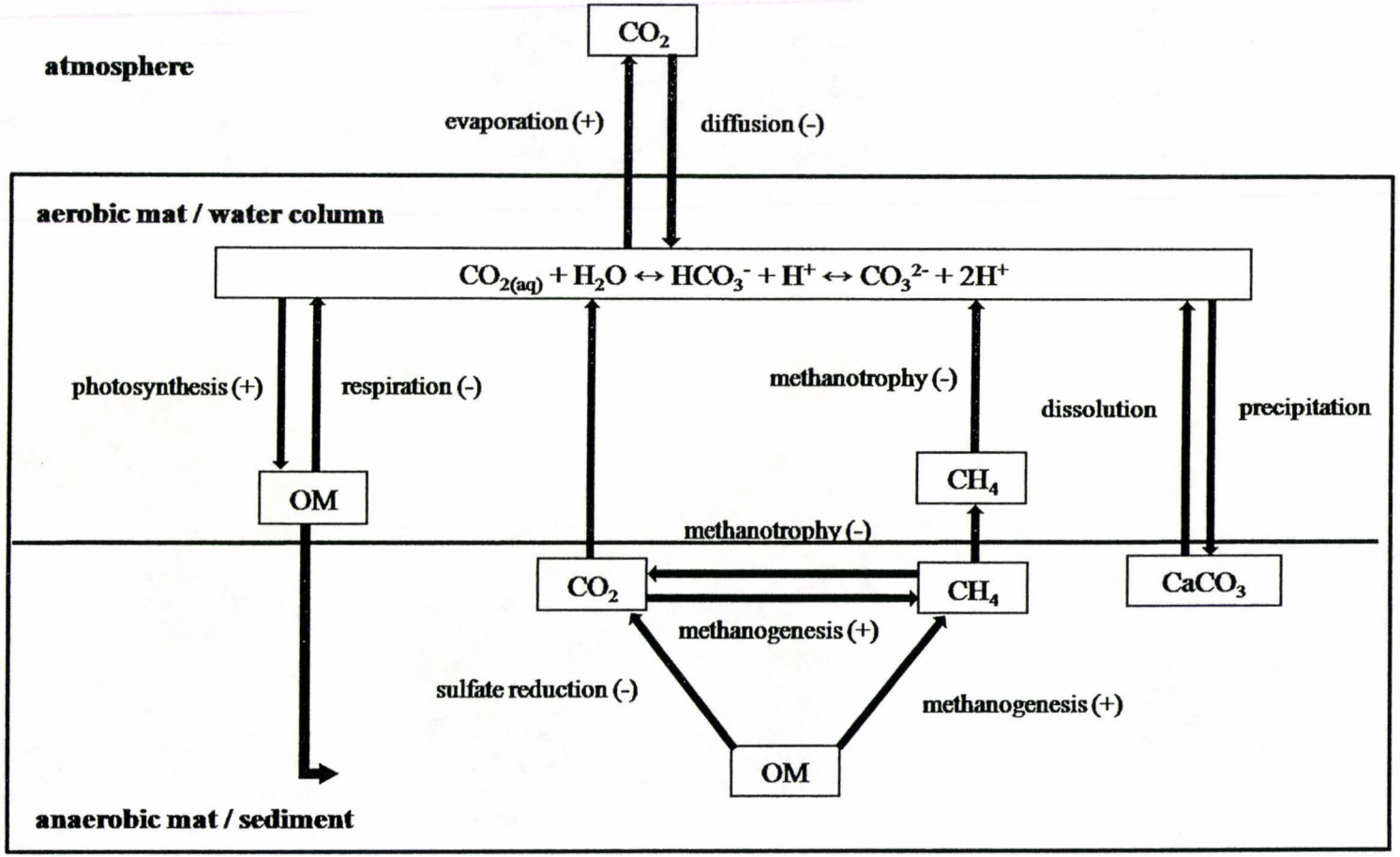

Figure 5.8: Illustration of the major carbon cycling pathways and whether each pathway would be expected to enrich (+) or depleted (-) the DIC in ^{13}C

Table 5.1: Water column chemistry including calculated pCO_2 and saturation indices from Cariboo mats. Sample names and time periods as described in text. Units in mg/L unless otherwise noted

