CARBON ISOTOPE BIOSIGNATURES

SPATIAL DISTRIBUTION AND PRESERVATION OF CARBON ISOTOPE BIOSIGNATURES IN FRESHWATER MICROBIALITE CARBONATE

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ABSTRACT

Modern microbialites provide the opportunity to explore the influences of biology on microbialite formation and understand how biosignatures can be preserved in these structures. In this study, we compared δ13Ccarb values from nodule and surface biofilm carbonates on microbialite structures across depths and locations throughout Pavilion Lake to evaluate whether variable light exposure produced limitations to biosignature formation. At depths below 21 m, vertical profiling of $\delta^{13}C_{carb}$ across colour transitions of surface biofilm on microbialite structures was performed to identify spatial arrangement of autotrophic and heterotrophic biosignatures. Finally, preservation of the photosynthetic biosignature over time was investigated by collecting carbonates beneath the microbialite surface. These investigations were performed in order to better characterize the factors controlling biosignature formation, distribution, and preservation within Pavilion Lake.

Decreasing trends of $\delta^{13}C_{carb}$ with depth across study sites indicated that attenuated sunlight in the water column is likely the primary control on biosignature formation. Below 21 m, photosynthetic enrichments representing biosignatures on microbialite surfaces were reduced and recorded $\delta^{13}C_{carb}$ values that fell within the predicted equilibrium range. Biosignature loss is suggested to result from the relative proportions of autotrophic and heterotrophic processes changing at depths and producing average $\delta^{13}C_{carb}$ values. Variability of where biosignatures are lost on the microbialite surface indicated that the spatial extent of photosynthetic communities producing enrichments is potentially influenced by variable incidences of light at these depths.

Although no definitive biosignatures of heterotrophy were identified, several interfaces were identified where the balancing proportions of autotrophic and heterotrophic processes influenced by light variability potentially mediate biosignature loss. Decreasing trends of $\delta^{13}C_{carb}$ beneath the microbialite surface and estimates of past microbialite growth rates indicated that

surface biosignatures are lost within 100 – 400 years. It is suggested that infilling processes overprint enrichments and deplete $\delta^{13}C_{carb}$ values due to heterotrophic abundance below the microbialite surface. This is supported by an isotopic mass balance that predicts smaller inputs of heterotrophically-depleted DIC are required to sufficiently overprint $\delta^{13}C_{carb}$ enrichments. These results concluded that the photosynthetic biosignature identified in Pavilion Lake is short-lived and mitigated by biological processes.

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To my friends and family, thanks for keeping me going. Mom and Dad, I could never put into words how much your love and support has been instrumental to everything I set out to accomplish. Baba, thank you for all your love and support, and for cheering me on right to the bitter end. Matthew, thank you for supporting my academic endeavours, even if you have no idea what I've been doing all this time. I'll be cheering you on when it's your turn! To all of my friends, especially Melissa, Amanda, and Jessica, thanks for all of your encouragement.

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PREFACE

This thesis entails one major research study that is currently being prepared for submission. The relevance of this study to microbialite research and astrobiology is detailed throughout the first chapter. The paper presented is:

> Chapter 2 – M. A. Belan, A.L. Brady, S.-T. Kim, C. Haberle, D.S.S. Lim, G.F. Slater (2015) Spatial distribution and preservation of carbon isotopes in microbialite carbonate from Pavilion Lake, British Columbia.

As first author, I conducted the majority of the research presented, such as manuscript writing, sample collection and analysis (primarily isotope analysis). Dr. Greg Slater was involved in discussing the results, editing, and formulating the direction of the research project. Dr. Allyson Brady was similarly involved, lending assistance in interpreting the results and editing. Martin Knyf prepared the protocols for the Gasbench and Finnigan MAT DeltaPlus XP and correction of δ13Ccarb values under supervision by Dr. Sang-Tae Kim, who was also involved in discussing the results. Collection, preparation of carbonates prior to analysis, and data interpretation was performed by myself. As the principle investigator of the Pavilion Lake Research Project, Dr. Darlene Lim was included as an author for her necessary assistance with site access and logistics. In addition, she provided valuable intellectual contributions towards the discussion. Chris Haberle provided the light data used in Chapter 2 that details surface light variability in Pavilion Lake. All coauthors will be involved in editing of the manuscript prior to submission.

CHAPTER 1
INTRODUCTION TO FUNDAMENTAL CONCEPTS

Microbialite research has made significant contributions to our understanding of geological and biological origins on Earth. "Microbialite" is a general term for organo-sedimentary structures whose growth and development are believed to have biogenic origins due to their associations with microbial mat communities (Burne & Moore, 1987). Stromatolites, a form of lithified microbialite, are ubiquitous throughout the geologic record and are purported to be the oldest geologic evidence for life on Earth (Walter *et al.*, 1980; Awramik, 1992; Riding, 2000). The ability to interpret the presence or activity of life in the geologic record relies on the identification of biologically produced mineral, morphological, organic, or isotopic features – known as biosignatures – that are preserved within the microbialite structure (Slater, 2009). Preservation is an important component to successfully interpreting life in unknown or unexplored environments because a variety of physical, chemical, geologic, and even biological processes can mask biosignatures over time (Boston *et al.*, 2001; Sherwood Lollar & McCollum, 2006; Summons *et al.*, 2008; Slater, 2009). Characterizing these biosignatures and their preservation potential throughout the geologic record is important in order to obtain clear insights to the rise of life on Earth and its evolutionary development throughout history. Likewise, investigating extraterrestrial geological systems for potential life relies on understanding biosignature dynamics and the processes that mitigate biosignature viability. In microbialite systems, biosignatures develop as a result of the interactions that occur concurrently with microbialite formation and biological processes. Carbon isotope ratios are an example of one type of biosignature that can identify metabolic effects responsible for facilitating the precipitation of carbonate necessary for the accretionary growth of microbialite structures (Burne & Moore, 1987; Schidlowski, 2000; Sumner, 2001; Brady *et al.*, 2009; Brady *et al.*, 2010; Brady *et al.*,

2014). Investigating carbon cycling and isotope dynamics in modern, analog microbialite systems helps not only to elucidate the dominating microbial communities that influence biosignature generation, but potentially provide insights to factors limiting their generation and preservation within the geologic record.

Chapter 1 introduces the fundamental concepts surrounding carbonate precipitation, mechanisms of microbialite formation, and the generation of carbon isotope biosignatures in order to demonstrate how modern microbialite research can be applied to ancient systems. In addition, the roles of autotrophic and heterotrophic microorganisms in biosignature formation are explored, as well as the challenges associated with these processes for constraining biogenicity in carbon isotope biosignatures.

1.1 SIGNIFICANCE OF MICROBIALITE RESEARCH

1.1.1 Carbonate precipitation

Accretionary buildup of microbialites is dependent on the precipitation of minerals. While microbialite mineralogy can vary greatly, most microbialites are comprised of carbonate (Dupraz *et al.*, 2011). The precipitation of calcium carbonate is a function of carbonate alkalinity and available free calcium, which is expressed in the saturation index. The saturation index (SI) is a parameter that describes the saturation conditions of a solution and is defined in Equation (1.1):

$$SI = \log (IAP/K_{sp}) \quad (1.1)$$

where IAP designates the ion activity product (the level of electrostatic interactions between Ca^{2+} and CO_3^{2-}), and K_{sp} is the solubility product of $CaCO_3$. The resultant net balance of these factors largely influences whether precipitation occurs (Dupraz *et al.*, 2009). In a solution,

supersaturation with respect to a mineral occurs when SI>0, however recent studies in marine environments have shown that spontaneous $CaCO_3$ precipitation occurs when SI>0.8 (Kempe & Kazmierczak, 1994). In non-marine environments, the SI generally needs to be greater than 1 for this to occur (Arp *et al.*, 2001). The concentration of ions depends on the carbonate equilibrium shown in Equation (1.2):

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

pH of the solution strongly influences the IAP and the proportion of dominating carbon species (Figure 1.1). With increased alkalinity, CO_3^{2-} is more readily available to precipitate as $CaCO_3$. This system of processes within which carbonate precipitation is controlled is referred to as the alkalinity engine, and is mediated by both abiotic and biotic effects (Dupraz *et al.*, 2009). In abiotic cases, various physicochemical processes such as evaporation and/or CO_2 degassing can increase the saturation index and induce carbonate precipitation. Accretionary buildup of precipitated carbonate eventually produces the lithified characteristics of carbonate structures in many lakes around the world, such as Lake Van in Turkey (Kempe *et al.*, 1991) or carbonate tufa at Mono Lake, California (Council & Bennett, 1993). When investigating microbialites, constraining biological influences to the alkalinity engine that can induce precipitation is necessary to understand the potential for biosignature generation.

1.1.2 Mechanisms of microbialite formation

Microbialites are normally hosts to extensive colonies of microbial life called biofilms, or "microbial mats" (Burne & Moore, 1987; Visscher *et al.*, 2000, Dupraz *et al.*, 2009).

Through the interactions between microbes and the environment, the precipitation of carbonate

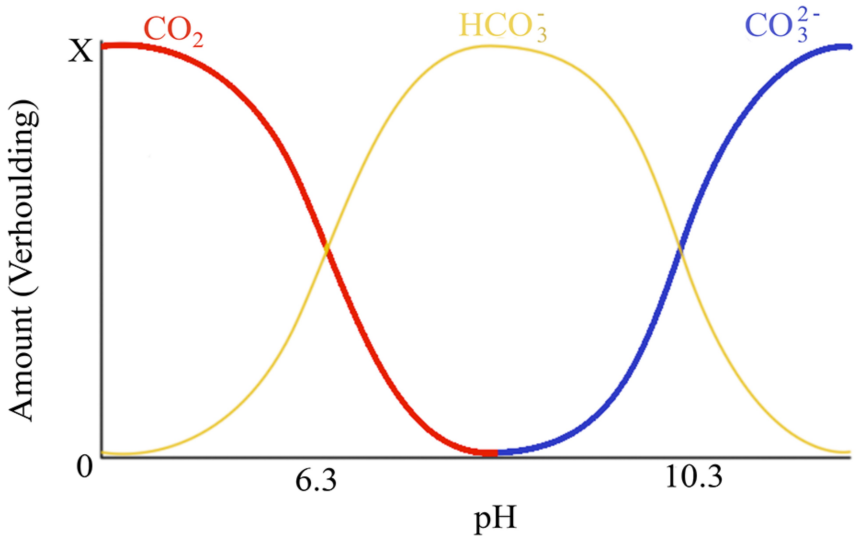

Figure 1.1: Verhoulding diagram of dominating carbon species existing at various pH levels. Low pH/acidic environments contain CO_2 as a dominating species, while high pH/alkaline environments contain CO_3^{2-} as dominating species.

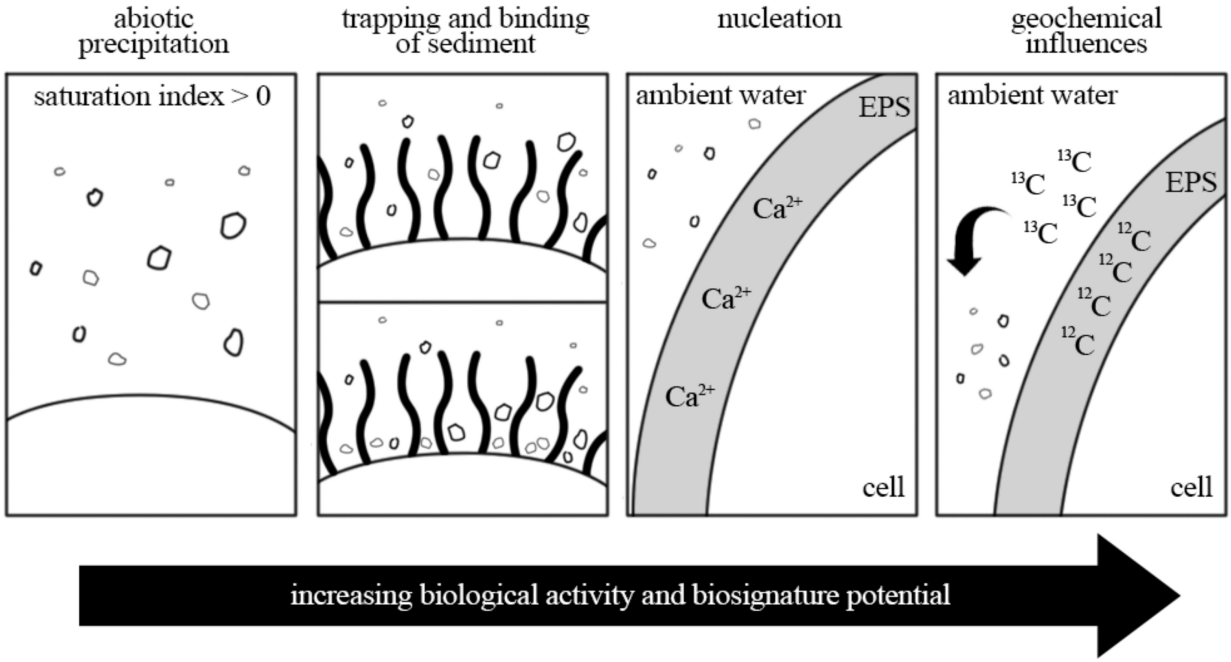

Figure 1.1: Four mechanisms of carbonate precipitation leading to microbialite development. Abiotic precipitation contains little to no biological involvement, making biosignature formation unlikely. However, geochemical influences can largely be mediated by microbial communities, and therefore demonstrates the greatest potential to generate a biosignature. Figure modified and adapted from Brady, 2009.

can be influenced in a variety of ways, such as trapping and binding of sediments (Dupraz & Visscher, 2005; Altermann, 2008), crystal nucleation on microbialite surfaces (Greenfield *et al.*, 1984; Léveillé *et al.*, 2000), and/or microbial influences to local geochemistry (Sumner, 2001; Brady *et al.*, 2010). These mechanisms of microbialite formation (Figure 1.2) differ in their potential to record a biosignature and are therefore relevant in the search for evidence of life in the geologic record.

1.1.2a Trapping and binding

Filamentous, microbial communities existing in mat systems can entrap ambient free detritus and create structure via their adhesive exocellular properties (Burne & Moore, 1987; Sprachta *et al.*, 2001; Bosak & Newman). Stratification in trapping and binding processes is accomplished by microbial responses to diurnal variations in light intensity and the growth of microbial filaments (Burne & Moore, 1987; Schneider & Le Campion- Alsumard, 1999; Dupraz & Visscher, 2005; Dupraz *et al.*, 2009). However, since the carbonate is usually precipitated in the external environment prior to any biological or metabolic intervention, most of this carbonate may be abiotically produced and therefore has little potential for producing a biosignature.

1.1.2b Nucleation

The mucilage barrier separating cells from the external environment, known as the exopolymeric substance (EPS), is responsible for a variety of functions that optimize cellular protection, stability, stress resistance, and surface adhesion (Golubic & Seong-Joo, 1999; Decho, *et al.*, 2003; Dupraz *et al.*, 2009). The EPS surface has a net negative charge resulting from a large number of negatively charged functional groups (Konhauser, 2007). Saturation of Ca^{2+} at these functional groups can create nucleation sites in which free anions, namely CO_3^{2-}, bind and rapidly induce mineralization.

1.1.2c Influence on geochemical environment

Microbial metabolic activity, such as autotrophy and heterotrophy, can induce changes to the local geochemical environment that promote carbonate precipitation (Merz, 1992; Merz-Preiß, 2000; Ludwig *et al.*, 2005). During photosynthesis, autotrophic cyanobacteria take up CO_2 and release O_2 in the overall reaction:

$$H_2O + CO_2 \rightarrow CH_2O + O_2 \quad (1.3)$$

Cyanobacteria have been shown to possess a carbon concentrating mechanism that enables the uptake of HCO_3- under low CO_2 concentrations (Miller and Coleman, 1980; Badger & Price, 2003) via the following reaction:

$$HCO_3\text{-} + H_2O \rightarrow CH_2O + OH^- + O_2 \quad (1.4)$$

OH- ions are released outside the cell and increase pH in the microenvironment, elevating CO32- concentrations and thus increasing the SI of $CaCO_3$, promoting precipitation (Thompson & Ferris, 1990).

Sulfate reducing bacteria (SRB), one of the prevalent heterotrophic metabolisms in marine microbialite mat communities (Visscher *et al.*, 2000; Dupraz & Visscher, 2005; Baumgartner *et al.*, 2006), oxidize organic matter to hydrogen sulfide and CO_2 through the following reaction:

$$SO_4^{2-} + 2\ CH_2O + OH^- \rightarrow HS^- + 2\ HCO_3\text{-} + H_2O \quad (1.5)$$

The release of HCO_3-, an inorganic ion, also leads to an increase in alkalinity and an increase in the saturation index (Visscher *et al.*, 2000; Altermann *et al.*, 2006; Baumgartner *et al.*, 2006). While these changes to SI are driven by metabolic effects, the method in which biosignatures are made and preservered is due to the biological partitioning of carbon's stable isotopes. The cycling of carbon isotopes within microbialite systems, specifically amongst metabolic reactions, constitutes the greatest potential for recording biosignatures within precipitated carbonate.

1.2 CARBON ISOTOPES AND BIOSIGNATURES IN MICROBIALITE SYSTEMS

Isotope biosignatures involve identifying and comparing the relative abundances of an element's stable isotopes (Figure 1.3A). Chemical reactions partition isotopes via a process called an isotope effect or fractionation, where the distribution of light and heavy isotopes within a system varies depending on energetic favourability or kinetic isotope effects. The larger and more variable isotope effects are generally associated with biological reactions (Zeebe & Wolf-Gladrow, 2001), while the former are generally associated with equilibrium precipitation (Figure 1.3B; Zeebe & Wolf-Gladrow, 2001). Isotopic abundances of carbon isotopes measure the ratio of ^{13}C vs. ^{12}C in a sample compared to that of an international standard such as PeeDee Belemnite, and is expressed as δ notation, in per mille (‰):

$$\delta^{13}C_{sample} = ((^{13}C/^{12}C_{sample} / {}^{13}C/^{12}C_{standard}) - 1) \bullet 1000 \quad (1.6)$$

In a microbialite system, both abiotic and biotic mechanisms can fractionate ^{13}C in the dissolved inorganic carbon (DIC) pool of ambient water (Sumner, 2001). These isotope effects can then be observed in the isotopic compositions of a variety of thermodynamic and

metabolic products that originate from DIC, such as microbialite carbonate and/or cellular biomass (Sumner, 2001; Brady *et al.*, 2010; Brady *et al.*, 2014). Equilibrium isotope effects to DIC largely result in carbonate containing $\delta^{13}C$ values that are reflective of abiotic conditions. Non-equilibrium isotope effects, however, such as the preferential uptake of ^{12}C vs. ^{13}C by microbial metabolic activity, can induce larger fractionations relative to DIC, which produces δ13C values in carbonate that are distinct from those expected at equilibrium (Sumner, 2001). Essentially, the larger and more variable fractionations produced by biological activity induce larger deviations to DIC compared to the smaller and more consistent fractionations seen in equilibrium precipitation of carbonate (Zeebe & Wolf-Gladrow, 2001). As a result, biogenicity can generally be determined when δ13C values of precipitated carbonate fall outside a range of predicted $\delta^{13}C$ values for equilibrium precipitation (Brady *et al.*, 2010; Brady *et al.*, 2014).

1.2.1 Abiotic (equilibrium) isotope dynamics

In the inorganic carbon system, carbon is fractionated multiple times between different molecular species in thermodynamic equilibrium (Figure 1.4). $\delta^{13}C_{DIC}$ values reflect the net fractionation of carbon throughout this system. One example of fractionation occurs between dissolved CO_2 and HCO_3- in the water column. The isotopic exchange reaction can be written:

$$^{13}CO_2 + H^{12}CO_3\text{-} \leftrightarrow {}^{12}CO_2 + H^{13}CO_3\text{-} \quad (1.7)$$

How much the stable isotopes of carbon are partitioned amongst the reactants and products of exchange reactions is described by the fractionation factor, α. The fractionation factor of reaction (1.7) is equal to its equilibrium constant, k:

$$k = \alpha_{HCO_3\text{-}\text{-}CO_2} = \frac{[^{12}CO_2][H^{13}CO_3\text{-}]}{[^{13}CO_2][H^{12}CO_3\text{-}]} = \frac{(^{13}C/^{12}C)_{HCO_3\text{-}}}{(^{13}C/^{12}C)_{CO_2}} = 1.009 \quad (1.8)$$

at 25 °C (Mook *et al.*, 1974; O'Leary *et al.*, 1992; Zeebe & Wolf-Gladrow, 2001). This fractionation results in 13C atoms concentrating more closely to the bicarbonate ion, which can be better understood by considering the relative zero-point energies of the molecules involved:

$$\ln k = (\Delta E1 - \Delta E2) / RT \quad (1.9)$$

where ΔE1 and ΔE2 are the differences between the zero-point energies of $H^{12}CO_3$- and $H^{13}CO_3$-, and $^{12}CO_2$ and $^{13}CO_2$, respectively (Figure 1.3C). When ln k > 0, or k > 1, the 13C/12C ratio in the bicarbonate ion is greater compared to CO_2. In this case, the isotopic exchange reaction of dissolved CO_2 and HCO_3- results in HCO_3- being enriched in 13C by approximately 9‰ at 25 °C relative to CO_2 (Mook *et al.*, 1974). Equation (1.9) demonstrates that changes to pressure and temperature in a thermodynamic system can fluctuate the level of fractionation in isotope exchange reactions. This variability can produce a range of $\delta^{13}C_{DIC}$ values expected for equilibrium DIC composition. During precipitation from DIC, additional fractionation may vary δ13C values of calcium carbonate based on the resultant polymorph (structural form). Calcite and aragonite are two common polymorphs of calcium carbonate that are chemically identical but exhibit distinct geometric orientations at the atomic level that contain specific fractionation effects (Rubinson & Clayton, 1969; Grossman & Ku, 1986; Romanek *et al.*, 1992; Deines, 2004). Calcite is enriched in 13C by +1.0 ± 0.2‰ above the bicarbonate from which it precipitates, while aragonite is enriched by +2.7 ± 0.6‰ (Romanek *et al.*, 1992). These additional enrichments must be considered when predicting δ13C values for carbonates precipitating from equilibrium DIC.