Sample	pH	T (°C)	Specific conductance (µS/cm)	DIC	pCO_2 (µatm)	Na	Ca	Mg	SO_4	S.I.$_{calcite}$*	S.I.$_{dolomite}$	S.I.$_{magnesite}$
PL-M ES2006	10.1	16.0	29500	3300	257	10500	5	9	n.d.	1.3	3.2	1.3
PL-M LS2006	10.2	20.0	36400	4430	191	14300	< 10	11	94	1.1	3.2	1.3
PL-M ES2007	10.0	16.2	22500	3270	363	11600	4	8	43	1.2	3.1	1.2
PL-M LS2007	10.1	22.2	38500	4760	302	16100	3	11	115	1.0	3.1	1.3
PL-M Sp2008	9.9	6.8	4150	386	132	n.d.	n.d.	n.d.	17	-0.8	-0.9	-0.7
PL-M ES2008	10.1	14.5	18800	1910	182	6260	2	6	29	0.9	2.7	1.1
PL-M LS2008	10.1	18.3	29300	3240	251	10500	4	8	43	1.1	3.1	1.3
PL-M F2008	10.0	5.4	42600	4910	457	16200	4	11	52	1.1	3.0	1.4
DL-M ES2006	10.3	17.8	22300	2330	93	7420	< 5	50	< 0.5	1.0	3.8	2.1
DL-M LS2006	10.3	19.7	26900	3100	110	9450	4	60	19	1.2	4.0	2.1
DL-M ES2007	10.1	13.8	24000	54	214	9700	3	48	< 30	1.0	3.7	2.0
DL-M LS2007	10.2	19.8	31900	3800	178	14000	4	70	45	1.1	4.0	2.2
DL-M Sp2008	10.0	7.6	2370	218	100	n.d.	n.d.	n.d.	6	-0.4	0.6	0.5
DL-M ES2008	10.2	15.3	20300	2070	132	6508	2	27	17	0.8	3.3	1.8
DL-M LS2008	10.1	19.2	25500	2690	224	8820	2	29	45	0.9	3.5	1.8
DL-M F2008	10.1	5.3	35800	3920	302	12400	3	72	23	1.0	3.7	2.2
GEL-M ES2006	10.2	17.3	42700	4650	200	16000	n.d.	55	n.d.	1.0	3.7	2.0
GEL-M LS2006	10.2	19.1	53500	4270	170	21300	3	58	2090	1.0	3.8	2.0
GEL-M ES2007	10.2	15.7	46400	5030	200	19900	3	50	2070	1.0	3.7	2.0
GEL-M LS2007	10.3	19.5	50290	3091	93	n.d.	n.d.	n.d.	n.d.	1.0	3.8	2.0
GEL-M Sp2008	10.4	11.7	27200	2300	93	n.d.	n.d.	n.d.	712	0.6	3.0	1.8
GEL-M ES2008	10.3	15.8	50200	5460	138	20600	3	57	2090	1.0	3.7	2.0
GEL-M LS2008	10.2	20.0	59100	6240	209	25400	3	64	2390	1.0	3.8	2.0
GEL-M F2008	10.1	5.5	77000	9200	417	32600	4	96	3460	1.1	3.8	2.2

n.d., not determined

*spring SI values based on estimates of Ca, Mg concentrations in relation to measured Cl ion

Table 5.2: Vertical profiles of porewater chemistry in Cariboo Plateau lakes including methane concentrations and $\delta^{13}C$ values. Corresponding surface water measures are included for comparison. Units in mg/L unless otherwise stated.

	Porewater samples 2006					*Porewater samples 2007*					
Depth (cm)	**pH**	**DIC**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**	**pH**	**DIC**	**$\delta^{13}C_{DIC}$ ‰**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**
Deer Lake											
surface	10.3	3100	0.2	n.d.	19	10.2	3800	3.4	4.1	n.d.	45
1		2800	781.9		100	9.9			0.3		
3	10.6	3040			112		3019	2.8	99.7		
5											10
7		3470		-66.7	116	10.0					
9	10.2	3360			n.d.		3524	3.1			
11			791.8								9
13		3560			12	10.5					
15	10.4	3650			12		4086	3.4	505.2		
17				-72.2							13
19		3790			12	10.0					
21			680.2				2708	3.4			
23	10.6	3760			14						10
25						10.3					
27		3910		-71.9	6		3478	3.5	434.5		
29	10.5	3890			7	10.1					
31			647.6								9
33		3650			11		3927	3.7			
35	11.0	3850		-71.6	n.d.	10.0					
37											9
39		4110			n.d.		2784	3.8	677.6		
Goodenough Lake											
surface	10.2	4270	0.0	n.d.	2090	10.3	3091	-1.5	0.3	n.d.	n.d.
1	10.5	6060			2290	10.6			3.8		
3			2.2				4642	-2.5			

Table 5.2 continued

	Porewater samples 2006					*Porewater samples 2007*					
Depth (cm)	**pH**	**DIC**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**	**pH**	**DIC**	**$\delta^{13}C_{DIC}$ ‰**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**
5		6490			2350						2480
7	10.3	6320			1800	10.1				-64.6	
9				-56.4							
11		7930			2170						2830
13	10.3	7520			2200	10.0			9.6		
15			13.4				6677	-2.5			
17		8000			3310					-76.5	1450
19	10.4	8080			2870	10.1					
21				-57.3			7490	-2.3			
23		7900			3260				13.8		2620
25	10.4	8020			3080	10.4				-95.9	
27			9.5				6374	-2.2			
29		8440			3300						
31	10.4	8870			n.d.						
33				-56.8							
35		8360			n.d.						
37											
Probe Lake											
surface	10.2	4430	1.7	n.d.	94	10.1	4760	0.0	3.7	n.d.	115
1		4640	934.1		53						
3		4600			60						
5	9.9					9.6			356.1		
7		4320		-68.6	61		3013	-1.6	761.2		
9		4350			28						49
11	9.8										55
13		4440			66	9.7					
15		4380			62		1663	-0.8			
17	9.7										58
19		4340			54	9.6			811.7		
21		4110		-72.896	2		2490	-0.4			

Table 5.2 continued

	Porewater samples 2006					*Porewater samples 2007*					
Depth (cm)	**pH**	**DIC**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**	**pH**	**DIC**	**$\delta^{13}C_{DIC}$ ‰**	**CH_4 (µM)**	**$\delta^{13}C_{CH4}$ ‰**	**SO_4**
23	9.7										58
25		3870			n.d.	9.6					
27							3350	0.3			
29	9.7	4090	579.0		25						40
31				-68.5		9.6			748.2		
33							2819	0.2			
35											29
37						9.7					
39							2273	0.5			
41											24
43							2465	0.5	561.6		

Table 5.3: $\delta^{13}C$ values of DIC, carbonate and bulk organic matter from Cariboo mats. Samples names and time periods as in text. Standard deviations are reported for triplicate analyses. All $\delta^{13}C$ values reported relative to PDB.