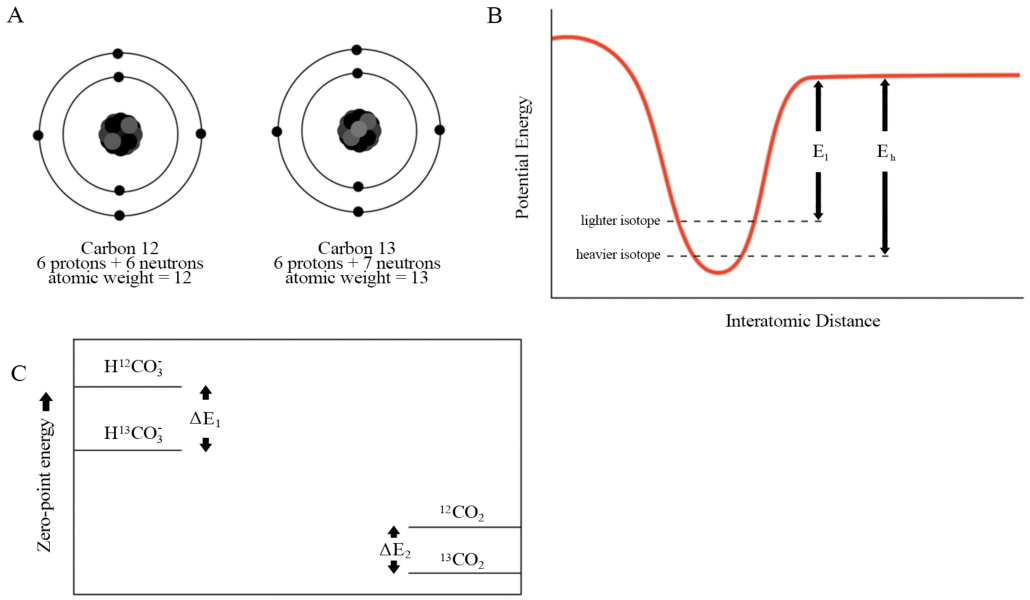

Figure 1.3: Carbon has two stable isotopes, ^{12}C and ^{13}C (**A**). These differences are the result of different atomic weights associated with the number of neutrons in an element. The preference for lighter carbon is a result of less energy being required to break bonds that are associated with lighter isotopes (**B**). This figure plots potential energy against interatomic distance and demonstrates that it is energetically favourable to break bonds between lighter isotopes than heavier ones. (**C**) ^{13}C atoms concentrate preferentially in the bicarbonate ion because the difference in the zero-point energy between $H^{12}CO_3^-$ and $H^{13}CO_3^-$ is greater than the difference between $^{12}CO_2$ and $^{13}CO_2$.

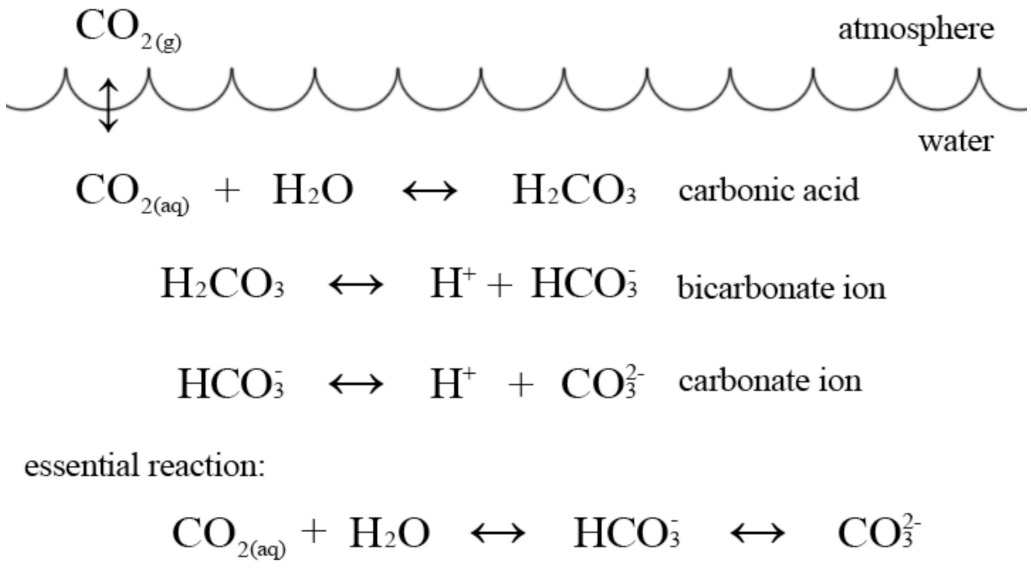

Figure 1.4: The inorganic carbon system.

1.2.2 Biotic (metabolic) isotope dynamics

In biological environments, both autotrophy and heterotrophy can promote precipitation (Visscher *et al.*, 2000), but the isotopic composition of resultant carbonate from each process can differ greatly due to different and opposing fractionation reactions. Photosynthetic fixation of inorganic carbon (CO_2) to organic carbon by autotrophs produces significant isotope differences between ambient DIC and cellular organic carbon. This difference is largely due to kinetic effects associated with the primary steps of photosynthesis, seen in Figure 1.5 (Schidlowski, 2001; Sumner, 2001). The first isotope effects, Φ1 and Φ2, describe the simple diffusion of CO_2 across the cell membrane, and produce marginal fractionations on the order of ~ 4.4‰ (Raven *et al.*, 1985; Espie *et al.*, 1991). The largest kinetic effect, Φ3, is observed in the subsequent enzymatic fixation of intracellular carbon, where the level of fractionation is highly variable depending on the kind of photosynthetic process undertaken by distinct autotrophic species. For example, the production of 3-carbon skeletons via the Calvin cycle in many photoautotrophic bacteria is one of the most common photosynthetic pathways and produces a fractionation of ~ -25‰ (O'Leary, 1981; Farquhar *et al.*, 1989), while the production of 4-carbon skeletons via a different carboxylation process produces fractionations between only -2 and -3‰ (Whelan & Sackett, 1973; O'Leary, 1981). Essentially, carbon processing into cell biomass demonstrates preferential uptake of ^{12}C. The abundance of ^{13}C in DIC increases, producing higher values of $\delta^{13}C_{DIC}$ in the residual pool resultant from photosynthetic fractionations. During equilibrium precipitation from this DIC pool, this metabolic effect is preserved in the isotopic composition of carbonate. This can be considered a biosignature of autotrophy if the $\delta^{13}C_{carb}$ value is greater than the predicted range of values for equilibrium precipitation from the bulk solution DIC. On average, the level of enrichment above equilibrium in carbonates produced by microbial photosynthetic activity is typically on the order of 2 -5‰ (Merz, 1992; Guo *et al.*, 1996; Thompson *et al.*, 1997; Brady *et*

al., 2010; Brady *et al.*, 2014).

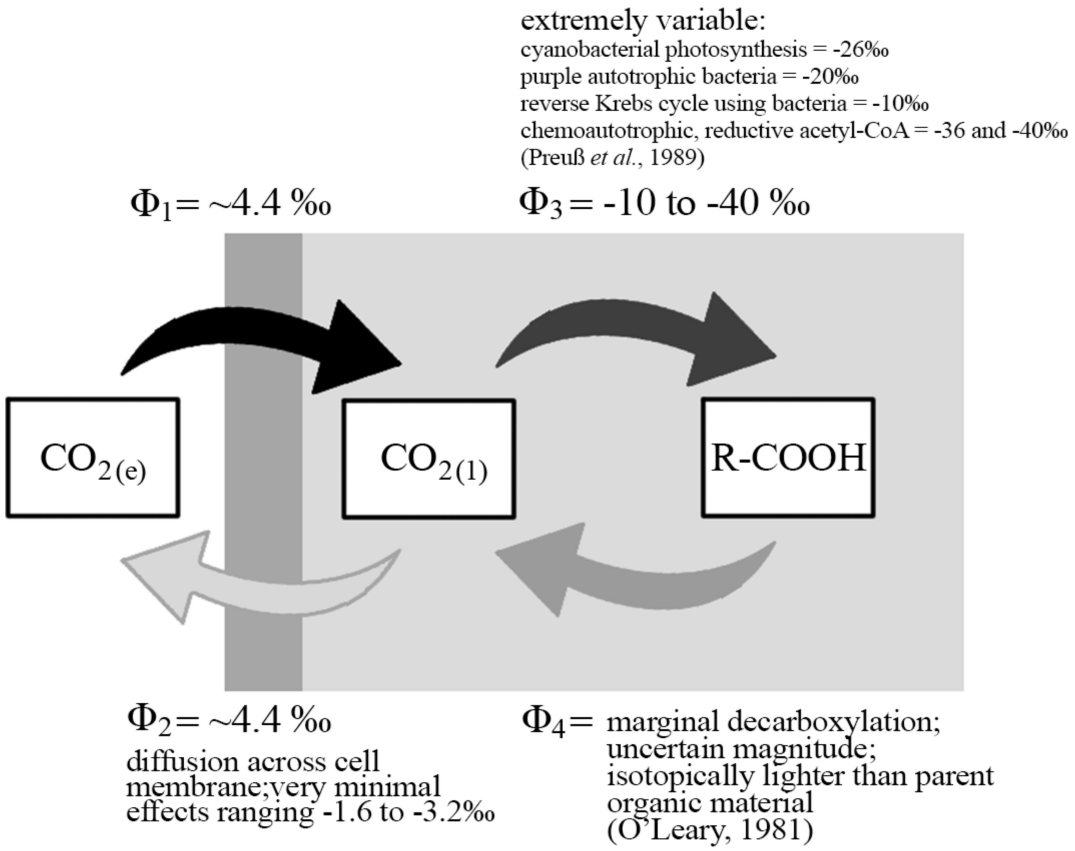

Figure 1.5: Fractionation effects associated with the uptake of carbon into the cell. Different autotrophic microbes contain various metabolic pathways that fractionate carbon differently and produce variable $\delta^{13}C$ values.

Conversely, biosignatures of heterotrophy can also be formed when $\delta^{13}C_{carb}$ values record below the predicted ranged of values for equilibrium precipitation (Andres *et al.*, 2006; Breitbart *et al.*, 2009). Heterotrophic organisms lack the ability to fix carbon and instead rely on consuming autotrophic communities to acquire organic carbon necessary for their metabolism. Since there is minimal isotopic fractionation associated with respiration of organic matter, the effects of heterotrophic metabolism largely lead to an output (respiration) of inorganic carbon depleted in ^{13}C (Sumner, 2001; Brady *et al.*, 2009). Sulfate reduction (1.4) is one of the most predominant heterotrophic processes occurring in microbialite communities and is largely

responsible for this increased ^{12}C output, although a multitude of other anoxygenic processes can contribute to this as well (Figure 1.6). The influx of ^{12}C back into the DIC pool results in decreasing $\delta^{13}C_{DIC}$ values, balancing the partition of carbon isotopes. Heterotrophic metabolisms can therefore jeopardize biosignature viability if significant outputs of ^{12}C result in net balance of precipitated carbonate recording $\delta^{13}C$ values consistent with the predicted range of values for equilibrium precipitation. However, sufficient inputs of ^{12}C may cause DIC composition to record below the equilibrium range, signifying a heterotrophic biosignature. While both autotrophy and heterotrophy are common biological roles in microbialite systems, they exhibit competing isotopic processes that can create and erase ^{13}C-enriched biosignatures, making the interpretation of biogenicity a difficult task.

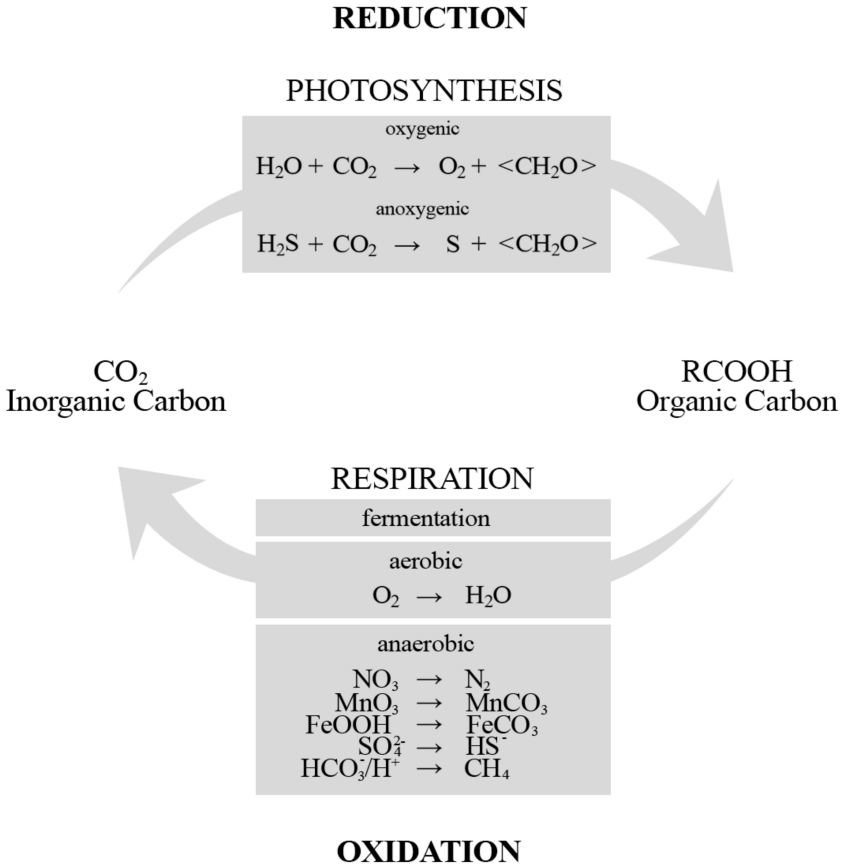

Figure 1.6: Reduction and oxidation processes of carbon in microbialite systems by autotrophy and heterotrophy. Adapted from Dupraz *et al.*, 2009.

1.3 INVESTIGATION OF MODERN MICROBIALITE ANALOGUES

Modern analogues of ancient microbialite systems present opportunities to understand biotic influences to both active microbialite development and biosignature generation. Stromatolites were largely thought to be restricted to the geologic record until the discovery of modern microbialites in Shark Bay, Australia in 1964 (Dupraz *et al.*, 2011). Since then, modern microbialites have been found in various marine, continental, freshwater, and hypersaline environments around the world. While Shark Bay remains an active site of microbialite research, additional field sites containing modern microbialites include Cuatro Cinénegas, Mexico (Breitbart *et al.*, 2009; Nitti *et al.*, 2012), Highborne Cay, Bahamas (Andres and Reid, 2006;Andres *et al.*, 2006; Myshrall *et al.*, 2009), Lake Van, Turkey (Kempe *et al.*, 1991; López-Garcí *et al.*, 2005) and Pavilion Lake, Canada (Laval *et al.*, 2000; Lim *et al.*, 2009; Brady *et al.*, 2010). Understanding biosignature formation and preservation in these terrestrial analogs evaluates their viability as astrobiological tools in identifying life in other planets and unknown geological systems.

1.3.1 Microbialites at Pavilion Lake, British Columbia, Canada

Pavilion Lake is a freshwater lake in south-central British Columbia, Canada (50°51' N, 121°44' W) that is an excellent example of a site investigating microbial biosignatures. It is slightly alkaline, with a mean pH of ~ 8.3, is slightly saturated with respect to calcite, and is comprised of three distinct basins (North, Central, and South) that are groundwater fed and characterized by low sedimentation rates (Lim *et al.*, 2009). The deepest parts of the lake are 65 m deep, with microbialites existing at depths between 5 to 60 m (Laval *et al.*, 2000). Mean concentrations of $CaCO_3$ and Ca^{2+} are 181.8 mg/L and 39.5 mg/L, respectively, with Ca^{2+} inputs from groundwater, making Pavilion a hard water lake (Lim *et al.*, 2009). Phosphorus

concentrations fall within the ultra-oligotrophic range (mean total phosphorous = 3.3 μg/L) and optical transmission is ~ 95% that of pure water throughout most of the water column (Lim *et al.*, 2009). Light levels vary throughout the water column, with depths beyond 55 m exhibiting as little as 0.1% of surface irradiance (Lim *et al.*, 2009). Pavilion Lake contains an abundance of actively accreting microbialite structures that exhibit a variety of different morphologies with depth (Laval *et al.*, 2000; Lim *et al.*, 2009; Omelon *et al.*, 2013). The microbialites are estimated to be younger than 12, 000 years based on uranium series dating (Laval *et al.*, 2000), and 14C analyses of microbialite carbonate have estimated rates of precipitation to be 0.05 mm year-1 (Brady *et al.*, 2009). At shallow-to-intermediate depths (~10 m), microbialites display short (centimeter to decimeter scale), friable aggregates of calcite covered by photosynthetic microbial communities (Figure 1.7A; Laval *et al.*, 2000). Large, dome-shaped structures (up to 3 m high) represent a large extent of morphological variation at intermediate (~ 20 m) depths. Closer aggregation of calcite results in denser (i.e. less friable) structures, seen in the unique, vertically oriented ridges of calcite clusters that form along the surface. In addition, small (several centimeter) cone-like structures can occasionally be found on the dome surfaces (Figure 1.7B; Laval *et al.*, 2000). At intermediate-to-deep depths (~ 20 –30 m), microbialites exhibit exaggerated versions of intermediate depth morphologies, with individual cones reaching ~ 20 – 30 cm in height and increased carbonate density (Figure 1.7C; Laval *et al.*, 2000). The deepest depths (> 30 m) display extremely dense structures (centimeter to meter scale) whose orientations closely resemble artichokes (Figure 1.7D; Laval *et al.*, 2000). Their surfaces also contain thinner microbial mats than at shallower depths, and variations in colour along the lateral regions of the structures are believed to illustrate spatial segregation of autotrophic and heterotrophic communities (Brady *et al.*, 2014). These differences in morphology have been hypothesized to result from different precipitation rates and processes brought about by community variation in

changing light regimes (Lim *et al.*, 2009; Omelon *et al.*, 2013). At shallow depths, biomass accumulation by photosynthesis is thought to outpace carbonate precipitation, creating loose aggregates that result in friable structures. At deeper depths, where photosynthetic rates are reduced, heterotrophy has been proposed as a dominant mechanism responsible for creating denser structures as a result of carbonate infilling between aggregates (Omelon *et al.*, 2013; Harwood Theisen *et al.*, 2015). Distinguishing the roles of autotrophy and heterotrophy in microbialite formation remains under intense study.

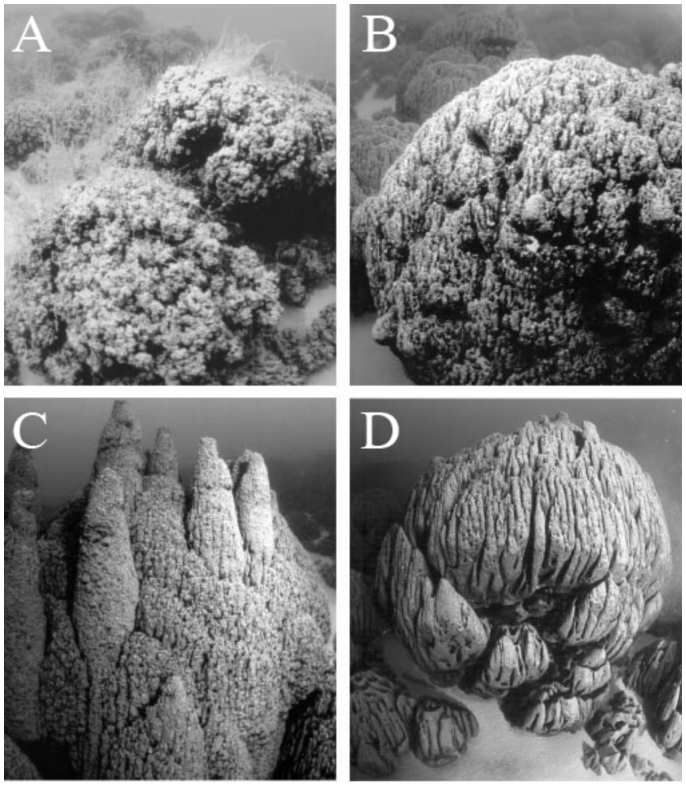

Figure 1.7: Pavilion Lake microbialite morphotypes (Laval *et al.*, 2000).

1.3.2 Biosignatures in Pavilion Lake microbialite carbonate

At Pavilion, microbial communities that have been identified comprising the microbial mats include filamentous (Calothrix, Fischerella, Oscillatoria) and unicellular (Gloeocapsa,

Synechococcus) cyanobacteria, diatoms, and heterotrophs (Laval *et al.*, 2000; Russell *et al.*, 2014; Brady *et al.*, 2014; Chan *et al.*, 2014). The potential for microbial metabolic activity from these communities to change local aqueous geochemistry and promote the precipitation of calcium carbonate represents the greatest potential for the generation of a biosignature (Merz, 1992; Shiraiwa *et al.*, 1993; Shiraishi *et al.*, 2008). Microbial effects on geochemical conditions may be restricted to microenvironments within the microbial mat (Jørgensen *et al.*, 1983; Revsbech *et al.*, 1983; de Beer *et al.*, 1997; Andres *et al.*, 2006). At Pavilion, the finding of ~ 1 cm green and purple nodules on the surface of microbialites from ~ 20 m depths represents one such microenvironment. Although the species of filamentous cyanobacteria differ between the nodules (Russell *et al.*, 2014), elevated levels of dissolved oxygen (DO) and pH within their microstructures suggest photosynthesis is an active mechanism driving precipitation (Brady *et al.*, 2010). The layout of precipitated carbonate within these nodules is surprisingly different. Cross-sectioned green nodules exhibit layering of organic matter and precipitated carbonate in alternate green- and grey- coloured bands. Purple nodules consist of clusters of carbonate randomly distributed throughout the nodule fabric. While the disparity of carbonate localization between nodule colours is unknown, carbonates within these nodule microenvironments have shown $\delta^{13}C_{carb}$ values enriched above the predicted range of values for equilibrium precipitation, designating a biosignature of autotrophy (Brady *et al.*, 2010). ^{13}C-enriched carbonates have also been identified in the thin (< 5 mm) surface biofilm covering the majority of the remainder of the microbialite surface. These findings were initially identified on microbialites situated along the eastern shorelines of Pavilion's central basin (Brady *et al.*, 2010). Surface biofilm enrichments from this site, called Three Poles, were found at depths up to ~ 20 m below the water surface, whereas carbonate biofilm associated with microbialites at deeper depths generally recorded $\delta^{13}C$ values within the predicated range of values for equilibrium precipitation (Brady *et al.*, 2014).