Sample	$\delta^{13}C_{DIC}$ ‰	$\delta^{13}C_{carb}$ ‰	$\delta^{13}C_{org}$ ‰	$\Delta^{13}C_{DIC\text{-}org}$
PL-M ES2006	-2.1	-1.5 ± 0.3	-22.8 ± 0.2	20.7
PL-M LS2006	-0.9	0.4 ± 0.1	-24.3	23.4
PL-M ES2007	-0.6	-1.2	-23.9	23.3
PL-M LS2007	0.0	-3.8 ± 1.7	-24.0	24.0
PL-M Sp2008	-3.9	0.6	-24.3 ± 0.1	20.4
PL-M ES2008	0.5	-0.9 ± 0.4	-24.3 ± 0.1	24.8
PL-M LS2008	0.7	-0.1	-23.9	24.6
PL-M F2008	1.1	0.1	-24.6	25.7
DL-M ES2006	0.7	0.4	-18.7	19.4
DL-M LS2006	2.7	4.3	-18.6	21.3
DL-M ES2007	3.2	3.7	-18.8	22.1
DL-M ES2007 UPPER	3.2	3.4	-19.1	22.3
DL-M ES2007 LOWER	3.2	-1.8 ± 1.8	-18.4	21.6
DL-M LS2007	3.4	1.1 ± 0.4	-18.8 ± 0.1	22.3
DL-M Sp2008	-3.6	8.0	-18.7	15.1
DL-M ES2008	4.2	3.6	-18.6	22.8
DL-M LS2008	4.2	3.2	-19.0	23.2
DL-M F2008	4.0	3.6 ± 0.4	-18.6 ± 0.3	22.6
GEL-M ES2006	-3.5	-1.5	-23.3 ± 0.1	19.8
GEL-M LS2006	-2.4	-1.7 ± 0.8	-25.4	23.0
GEL-M ES2007	-2.1	2.2 ± 0.1	-25.4	23.3
GEL-M LS2007	-1.5	-1.8	-25.4	23.9
GEL-M Sp2008	-0.4	2.4 ± 0.1	-26.5	26.1
GEL-M ES2008	-0.2	-0.1	-24.8	24.6
GEL-M LS2008	-0.3	-0.3	-26.4 ± 0.2	26.1
GEL-M F2008	1.1	-0.3	-25.7	26.8

Table 5.4: PLFA profiles expressed in mol % for Probe Lake (PL-M) mat samples collected from 2005 - 2008. Sampling time periods are as described in text.

PLFA I.D.	PL-M LS2005	PL-M ES2006	PL-M LS2006	PL-M ES2007	PL-M LS2007	PL-M Spr2008	PL-M ES2008	PL-M LS2008	PL-M F2008
br14:0	0.7	0.0	1.0	0.0	3.3	0.9	0.9	0.5	1.7
14:0	1.2	2.0	1.3	0.0	3.3	2.1	1.7	2.3	2.3
i15:0	4.4	0.8	7.4	3.5	5.4	5.3	3.5	3.8	6.9
a15:0	2.8	0.6	4.1	2.7	6.8	4.8	3.5	2.1	5.0
15:1	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.4	1.8
15:0	1.1	0.5	0.0	0.0	3.0	0.9	0.6	0.7	1.6
i16:0	1.1	0.3	3.0	0.0	3.7	1.3	2.5	1.0	2.1
16:2	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.6	0.0
16:1	17.0	15.7	10.6	9.6	13.6	18.6	19.5	22.4	16.6
16:0	17.3	17.5	17.5	14.5	4.8	15.6	17.2	18.2	13.9
br17:1	0.7	0.0	1.2	0.0	3.0	0.9	0.0	0.6	1.3
10me16:0	0.7	0.0	1.7	0.0	3.0	1.3	0.8	1.4	1.8
i17:0	1.1	0.6	1.2	0.9	3.0	0.9	0.8	0.7	1.4
a17:0	1.2	0.4	2.3	1.1	5.2	1.3	1.6	0.8	1.9
17:1	0.0	0.0	2.6	0.0	11.6	2.3	1.9	1.0	2.3
cy17:0	0.0	0.8	0.0	1.3	1.4	0.8	0.9	1.6	1.4
17:0	1.2	0.7	3.9	0.8	1.5	0.9	1.1	0.7	1.4
tetramethyl16:0	0.0	0.0	3.1	9.2	1.5	0.8	0.6	0.0	1.4
18:3	0.0	3.5	0.0	0.0	0.0	0.0	0.6	0.8	0.0
18:2	1.6	4.7	4.6	0.0	1.6	3.0	2.0	6.9	3.1
18:1	44.5	34.9	43.7	44.3	15.5	31.2	35.9	29.0	23.2
18:0	2.0	5.6	6.1	12.0	1.8	1.9	2.1	1.5	2.1
br19:0	0.0	0.0	2.9	0.0	4.0	0.0	0.0	0.0	0.0
19:1	0.0	0.0	4.4	0.0	1.6	1.0	1.2	0.8	1.5
cy19:0	1.3	0.0	4.8	0.0	1.5	0.8	0.7	0.6	1.4
20:5	0.0	11.5	0.0	0.0	0.0	1.3	0.0	0.6	1.3
20:4	0.0	0.0	0.0	0.0	0.0	0.7	0.6	0.4	1.3
20:3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
20:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1

Table 5.5: PLFA profiles expressed in mol % for Deer Lake (DL-M) mat samples collected from 2005 - 2008. Sampling time periods are as described in text.