The loss of ^{13}C-enrichment at depths below ~ 20 m has hypothesized by Brady *et al.* (2014) to result from reduced light availability with depth controlling photosynthetic activity. Within Pavilion, depths of 55 m receive as little as 0.1% of surface sunlight according to previous measurements of photosynthetically active radiation (PAR) throughout the water column (Lim *et al.*, 2009; Brady *et al.*, 2014). This decrease in the water column suggests that photosynthetic activity at deeper depths may not be sufficient to record large enrichments indicative of this process. Alternatively, known increases to heterotrophic abundance at deeper depths (Brady *et al.*, 2010; Brady *et al.*, 2014; Russell *et al.*, 2014) may also suggest depletions to DIC isotopic composition that results in average $\delta^{13}C_{carb}$ values. Loss of measureable enrichment with increasing depth is therefore hypothesized to result from changing light levels influencing the balance of autotrophic and heterotrophic processes shifting $\delta^{13}C_{DIC}$. Changes to the relative abundances of autotrophic and heterotrophic processes have been previously identified at Pavilion. On microbialite structures below 20 m, gradual transitions in surface biofilm colour and biomass concentrations have indicated potential segregation of autotrophic and heterotrophic communities in a spatial manner. Biofilm on upper regions of deep microbialites are commonly green in colour, and are inhabited by a greater proportion of photoautotrophic communities. This has further supported hypotheses suggesting the perceived phototaxic orientation of deep microbialite morphologies is due to active accretionary precipitation by these communities on peak surfaces. Below this region, gradual transitions into brown biofilm, which contains increased abundances of heterotrophic communities, occur along the sides of the microbialite towards the sediment-water interface (SWI), after which point biomass is substantially reduced (Brady *et al.*, 2014). While previous ^{13}C analysis did not reveal substantial differences in $\delta^{13}C_{carb}$ values between these regions, it is not clear if a higher resolution vertical sampling of biofilm carbonates would reveal the gradual onset of a heterotrophic biosignature. Since PAR is

significantly decreased at these depths, the likelihood of identifying a heterotrophic biosignature, previously unseen at Pavilion, is expected. In addition, identifying a heterotrophic biosignature may be possible in other regions of the lake where surface light variation exists. Up until recently, biosignature characterization at Pavilion has only been done at the Central basin. Surface light irradiation models suggest that that the Western side of Pavilion's South basin receives less surface sunlight per month in comparison to the Eastern side (Haberle, unpublished data). While photosynthetic biosignatures are known on the eastern side of the Central basin, the spatial distribution of these biosignatures within the lake has not been addressed. In addition, the presence and extent to which surface nodules are distributed within the lake is also unknown. The effects of different total surface irradiation and how this may affect nodule presence, biosignature development, and the abundances of autotrophic and heterotrophic communities in microbialites at the same depths but different locations within the lake is not well constrained. Photosynthetic biosignatures may be limited to the eastern side of the lake where increased light availability exhibits higher photosynthetic activity of phototrophic communities. Conversely, less light throughout the water column on the western side may result in either a smaller and/or less active population that is unable to generate a detectable photosynthetic biosignature, or increased heterotrophic activity that leads to the onset of a heterotrophic biosignature, especially on brown biofilms surfaces near the SWI at depths below 20 m. Investigating $\delta^{13}C_{carb}$ values from microbialite surfaces at a variety of lake locations and depths can help elucidate the extent to which changing light levels control biosignature formation within Pavilion Lake.

Beneath the microbialite surface, nodule structures have been identified as fossilized microfabrics within the bulk carbonate, with preservation of filamentous features capturing the arrangement of microbial life in a hemi-spherical fashion (Harwood Theisen *et al.*, 2015). Organic inclusions demonstrate that subsequent accretionary buildup has the ability to entomb

microbial features with varying degrees of preservation, and potentially preserve isotopic compositions of carbonates closely associated with these microfabrics. Surface-dominating communities from the past may be well preserved beneath the surface and record photoautotrophic enrichments. The preservation of these enrichments is important when interpreting the presence of life in the geologic record. Degradation of organic matter within the microbialite fabric produces void spaces that are susceptible to infilling of isotopically lighter carbonate brought about by heterotrophic metabolisms (Omelon *et al.*, 2013). This infilling process overprints $\delta^{13}C_{carb}$ values and decreases their ^{13}C content. As a result, interpreting the geologic past for biogenic origins of carbonate structures may be difficult if heterotrophic infilling compromises $\delta^{13}C_{carb}$ values to fall within the predicted equilibrium range and become indistinguishable from metabolic processes.

Pavilion Lake offers an opportunity to investigate the distribution of surface enrichments in areas and depths of the lake with different light regimes, and their potential for preservation beneath microbialite surfaces in a dynamic environment with both autotrophic and heterotrophic activities contributing to microbialite formation. This thesis aims to investigate the presence of the photosynthetic biosignature previously identified at Three Poles in other regions of the lake where light exposure differs, specifically with respect to variation with depth and opposite sides of the lake. Nodule distribution within the lake is also investigated. It is hypothesized that $\delta^{13}C_{carb}$ values will reflect decreasing trends correlated to increasing depth as a result of changing light exposure. Vertical profiling of $\delta^{13}C_{carb}$ across colour variations of the microbialite surface is hypothesized to reveal heterotrophic biosignatures that are expected to be present on deep microbialite structures below 20 m, especially on surfaces closer to the SWI. Finally, decreasing $\delta^{13}C_{carb}$ trends leading to identification of a heterotrophic biosignature is also expected within microbialite interiors. Correlating the distance from the microbialite surface in which

photosynthetic biosignatures are lost with previous estimates of microbialite growth rates can suggest the length of time these signals are preserved.

1.4 THESIS STRUCTURE

This thesis focuses on the carbon isotope systematics that contribute to biosignature generation in precipitated carbonate to address the spatial distribution and preservation of isotope biosignatures across sites, depths, and time. Given that Pavilion Lake demonstrates an actively accreting microbialite system with both autotrophic and heterotrophic communities affecting carbonate precipitation, understanding the nature and extent of biosignature generation is important for determining its success as a tool for identifying presence of (past) life in unexplored environments.

Chapter 1 provides an overview of the importance of modern microbialite research and the process of isotope biosignature generation in actively forming microbialite systems. It also addresses the biological roles affecting carbon isotope dynamics in the microbialite system, and the potential for them to record and preserve surface isotope biosignatures over time.

Chapter 2 assesses the extent to which photoautotrophic and heterotrophic processes affect biosignature generation and preservation in microbialite carbonate from Pavilion Lake. This is explored by characterizing ^{13}C content in precipitated carbonate from microbialite surfaces in different lake sites and depths. In addition, it investigates the preservation of these surface $\delta^{13}C$ values beneath the microbialite surface, fundamentally gaining insight to $\delta^{13}C$ preservation with continued accretionary growth.

Chapter 3 contains a summary of the significant findings of this dissertation. It identifies general conclusions that can be drawn from this work, and includes considerations for future research.

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CHAPTER TWO

Spatial distribution and preservation of carbon isotope biosignatures in freshwater microbialite carbonate

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ABSTRACT

Carbon isotope biosignatures in carbonate microbialites present in Pavilion Lake, British Columbia were found to be spatially widespread and correlated with depth. These findings imply that the extent of photosynthetic productivity as related to light levels was controlling their formation. Carbonates on the surfaces of the microbialites, associated with either nodular microbial communities or thin surface biofilms, at depths ≤ 18 m were consistently enriched in ^{13}C ($\delta^{13}C_{carb}$ = +1.9 to +2.8‰) above the predicted range of values for equilibrium precipitation from the bulk ambient water ($\delta^{13}C_{carb}$ = - 0.2 ± 1.3‰). At 21 m and below, vertical profiling of $\delta^{13}C_{carb}$ across individual microbialite surfaces showed isotopic enrichments above the predicated equilibrium range were concentrated at or near the apex of the structures. Elsewhere on the structure, $\delta^{13}C_{carb}$ values recorded isotopic compositions within the predicted range of values for equilibrium precipitation. Surface biosignatures were found to be preserved 0.5 – 2 cm below the surface, that is, within the microbialite structures. Beyond this interior depth, $\delta^{13}C_{carb}$ values were consistent with the predicted range of values for equilibrium precipitation, possibly due to heterotrophic overprinting by carbonate infilling processes. An isotopic mass-balance calculation indicated that if such infilling precipitation had a $\delta^{13}C_{carb}$ value equivalent to predicted equilibrium precipitation values (i.e. -1.5‰), ~ 40% of total carbonate mass present would have had to precipitate in this manner to overprint the original surface photosynthetic biosignatures. In

contrast, if infilling carbonate had a

$\delta^{13}C$ = -25 ‰, the end member value that might be expected for DIC inputs from heterotrophic respiration, only 13% of the total carbonate would have to precipitate by heterotrophic infilling. Based on previously determined growth rate estimates, the ~ 0.5 to ~ 2 cm depth of surface biosignature preservation suggests these processes can completely overprint photosynthetic signatures in 100 – 400 years.

2.1 INTRODUCTION

Large-scale geochemical change on Earth has long been contemporaneous with the evolution of life. Through the interactions of biology and the environment, chemical and isotopic signatures of biological activity can be produced and preserved within the geologic record. Known as biosignatures, identifying these unambiguous signals of life within the geologic record has helped to interpret a variety of important geochemical events throughout history, such as the rise of early life on Earth (Schidlowski, 1988; Dauphas *et al.*, 2004; Horita, 2005) or the Great Oxidation Event (Farquhar & Wing, 2003; Thomazo *et al.*, 2009; Kump *et al.*, 2011). Stromatolites are ubiquitous throughout the geologic record and are some of the earliest records of life on Earth (Walter *et al.*, 1980; Awramik, 1992; Riding, 2000). Microbialites are a general term for stromatolite- like, organo-sedimentary structures that are believed to have biogenic origins due to their associations with microbial mat communities (Burne & Moore, 1987). The principle mechanism by which microbialites are formed is via gradual buildup of carbonate brought about by in situ precipitation from both abiotic (Grotzinger *et al.*, 2000) and biotic processes (Webb, 2001; Dupraz et al, 2009). While biological mechanisms of formation can include trapping and binding (Dupraz & Visscher, 2005; Altermann, 2008) and crystal nucleation processes (Greenfield *et al.*, 1984; Léveillé *et al.*, 2000), microbially induced changes to local

geochemistry represent the largest potential to record an isotopic biosignature of microbial influence on precipitation (Merz-Preiß & Riding, 1999; Merz-Preiß, 2000; Reid *et al.*, 2000; Dupraz *et al.*, 2009). Preservation is an important component to successfully interpreting life in unknown or unexplored environments because a variety of physical, chemical, and even biological processes can mask biosignatures over time. Modern microbialites are analogues for ancient stromatolite-forming systems and provide a means to investigate the abiogenic versus biogenic processes contributing to both biosignature formation and preservation. Pavilion Lake in British Columbia, Canada, contains an abundance of actively accreting microbialite structures (Laval *et al.*, 2000; Lim *et al.*, 2009). These microbialites provide an opportunity to test hypotheses related to the generation and preservation of microbial biosignatures associated with microbialite formation.

Isotopic biosignatures observed within precipitated carbonates can be related to either autotrophic or heterotrophic microbial process through alterations to the isotopic composition of the dissolved inorganic carbon (DIC) pool out of which carbonate precipitates (Sumner, 2001; Dupraz *et al.*, 2009; Brady *et al.*, 2010). Depending on the dominant metabolisms, these alterations may induce either enrichments or depletions to DIC isotopic composition that are recorded as $\delta^{13}C_{carb}$ values outside the predicted range of values for equilibrium (abiotic) precipitation. Enrichments above this equilibrium range are attributed to photosynthetic activity, which increases ^{13}C composition in DIC and carbonates due to preferential intake of ^{12}C during C fixation. These enrichments have been identified in the actively precipitating biofilm communities that dominate microbialite surfaces at Pavilion Lake (Brady *et al.*, 2010; Brady *et al.*, 2014), Kelly Lake (Ferris *et al.*, 1997), and others (Hollander & McKenzie, 1991; Merz, 1992; Thompson *et al.*, 1997). In contrast, heterotrophic oxidation of organic matter inputs ^{13}C-depleted CO_2, resulting in $\delta^{13}C_{carb}$ values that are lower than equilibrium. The potential for

photosynthetic enrichments to no longer be resolved due to inputs of ^{13}C-depleted carbonate represents one mechanism contributing to isotopic variability amongst carbonates. The resulting isotopic composition of precipitated carbonate and its relationship to the predicted range of equilibrium values reflects the balance of autotrophic to heterotrophic processes (Andres *et al.*, 2006; Breitbart *et al.*, 2009; Brady *et al.*, 2010; Brady *et al.*, 2014).

At Pavilion Lake, biosignatures of photosynthesis have been identified in carbonates from biofilm covering microbialite surfaces, especially within distinct, ~ 1 cm sized micro-stromatolitic (green) and non-laminated (purple) nodules that cover the surfaces of microbialites at ~ 20 m depths (Figure 2.1A-D; Brady *et al.*, 2010). While the microbial communities consist of different cyanobacterial species and other microbes (Russell *et al.*, 2014), both communities produce ^{13}C enrichments in the associated carbonate as a result of photosynthetic activity. These biosignatures were initially identified on microbialites situated along the eastern shoreline of Pavilion's central basin (Brady *et al.*, 2010). Surface enrichments from this site, called Three Poles, were found at depths up to ~ 20 m below the water surface, whereas carbonate biofilm associated with microbialites at deeper depths generally recorded $\delta^{13}C$ values within the predicated range of values for equilibrium precipitation (Brady *et al.*, 2014). Variable light levels with depth are believed to control the level of photosynthetic activity producing these biosignatures. Photosynthetically active radiation (PAR) measurements within Pavilion indicate that sunlight attenuates throughout the water column, where depths of 55 m receive approximately as little as 0.1% of surface light (Lim *et al.*, 2009; Brady *et al.*, 2014). It is hypothesized that this decrease in available light leads to decreased photosynthetic activity with depth and a corresponding decrease in the extent of autotrophic influence on the local DIC. However, while at deeper depths photosynthetic shifts to DIC may be significantly reduced and fail to produce measureable enrichments, known heterotrophic activity within Pavilion (Brady *et*

al., 2010; Brady *et al.*, 2014; Russell *et al.*, 2014) can also deplete DIC isotopic composition via inputs of ^{13}C-depleted CO_2. This loss of measurable enrichment with increased depth in the lake is therefore hypothesized to result from changing light levels in the water column influencing the balance of autotrophic and heterotrophic processes affecting $\delta^{13}C_{DIC}$ (Brady *et al.*, 2014). Changes in the relative abundances of autotrophic and heterotrophic processes associated with variations in surface biofilm colour and biomass concentrations on microbialite structures below 20 m have also been identified. Biofilm on the upper regions of deep microbialite structures are commonly green in colour, and are inhabited by a greater proportion of photoautotrophic communities. Below this region, gradual transitions into brown biofilm, which contains increased proportions of sulfate-reducing heterotrophic bacteria, occur along the sides of the microbialite towards the sediment-water interface (SWI), after which point biomass is substantially reduced (Figure 2.2; Brady *et al.*, 2014). While previous 13C analysis did not reveal substantial differences in $\delta^{13}C_{carb}$ values between these regions, it is not clear if a higher resolution vertical sampling of biofilm carbonates would reveal the onset of a heterotrophic biosignature. The likelihood of identifying the presence of a heterotrophic biosignature, previously unseen at Pavilion Lake, along these structures is expected due to reduced surface light at these depths. Likewise, variations in surficial light are expected to exist in other regions of the lake that have not been thoroughly investigated. Surface light irradiation models demonstrate that the Western side of Pavilion's south basin receives less surface sunlight per month in comparison to the Eastern side (Haberle, unpublished data). While photosynthetic biosignatures have been detected on the eastern side of the Central basin, the spatial distribution of these biosignatures within the lake has not been addressed. Specifically, the effects of differences in total surface irradiation and how this may affect the formation of photosynthetic biosignatures in microbialites at the same depths but different locations within the lake is not well constrained. It is possible that the

formation of the photosynthetic biosignatures in Pavilion Lake may be limited to the Eastern side of the lake where autotrophic communities may be more active. On the Western side, less surface sunlight throughout the water column may result in a smaller and/or less active population that is unable to generate a detectable photosynthetic biosignature. Alternatively, identification of a heterotrophic biosignature, especially on brown biofilm surfaces near the SWI at depths below 20 m, may be more likely at this location given hypothesized increased heterotrophic activity in light-reduced areas.

The use of isotopic biosignatures to interpret the geological record relies on the potential for these signatures to be preserved in a recognizable way. Temporal and/or spatial changes in the balance of autotrophic and heterotrophic processes may potentially affect the preservation of surface biosignatures at Pavilion over geologic time. Beneath the microbialite surface, remnants of nodule structures have been identified as fossilized microfabrics within the bulk carbonate, with preservation of filamentous features capturing the arrangement of microbial life in a hemi-spherical fashion (Harwood Theisen *et al.*, 2015). Organic inclusions demonstrate that subsequent accretionary buildup during the growth of the microbialite has the ability to entomb microbial features with varying degrees of preservation, and potentially preserve isotopic enrichments in carbonates closely associated with these microfabrics. In these cases, photosynthetic enrichments may be preserved well beneath the surface and record biosignatures of surface-dominating communities from the past. However, degradation of this organic matter within the microbialite fabric produces void spaces that are susceptible to infilling of isotopically lighter carbonate. Infilling by depleted carbonates can derive from DIC that is either within equilibrium or altered by increased heterotrophic activity beneath the microbialite surface (Visscher *et al.*, 1998; Andres *et al.*, 2006; Dupraz *et al.*, 2009; Omelon *et al.*, 2013). Identification of a heterotrophic biosignature may be possible if sufficient heterotrophic infilling

can deplete $\delta^{13}C$ values below the predicted equilibrium range. Otherwise, the distance from the microbialite surface at which photosynthetic biosignatures are lost can represent the interface where autotrophic enrichments are balanced by heterotrophic depletions making the detection of biological influences on precipitation impossible. The extent of this overprinting mechanism is therefore hypothesized to control the preservation of the photosynthetic biosignature over time. Given previous microbialite growth estimates of 0.05 mm year-1 (Brady *et al.*, 2009), it is possible to approximate the length of time required for infilling processes from DIC that is either in equilibrium or altered by heterotrophy to overprint photosynthetic surface biosignatures.

This study aims to investigate the presence of the photosynthetic biosignature previously identified at Three Poles in other regions of the lake where light exposure differs, specifically with respect to variation with depth and on opposite sides of the lake. Nodule distribution within the lake is also investigated, since they represent actives sites of carbonate precipitation and contain known photosynthetic biosignatures. Comparing $\delta^{13}C$ values from nodule and biofilm carbonates within Pavilion may potentially reveal: i) whether the photosynthetic biosignature is more common in nodules compared to surface biofilm, and ii) the sites and depths to which the photosynthetic biosignature forms and disappears which may reflect differing light levels. At depths below 20 m, vertical profiling of $\delta^{13}C_{carb}$ across colour variations of the microbialite surface can potentially identify heterotrophic biosignatures near the SWI. In addition, the preservation potential of these isotopic biosignatures during microbialite growth as carbonate accretes was investigated.

2.2 SAMPLING AND ANALYTICAL METHODS

2.2.1 Study site

Pavilion Lake is located in south-central British Columbia, Canada, approximately 450

km northeast 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 mean pH of 8.3, is slightly supersaturated with respect to calcite, develops a seasonal thermocline at approximately 10 m deep, and is comprised of three basins (North, Central, and South; Lim *et al.*, 2009). Pavilion is host to an abundance of actively accreting microbialite structures that exhibit a wide range of morphologies varying with depth (Figure 2.3; Laval *et al.*, 2000). This morphological variation has been hypothesized to result from changes in biological activity in different light regimes influencing precipitation (Lim *et al.*, 2009; Omelon *et al.*, 2013; Brady *et al.*, 2014). Figure 2.4 shows surface light distribution across Pavilion Lake.

2.2.2 Microbialite and water chemistry collection

Sampling of microbialites was performed at three distinct locations in Pavilion Lake: Three Poles (TP; 50.866N, 121.736W), South Basin East (SBE; 50.857N, 121.727W) and South Basin West (SBW; 50.853N, 121.725W; Figure 2.4). SCUBA divers collected microbialite samples during the June 2014 field season at approximately 10 m, 18 m, and 26 m depths (the deepest sample taken at SBE was from 21 m). Microbialites from 10 m at all sites were selected without preference. However, based on previous findings (Brady *et al.*, 2010), microbialites with green and purple nodules at 18 m were targeted at all locations to examine the spatial distribution of ^{13}C-enrichments as previously identified at this depth. In addition, microbialites from depths below 21 m displaying vertical colour transitions along their surfaces were chosen. Pieces of microbialite were sectioned and stored for resin application (explained below), while the remaining parent microbialites were frozen on-site and transported to McMaster University on dry ice for further analysis. Water samples for ^{13}C analysis were syringe pulled via SCUBA divers as close to the microbialite surface as possible at each location and depth within the lake.