PLFA I.D.	DL-M LS2005	DL-M ES 2006	DL-M LS2006	DL-M ES2007	DL-M LS2007	DL-M Spr2008	DL-M ES2008	DL-M LS2008	DL-M F2008
br14:0	1.4	0.0	5.5	0.0	3.6	1.5	0.9	0.6	5.3
14:0	1.7	5.1	6.3	2.0	3.6	1.8	2.0	1.1	6.2
br15:1	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0
i15:0	7.8	6.0	6.9	5.2	5.1	6.9	4.0	2.5	6.8
a15:0	9.8	7.1	9.6	4.1	6.5	6.9	3.4	2.0	8.1
15:1	0.8	0.0	0.0	0.0	0.0	0.8	0.7	0.0	0.0
15:0	1.1	0.0	5.3	0.0	3.2	1.1	1.0	0.4	0.0
i16:0	2.2	4.8	5.1	2.1	3.5	2.8	1.6	1.0	6.1
16:2	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
16:1	16.2	5.9	11.5	7.7	8.1	13.8	21.9	12.5	11.5
16:0	10.3	8.7	12.8	9.6	6.6	11.9	18.9	12.2	9.2
br17:1	2.2	0.0	0.0	0.0	0.0	0.9	0.6	0.0	0.0
10me16:0	0.7	0.0	0.0	0.0	0.0	1.0	1.3	0.3	0.0
i17:0	1.3	0.0	0.0	0.0	3.0	1.2	1.1	0.4	5.1
a17:0	1.9	4.5	5.3	1.9	3.3	2.4	1.5	0.7	5.7
17:1	2.2	0.0	0.0	1.7	0.0	3.1	1.4	0.9	0.0
cy17:0	1.4	0.0	0.0	0.0	3.0	1.0	0.8	1.0	0.0
17:0	1.1	4.4	4.3	0.0	2.9	1.3	1.0	0.6	5.1
tetramethyl16:0	0.0	0.0	4.3	0.0	5.5	1.1	0.7	0.0	6.0
18:2	1.7	0.0	0.0	7.9	6.2	1.3	3.7	2.3	0.0
18:1	32.5	42.4	12.0	41.4	20.8	31.0	27.2	25.9	13.3
18:0	1.7	11.0	6.5	9.3	6.1	2.2	1.9	1.8	6.3
br19:0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
19:1	1.1	0.0	0.0	0.0	0.0	1.5	0.9	0.4	0.0
cy19:0	1.2	0.0	0.0	7.3	5.8	1.3	0.6	0.4	5.3
20:5	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.8	0.0
20:4	0.0	0.0	0.0	0.0	0.0	1.0	0.7	0.3	0.0
20:1	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0

Table 5.6: PLFA profiles expressed in mol % for Goodenough Lake (GEL-M) mat samples collected from 2005 - 2008. Sampling time periods are as described in text.

PLFA I.D.	GEL-M LS2005	GEL- M ES2006	GEL-M LS2006	GEL-M ES2007	GEL-M LS2007	GEL-M Spr2008	GEL-M ES2008	GEL-M LS2008	GEL-M F2008
br14:0	2.7	0.9	0.0	0.0	1.7	0.6	0.6	1.1	1.4
14:0	3.1	1.5	1.4	4.0	2.0	1.1	1.1	1.6	1.6
i15:0	4.5	4.0	4.3	6.0	4.9	3.2	3.2	1.4	5.3
a15:0	3.4	3.2	2.5	4.7	3.0	1.8	1.8	6.4	3.6
15:1	0.0	0.0	0.0	3.8	2.9	0.7	1.2	1.4	0.9
15:0	2.5	1.6	1.5	4.0	2.6	0.9	0.9	1.4	1.6
i16:0	2.4	0.9	0.7	3.6	1.9	1.0	1.2	1.4	1.6
16:2	2.4	0.0	0.0	0.0	0.0	3.5	1.4	6.2	0.0
16:1	13.2	3.9	7.2	9.4	8.4	20.6	19.5	29.0	13.0
16:0	12.7	8.5	18.0	8.3	9.5	13.9	20.2	14.3	16.4
br17:1	0.0	0.0	0.0	3.4	1.6	1.2	1.2	0.0	1.8
10me16:0	0.0	1.7	3.0	4.3	4.6	4.1	6.0	3.0	3.7
br17:0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
i17:0	2.6	0.0	0.6	3.4	1.6	0.9	0.9	1.2	1.3
a17:0	2.3	1.0	0.7	3.5	1.7	1.1	1.2	1.4	1.4
17:1	0.0	0.0	1.4	3.8	2.8	1.5	1.9	1.3	2.2
cy17:0	2.4	0.9	0.0	3.4	1.7	0.9	0.9	0.0	1.3
17:0	2.3	1.0	3.4	3.4	1.6	0.9	0.9	1.1	1.2
tetramethyl16:0	0.0	8.4	2.5	1.6	2.4	0.8	0.5	1.0	1.0
18:3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:2	3.1	9.4	5.8	3.1	3.6	6.2	4.5	2.6	6.0
18:1	35.4	29.9	33.8	19.7	22.2	30.5	26.6	19.8	27.5
18:0	2.5	10.9	4.9	2.9	3.3	1.9	1.8	1.7	2.8
br19:0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0
19:1	0.0	0.0	0.0	0.0	2.5	1.2	0.8	1.2	0.0
cy19:0	0.0	12.0	6.1	3.7	4.9	1.5	1.4	1.5	2.6
20:5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
20:4	0.0	0.0	2.4	0.0	2.3	0.0	0.0	0.0	1.0
20:1	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0
20:0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0

Table 5.7: PLFA distribution expressed in mol % and $\delta^{13}C$ values reported relative to PDB for Deer Lake microbial mat subsamples. 'Upper' corresponds to the oxygenated zone identified via microelectrode profiling while 'lower' corresponds to the unoxygenated, sulfidic zone. PLFA $\delta^{13}C$ values are reported for triplicate analyses.

	DL-M LS2007 upper		DL-M LS2007 lower	
PLFA I.D.	**mol %**	**$\delta^{13}C$ ‰**	**mol %**	**$\delta^{13}C$ ‰**
br14:0	0.8		1.2	
14:0	2.1	-22.2 ± 0.1	1.9	
br15:0	0.0		0.0	
i15:0*	5.2	-22.2 ± 0.3	7.6	-25.4 ± 0.1
a15:0*	3.0		8.2	
15:1	1.1		0.0	
15:0	0.8		0.9	
i16:0	1.0		1.7	
16:2	0.4		0.0	
16:1	14.4	-24.4 ± 0.4	13.4	-22.2 ± 0.3
16:0	14.9	-27.1 ± 0.2	9.2	-22.9 ± 0.3
br17:1	1.5		1.3	
i17:0	0.9		0.7	
a17:0	0.7		1.2	
17:1	1.2		1.3	
cy17:0	0.5		2.2	
17:0	0.6		0.8	
tetramethyl16:0	0.9		1.2	
18:2	4.3		2.2	
18:1	41.4	-24.2 ± 0.1	40.4	-22.1 ± 0.1
18:0	1.9		2.2	
19:1	1.0		1.8	
cy19:0	0.8		1.3	
20:4	0.6		0.0	

*$\delta^{13}C$ value reported as single value as peaks were not completely resolved

Table 5.8: PLFA $\delta^{13}C$ values for Cariboo Plateau microbial mat samples collected from 2005 – 2008 in late summer (LS) and early summer (ES). Mean and standard deviations are reported for triplicate unless otherwise noted.

	LS2005	ES2006	LS2006	ES2007	LS2007	LS2008
Probe Lake						
14:0		-25.7 ± 0.2		-26.1 ± 0.2	-27.7 ± 0.4	-25.6 ± 0.2
i15:0			-26.7 ± 0.2	-27.5 ± 0.0	-26.4 ± 0.6	-27.0 ± 0.1
a15:0						
16:1	-31.6 ± 0.6	-29.0 ± 0.7	-29.8 ± 1.4	-28.0 ± 0.2	-29.0 ± 0.3	-27.5 ± 0.2
16:0	-32.5 ± 0.6	-29.2 ± 0.1	-29.9 ± 0.1	-30.1 ± 0.3	-29.7 ± 0.6	-26.8 ± 0.1
cy17:0					-22.5 ± 0.7	
18:1	-27.4 ± 0.3	-29.0 ± 0.1	-28.6 ± 0.3	-26.6 ± 0.6	-28.7 ± 0.8	-23.1 ± 0.1
cy19:0					-26.7 (n =1)	
Deer Lake						
14:0					-29.4 ± 0.1	
i15:0	-28.3 ± 0.2	-26.5 ± 0.1		-22.6 ± 0.1	-22.5 ± 0.4	-30.1 ± 0.0
a15:0						
16:1	-26.4 ± 0.4	-21.7 ± 0.1		-24.2 ± 0.2	-29.3 ± 0.4	-33.4 ± 0.7
16:0	-27.3 ± 0.3	-23.3 ± 0.1		-26.5 ± 0.2	-25.5 ± 0.2	-33.0 ± 0.1
18:1		-20.1 ± 0.2		-23.8 ± 0.1	-25.6 ± 0.7	-29.0 ± 0.0
Goodenough Lake						
14:0			-28.1	-28.4		-30.5 ± 0.1
i15:0			-28.6 ± 0.1	-29.1 ± 0.1	-28.2 ± 0.0	-30.2 ± 0.2
a15:0						
15:0					-27.5 ± 0.4	-31.3 ± 0.3
16:1	-31.4 ± 1.4		-27.7 ± 0.2	-30.7 ± 0.2	-27.1 ± 0.8	-33.3 ± 0.1
16:0	-29.3 ± 0.4		-31.3 ± 0.2	-31.5 ± 0.1	-31.2 ± 0.1	-34.6 ± 0.1
10me16:0					-28.4 ± 1.2	-29.9 ± 0.2
cy17:0				-31.3 (n = 1)		
18:1	-23.1 ± 0.4		-28.2 ± 1.8	-29.1 ± 0.4	-29.0 ± 0.2	-32.2 ± 0.1
cy19:0				-30.1 ± 0.4	-28.9 ± 0.3	-30.7 ± 0.3

*$\delta^{13}C$ value reported as single value as peaks were not completely resolved