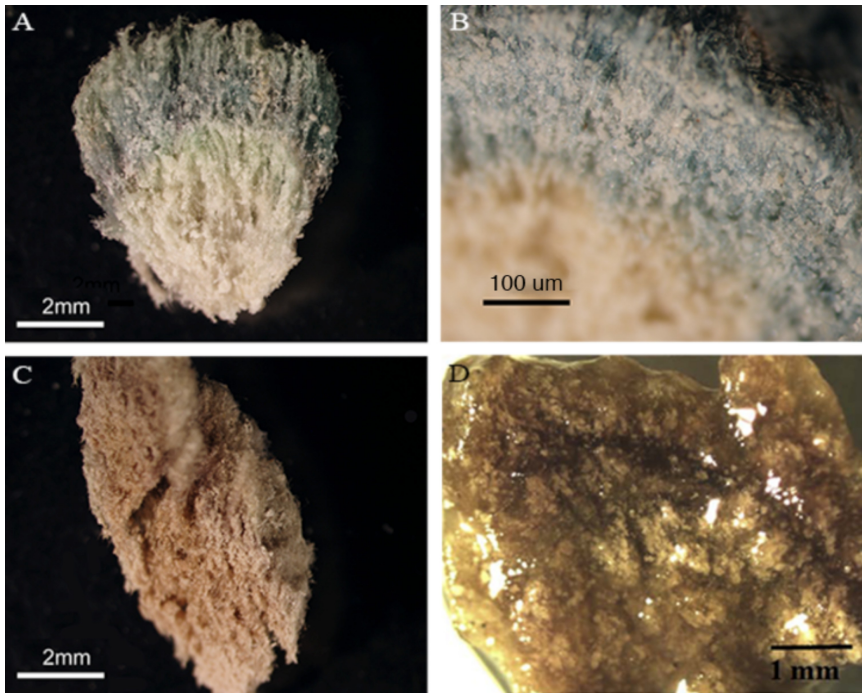

Figure 2.1: Nodule microstructures from microbialite surfaces at ~ 20 m depths are comprised of different filamentous cyanobacterial species (Russell *et al.*, 2014), but both produce photosynthetic biosignatures in precipitated carbonate. Green nodules (**A**) demonstrate banding of organic matter with precipitated carbonate bands clumped in between cyanobacterial filaments (micro-stromatolitic; **B**). Purple nodules (**C**) contain randomized concentrations of precipitated carbonate within the nodule fabric (**D**; Brady *et al.*,2010).

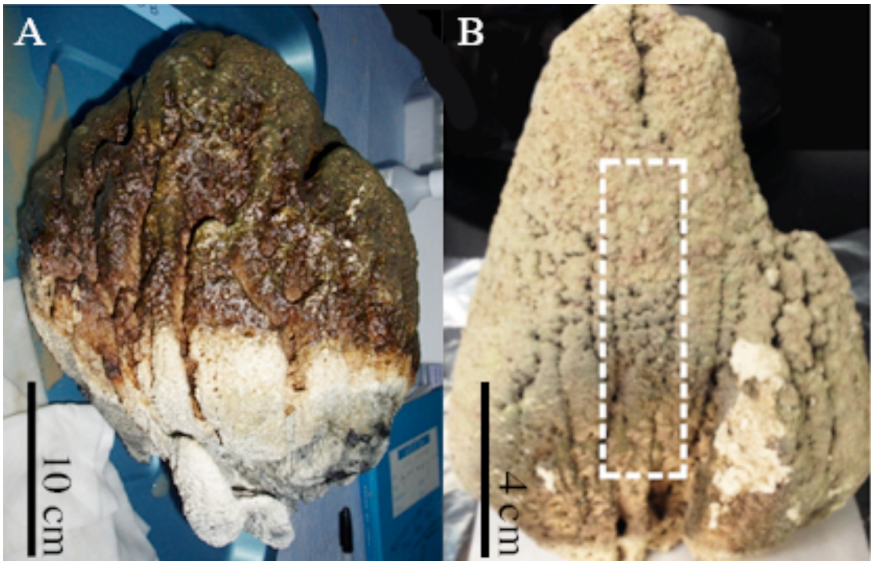

Figure 2.2: (**A**) Microbialite recovered from 33 m in 2008 shows distinct colour transitions from green, to brown, to the grey sediment-water interface (SWI; Brady *et al.*, 2014). In this study, microbialite from 26 m at TP (**B**) indicates similar transitions from green to brown along the lateral region of the structure. The dashed box outlines one such region.

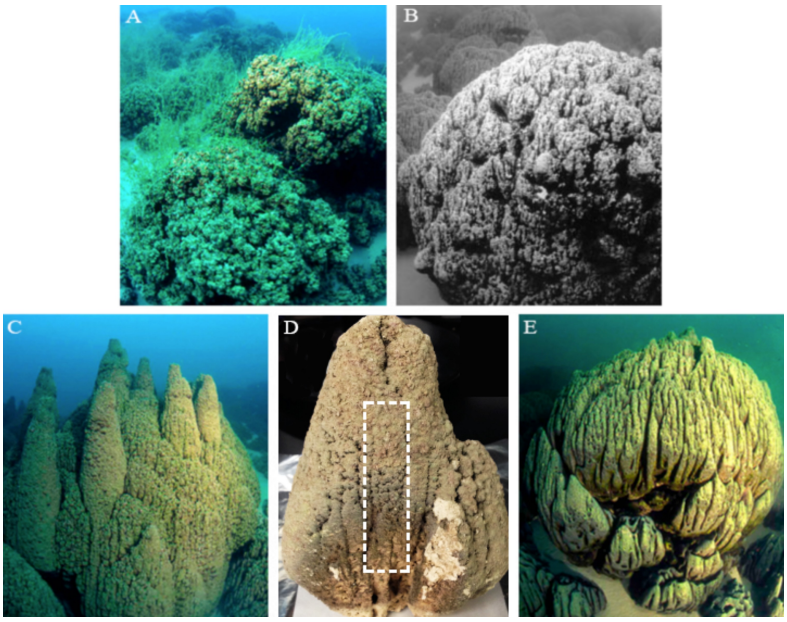

Figure 2.3: Microbialites at Pavilion Lake exhibit different morphologies that correlate with depth. (**A**) Shallow-to-intermediate depths (~ 10 m) microbialites display short (centimeter to decimeter scale) friable structures. (**B**) Large, dome-shaped structures (up to 3 m high) represent a large extent of morphological variation at intermediate (~ 20 m) depths. (**C**) At intermediate-to- deep depths (~ 20 - 30 m), microbialites exhibit taller versions of intermediate depth morphologies. (**D**) Biofilm on these structures begin to show distinct colour transitions along their lateral regions, usually from green at the surface towards brown and grey regions near the sediment-water interface. The dashed box indicates an example of one such region. (**E**) The deepest depths (> 30 m) display extremely dense structures (centimeter to meter scale) whose orientations closely resemble artichokes. Adapted from Laval *et al.*,2000.

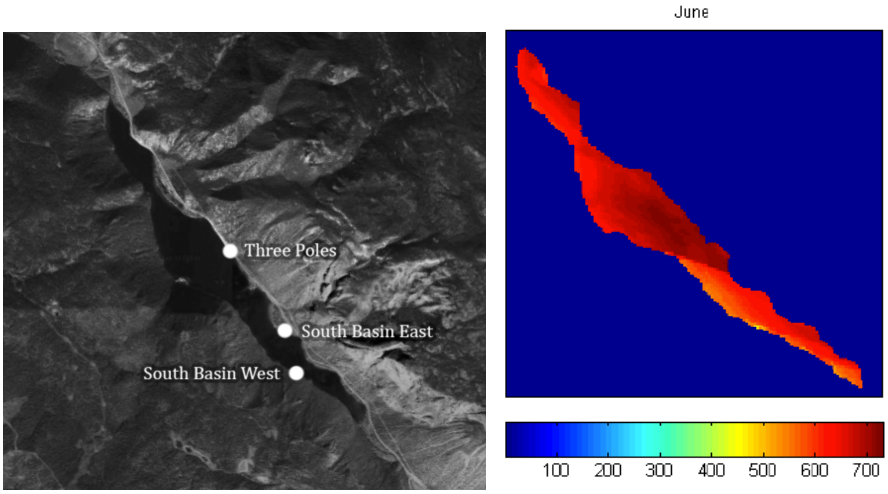

Figure 2.4: Pavilion Lake, British Columbia and sampling locations examined in this study (**A**). Sampling locations were determined based on surface light variation (**B**) that may limit biosignature presence. Light data displays the number 15 minute intervals of surface exposure to direct sunlight for the month of June. Mountains along the south-western shore cause ~ 5 hours less sunlight exposure per day during sunrise.

Water samples were collected in screw cap glass bottles with no headspace and fixed with mercuric chloride to prevent microbial activity.

2.2.3 Microbialite surface sampling

Green and purple nodules were sampled from microbialite surfaces using solvent- rinsed forceps and were bisected, cut to constrain a purported direction of accretionary growth, and sectioned into three parts representing the surficial, internal, and basal sections of the nodule structure (Figure 2.5). This was performed in order to investigate potential decreasing $\delta^{13}C_{carb}$ within the nodule microstructure. All three sections from TP nodules were tested, while only surficial and basal sections from SBE and SBW were analyzed.

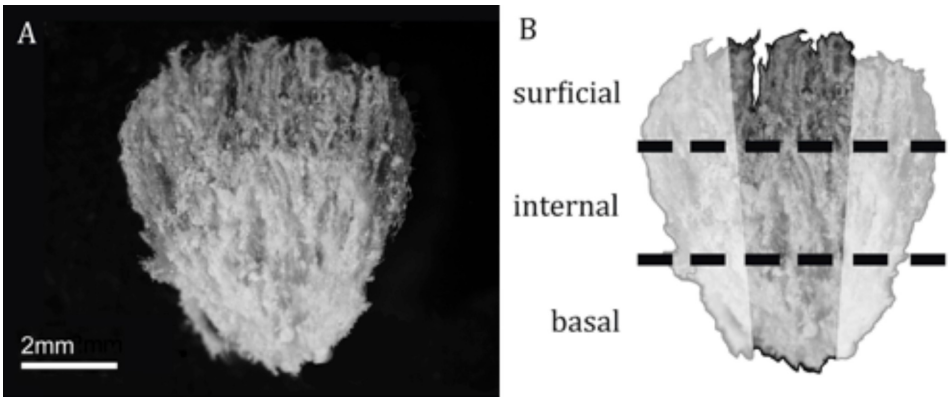

Figure 2.5: Bisected green nodule from South Basin West demonstrates banding of organic matter with precipitated carbonate bands clumped in between cyanobacterial filaments (**A**). Nodules were sectioned to constrain their growth (**B**) and cut into three sections representing the surface, interior, and basal components.

Surface biofilm carbonates from all sites and depths were also analyzed, but collected with different sampling methods. Surface biofilm from 10 and 18 m depths were collected from transects investigating carbonates of the microbialite interior (collection explained below). At 21, 26, and 27 m depths, three vertical transects of surface biofilm samples were collected along three different sides of individual microbialite structures at all sites. Transects traversed along the

microbialite surface from the apex towards the SWI (Figure 2.6). 1-3 mm-sized samples of biofilm were removed from microbialite with a solvent-rinsed scalpel.