CHAPTER 6

GENERAL CONCLUSIONS AND FUTURE RESEARCH

This dissertation applied stable and radiocarbon isotopes in conjunction with organic geochemical biomarker approaches to investigations of freshwater microbialites and carbonate-rich microbial mats in order to better understand carbon cycling, microbial influences on carbonate precipitation and associated isotopic biosignatures. The overarching hypotheses that directed this work were: 1) biology plays a role in the formation of Pavilion Lake microbialites 2) isotopic biosignatures of microbial metabolic activity are present in association with the microbialites and are related to environmental parameters and 3) isotopic biosignatures of microbial metabolic activity and carbonate precipitation are present within carbonate rich microbial mats on the Cariboo Plateau. This research has proved to be a valuable contribution to the general knowledge regarding microbialite formation and microbial influences on carbonate precipitation and isotopic composition. Not only does this research represent the isotopic characterization of unique, previously undescribed systems but this research plays a significant role in understanding microbial influences on carbonate precipitation and associated isotope biosignatures. With respect to the main hypotheses outlined for this research, the papers presented in this thesis indicate that there are detectable biosignatures of microbial activity and microbial influence on carbonate precipitation in Pavilion Lake microbialites and Cariboo microbial mats. The examination of seasonal and spatial variability in these biosignatures and the findings related to the individual study hypotheses as outlined in

Chapter 1 elucidate modern processes and provide direct evidence of photosynthetic influences on carbonate precipitation that strongly supports the role of biology in the formation of microbialites. These findings provide a tool that can be applied to future studies examining the geologic record.

6.1. Research summary

While the morphology of the Pavilion Lake microbialites had been characterized previously, the biogeochemical processes leading to the formation of these structures and potential biosignatures had not been investigated (Laval et al., 2000). This body of work represents the first isotopic investigation of the Pavilion Lake and provides fundamental knowledge regarding the carbon cycling within this system and the putative role of biology in the formation of the microbialites.

A primary question concerning the Pavilion Lake microbialites, and microbialites in general, is whether the structures are biogenic in origin. As indicated in Chapter 1, abiotic mechanisms of microbialite formation identify mixing of groundwater and lake water, creating localized zones of saturation as primary mechanisms of carbonate precipitation e.g. Council and Bennett, 1993. The study outlined in Chapter 2 applied ^{14}C and ^{13}C analysis to test the hypotheses that microbialites are actively growing and forming abiotically due to mixing of lake water and groundwater. This chapter investigated carbon sources and cycling within the lake in order to constrain potential groundwater carbon inputs and address the hypothesis that the microbialite isotopic compositions reflect groundwater carbon inputs. Characterization of the isotopic

compositions of the carbon pools in this system demonstrated that atmospheric CO_2 was ultimately the dominant source of carbon input to the dissolved inorganic carbon (DIC), carbonate structures or associated microbial communities.

Mass balance estimates of the relative contribution of ^{14}C-enriched atmospheric CO_2 and ^{14}C-depleted groundwater sources, constrained regional and/or local groundwater DIC inputs to a maximum of 9 –13 % of carbon input to ambient water DIC. Groundwater inputs to microbialites recovered from depths of 20 to 29 m were estimated at 10 to 16 %. In contrast, deep (> 32 m) microbialite samples showed greater ^{14}C depletion. It was hypothesized that this difference could be accounted for by either decreased growth rates due to low light levels at these depths, or greater inputs of ^{14}C-depleted groundwater carbon at these depths. Light levels at this depth were below 1 % surface PAR and the ^{14}C depletion was hypothesized to result from decreased photosynthetic activity and consequently, decreased growth rates. The unique nature of the deep microbialite samples in Pavilion Lake was further explored in Chapter 4 of this thesis. Minimal inputs of groundwater carbon to microbialite carbonate and associated microbial communities are consistent with proposed biological influences on carbonate precipitation and microbialite formation but do not rule out a role for abiotic processes in microbialite formation.

The role of microbial activity in carbonate precipitation and the identification of associated biosignatures were explored further in studies that address the specific hypotheses described for the additional chapters. Chapter 3 examined the hypothesis that photosynthetic influence on carbonate precipitation would result in a detectable

biosignature of activity. This study identified photosynthetic isotopic biosignatures in small, nodules on the surface of Pavilion Lake microbialites presenting evidence directly linking photosynthetic activity with carbonate isotopic biosignatures in a freshwater microbialite. Organic matter $\delta^{13}C$ values within the nodules (-30.6 to -21.1 ‰) and an average inorganic to organic carbon $\Delta\delta^{13}C$ value of 26.8 ‰ illustrated the preferential uptake of ^{12}C during photosynthesis. Uptake of ^{12}C supporting the generation of ^{13}C-enriched DIC that was reflected in carbonate within the nodules had $\delta^{13}C$ values that were enriched by up to +3.6 ‰ as compared to predicted abiotic carbonate $\delta^{13}C$ values from measured DIC (mean -1.2 ‰, n = 13), consistent with microbial photosynthetic influence on precipitation within the nodule microenvironment. Further support for photosynthetic influences on the microenvironment within the nodules was seen in microelectrode profile showing oxygen supersaturation of up to ~ 275 % and elevated pH compared to ambient water consistent with the hypothesis that microbial activity would alter the local geochemistry. Nodule carbonate $\delta^{18}O$ values were used to estimate temperature of formation and it was determined that the predicted temperature range of formation was consistent with summer temperatures observed in the lake. As higher levels of photosynthetic activity would be expected in the summer, these results provide further support for the role of biology in carbonate precipitation. The results of this study not only provide additional support for the hypothesis of a biological origin for the microbialites but contrasts previously studied marine microbialites in which heterotrophic activity was identified as a significant process in microbialite formation.

As the nodules described in Chapter 3 were predominantly collected during the summer and at similar depths, questions remained regarding variability in the level of photosynthetic activity and the relative role of heterotrophy with respect to varying light and temperature regimes that exist seasonally and spatially within the lake. Chapter 4 tested the hypothesis that seasonal and spatial changes in light and temperature would affect the relative autotrophic and heterotrophic dominance and corresponding isotope biosignatures. In contrast to the small, sporadic nodules investigated in Chapter 3, Chapter 4 examined variability in the autotrophic and heterotrophic community and associated biosignatures present in the thin (5 mm) surface microbial mat covering the majority of the microbialite surface. Previous studies of modern microbialites had found that microbial communities did not exhibit seasonal variation and that morphological variation in structure was related to physical conditions such as space for growth and sediment depth (Andres and Reid, 2006; Reid et al., 2000). For this study, microbialites were collected at depths ranging from 11 to 55 m and during different seasonal periods. To investigate the relative role of autotrophy and heterotrophy on isotopic biosignatures within an individual microbialite, samples were also selected for analysis that showed distinct colour variation that was hypothesized to represent different microbial communities. Autotrophic and heterotrophic dominated regions were identified on an individual microbialite through variability in colour, biomass estimates, PLFA profiles and PLFA $\delta^{13}C$ value. In addition, experiments designed to assess effects of light limitation on the microbialite communities did not show any trends in PLFA profiles or

detectable microbial influences on $\delta^{13}C$ values after periods of up to one month of limited light.

Variations in PLFA distribution and biomass estimates enabled the separation of the microbialites into three groups: above 20 m, 20 to 33 m, and below 46 m. The PLFA profiles of the surface microbial mat communities that covered the majority of the microbialite surface were primarily consistent with photosynthetic dominance and were stable over seasonal and depth profiles to 33 m. However, biomass estimates in the 11 and 26 m samples did show seasonal variability. In particular, the 11 m samples showed an increase in biomass during the summer. Concurrent with this observed increase in biomass during the summer, carbonate $\delta^{13}C$ values in the shallow 11 m samples were significantly different (Student's t-test, $p = 0.013$) than those collected in the same season from 26 m and were enriched relative to the predicted equilibrium mean by up to 2.4 ‰. In contrast, samples collected from below 20 m were within the range predicted for abiotic equilibrium with the DIC. This ^{13}C-enrichment is consistent with photosynthetic activity is hypothesized to result from high rates of photosynthesis and growth in these shallow samples supported by high summer temperatures and light levels at this depth.

Biomass estimates in the two samples below 46 m were 0.3 and 0.5 μg PLFA/g, in contrast to the highest value of 17.0 μg PLFA/g recovered from the 11 m samples from the same season, suggesting a decrease in microbial growth and production at depths below 46 m. Microbialite samples below 46 m showed different PLFA and isotopic profiles as compared to samples above 33 m. These differences were hypothesized to result from decreased levels of photosynthesis and growth rates at these depths due to low

light levels. In contrast to the commonly investigated marine systems, this study represents an investigation of the relationship between changes in environmental parameters and biosignatures in a system that shows seasonal and spatial variations in light and temperature. The results of this study indicate that changes in microbial activity levels and growth rate, rather than significant changes in microbial community plays an important role in formation of isotope biosignatures. These findings are consistent with the hypothesis in Chapter 2 that observed ^{14}C-depleted $\Delta^{14}C$ values in deep samples are due to low light levels and corresponding slower growth rates.

The role of microbial activity on isotope biosignatures was further investigated in the Cariboo Plateau carbonate rich microbial mats as detailed in Chapter 5. Within these mats it was hypothesized that photosynthetic activity would result in ^{13}C-enriched carbonate and ^{13}C-depleted organic matter reflecting uptake of ^{12}C. High levels of photosynthetic activity resulted in pCO_2 below equilibrium during the summer and DIC $\delta^{13}C$ values generally enriched above values predicted for isotopic equilibrium with atmospheric CO_2. Both DIC and carbonate $\delta^{13}C$ values showed variability, within an individual lake, carbonate $\delta^{13}C$ values had an overall range of ~ 4 to 8 ‰ over the course of this study. Variability in DIC and carbonate $\delta^{13}C$ values was attributed to shifts in the balance between the influences of photosynthesis and heterotrophy on the DIC within the lake and within the microbial mat. Despite extensive photosynthetic drawdown of CO_2 in the lakes, bulk organic values in the Cariboo lakes ranged from a mean of -18.7 to -25.3 ‰ with mean $\Delta^{13}C_{inorg\text{-}org}$ values that ranged from 21.1 to 24.2 ‰, consistent with non-CO_2 limited photosynthesis. These observations are in contrast to the saline, organic rich

mat and hot spring microbial mats that have been extensively studied that have ^{13}C-enriched bulk organic matter that has been attributed to CO_2 limitation or non-Calvin Benson photosynthetic pathways, e.g. Des Marais et al., 1989; van der Meer et al., 2000. It was proposed that the high levels of photosynthesis within the Cariboo microbial mats was due to high DIC concentrations and carbonate content that provided an extensive non-limiting supply of inorganic carbon. The high levels of photosynthetic activity supported by high concentrations of DIC and carbonate content within the mats provides an alternate explain for observations of ^{13}C-enriched bulk organic matter in modern systems and for ^{13}C-depleted organic matter recovered from the geologic record.

6.2. Directions for future research

As both Pavilion Lake and the Cariboo Plateau represent systems that have not been extensively studied, many possibilities exist for future research. More detailed characterization of the microbial communities associated with the microbialites and with the Cariboo mats will allow for a more comprehensive comparison of these systems to other modern analogues. As biosignatures of microbial activity were found that contrast other microbial systems, it is reasonable to hypothesize that the microbial communities on the microbialites and within the Cariboo microbial mats are distinct from other comparative modern systems. Genetic analysis can be used to address this hypothesis and provide more in-depth information regarding the organisms that are present and contributing to the observed biosignatures. It would also be of interest to see if the bacteria associated with the Pavilion Lake microbialites are similar to those present in the

carbonate rich Cariboo microbial mats as evidence of photosynthetic influences on DIC and carbonate was observed in both systems.

To fully understand the degree to which variability in microbial activity is related to seasonal changes in light and temperature, future studies are warranted that characterize the rates of photosynthesis within the microbialite surface microbial mat communities. Specifically, to address the hypothesis that higher levels of photosynthetic activity were present in shallow 11 m microbialite samples during the summer that correlated to elevated $\delta^{13}C$ values. This information will enhance our understanding of the potential influences of biology on the morphology of Pavilion Lake microbialites. Similarly, further refinement of the carbon cycling model discussed in relation to the Cariboo Plateau microbial mats is needed in order to fully understand these systems and the relative balance of autotrophy versus heterotrophy, including the magnitude of the various inputs and outputs. As microelectrode profiling of Pavilion Lake nodules and Cariboo mats was performed only during the day in the described studies, diel investigations of both the microbialite and microbial mat systems would contribute substantially to understanding the variation in microbial activity. Identification of the rate of photosynthesis within the microbial mats will aid in the determination of the relationship between inorganic carbon uptake and replenishment from the atmosphere. Understanding the potential for inorganic carbon resupply due to heterotrophic activity will also provide information that may be used to refine the proposed model. In particular, investigations of the role of sulfate reduction in carbon cycling are warranted

as it has been identified as an important process in microbial mats and in carbonate precipitation.

In Chapter 2 of this thesis, the use of ^{14}C analysis to test the hypothesis that microbialites are actively growing indicates that ^{14}C analysis can be used to determine microbialite growth rates. However, the range in sizes of microbialites within the lakes indicates that this initial growth rate is too slow to account for all of the observed variability. Additional ^{14}C analysis of microbialite growth in association with datable structures is needed to further understand microbialite growth rates within the lake. Determination of the variations in growth rate of microbialites throughout the lake including at different depths will address the hypothesis that higher levels of biological activity result in faster growth rates and a greater potential to preserve detectable isotopic biosignatures within carbonate structure. Additional investigations of growth rate will also further explore the hypothesis presented in Chapter 4 that biological control on microbialite formation is related to the overall morphology and friability of the structures.

Further characterization of the unique, deep microbialite structures will provide additional information regarding the source of the ^{14}C-depleted nature of these structures explore the hypothesis that growth rate at these depths are slower than at shallower depths and that this decreased growth may influence the size and morphology of these particular structures. It is evident based on the findings in Chapter 2 and Chapter 4 that the deep microbialites in Pavilion Lake are unique in comparison to other structures. Identification of the microbial communities associated with these structures and further

detailed isotopic analysis on additional samples is important to understanding the genesis of the unique characteristic of deep microbialites.

Critical to the use of isotopic biosignatures in the interpretation of the geologic record is the degree of preservation of these signals. Photosynthetic influences on recent carbonate precipitation may not be preserved throughout the system, implying that such signals would not be easily identified in ancient stromatolites. As the microbialite structures are porous in nature, diagenetic loss of isotopic signatures during mineral recrystallization may be occurring within the structures over time. To test the hypothesis that surface microbial mat and nodule photosynthetic biosignatures are preserved within the carbonate interior of the microbialites, the carbonate from the interior of the structures can be sampled and these carbonate $\delta^{13}C$ values compared to the range of elevated $\delta^{13}C$ values identified in this study. Likewise, organic and inorganic isotope biosignatures within the Cariboo microbial mats may not preserve in the underlying sediment. During the course of this thesis, sediment core samples were collected from the Cariboo lakes to address this question of preservation. Characterization of these cores is currently on-going to test the hypothesis that isotopic biosignatures will be recovered from lake sediments that will allow for identification of microbial activity, providing important information regarding the level of preservation of the biosignatures that were identified in the mat.

With respect to identifying biosignatures preserved in the geologic record, investigation of the potential for other microbial biomarkers in the microbialite and Cariboo microbial mat systems that could be used in conjunction with isotopic signals

would prove useful. PLFA degrade relatively quickly upon death of the organisms, therefore although PLFA provide a useful characterization a viable microbial community, other biomarkers have a higher potential for preservation in the geologic record. 2α-methylhopane is one such distinctive cyanobacteria marker that has been identified in both modern mats from Yellowstone and in sediments as old as 2700 Ma (Summons et al., 1999). Future research examining the hypothesis that these and other biomarkers exist in Pavilion Lake and the Cariboo microbial mats would provide additional information linking organic and inorganic biosignatures, and contributes to identifying multiple lines of evidence that would enhance interpretation of biosignatures in the geologic record.

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