2.2.4 Microbialite interior carbonate drilling

To investigate the isotopic composition of internal microbialite carbonate, 3 – 6 cm pieces of microbialite containing nodules (both green and purple) and surface biofilm were sectioned and prepared for resin application. Pieces were placed within 50 mL Falcon® conical centrifuge tubes and oriented to best capture a vertical profile representing the hypothesized direction of in situ growth (with bottom-most part of structure representing primary carbonate deposits and top-most representing active growth at the surface). Due to the friability of the microbialites, especially the shallower microbialites in particular, pieces were treated with Epotek® 301 epoxy resin to preserve structural integrity of the samples and then cross-sectioned with a tile saw. Carbonates were vertically sampled at 0.5 cm intervals along intra-microbialite transects starting from the microbialite surface to approximately 3 – 5 cm deep into the structure. Intra-microbialite sampling transects began directly at the microbialite surface, with exception to transects beneath nodular surfaces, which began at the interface where the base of the nodule microstructure meets the general microbialite surface. 2 mm x 2 mm holes were drilled with a micro electric hand drill, and approximately 0.1 to 0.3 mg of microbialite carbonate was collected in preparation for stable isotope analysis.

2.2.5 Stable isotope analysis

DIC isotopic composition was determined by acidification and conversion to CO_2 by an automated continuous flow isotope ratio mass spectrometer at the G.G. Hatch Laboratory in Ottawa, Canada (St-Jean, 2003). Carbonate ^{13}C analyses were performed on a Gasbench and

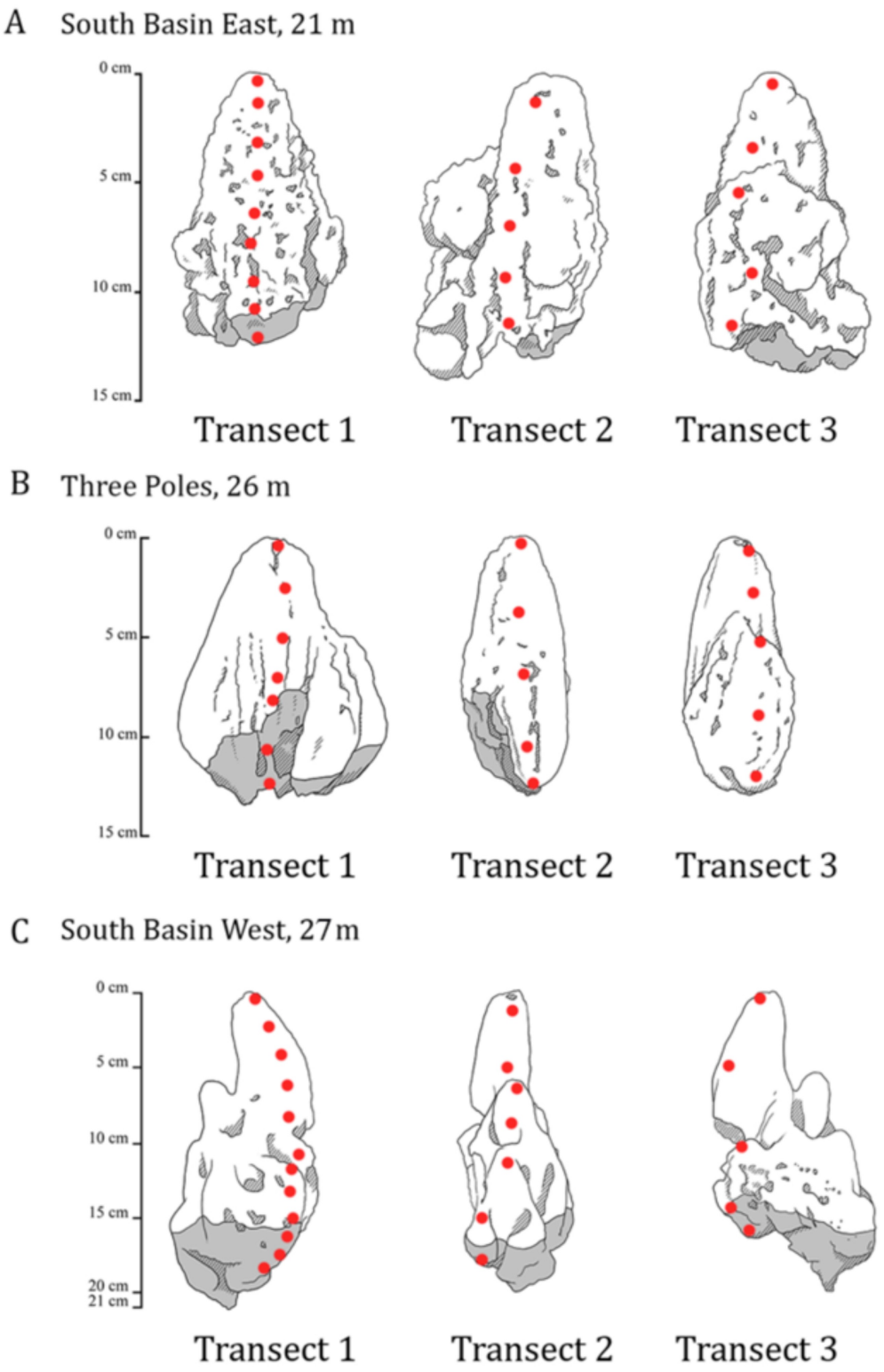

Figure 2.6: Surface biofilm sampling transects (shown with red dots) on 21 m (**A**), 26 m (**B**), and 27 m (**C**) microbialite structures. Grey regions represent portion of microbialite beneath SWI.

Finnigan MAT DeltaPlus XP at 25° C at McMaster University. Carbonates were not treated to remove organics prior to analysis there is no consensus regarding proper pre-treatment of biological carbonates, and to remain consistent with previous studies on Pavilion Lake microbialites that did not pre-treat carbonates. Testing of epoxy resin with Grenville Calcite Standard revealed $\delta^{13}C$ contributions of +0.023 ± 0.008‰, considered negligible for the purposes of this study. All ^{13}C values are reported in standard delta notation in reference to PeeDee Belemnite (PDB).

2.3 RESULTS

2.3.1 Isotopic composition of DIC

$\delta^{13}C_{DIC}$ values for all three sites and depths averaged -1.6 ± 0.3‰ (Table 1). As calcite is enriched in ^{13}C by +1.0 ± 0.2‰ above the bicarbonate from which it precipitates (Romanek *et al.*, 1992), the predicted equilibrium carbonate $\delta^{13}C$ value based on the mean measured lake DIC was -0.6 ± 0.3‰. This value falls within the range of predicted values for equilibrium precipitation originally proposed by Brady *et al.*, (+1.1 to -1.5‰; 2010). As the range proposed by Brady accounts for season and temporal variation, it was used as the basis of comparison in this study.

Table 1
Isotopic composition of aqueous DIC from Three Poles, South Basin East, and South Basin West at different depths. Analytical precision was <0.30‰ (s.d., standard deviation).

Sample	Measured $\delta^{13}C_{DIC}$ (‰ PDB)	Predicted $\delta^{13}C_{carb.}$ (‰ PDB)
TP160614_11	-1.9	-0.9
TP160614_18	-1.7	-0.7
TP220614_26	-1.7	-0.7
SBE220614_13	-1.6	-0.6
SBE062214_16	-1.1	-0.1
SBE062214_21	-1.5	-0.5
SBW062414_11	-1.2	-0.2
SBW062314_18	-1.4	-0.4
SBW062414_26	-1.9	-0.9
Mean	-1.6	-0.6
S.d.	0.3	0.3

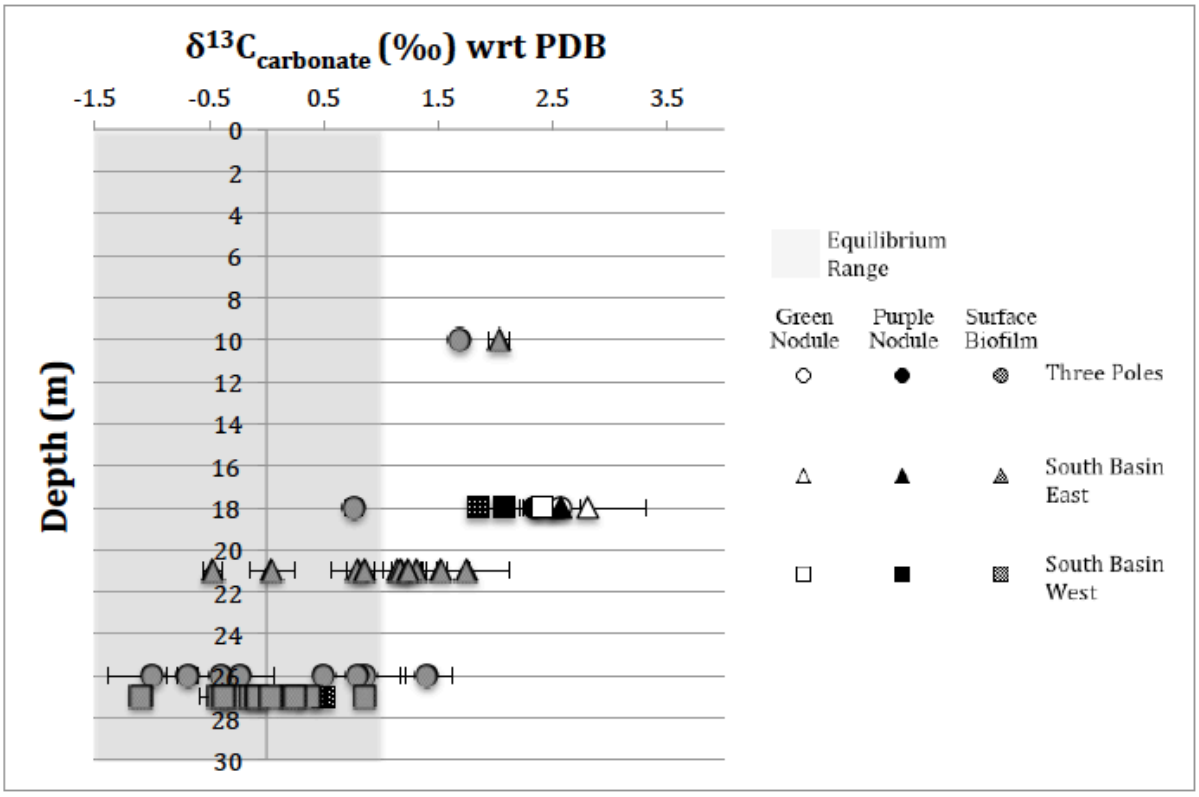

Figure 2.7: Measured $\delta^{13}C$ values of carbonate from microbialite surfaces at all sites and depths. Green and purple nodules (white and black data; $n_{TP\ green}$= 6, $n_{TP\ purple}$ = 4, $n_{other\ sites}$ = 2) are most common at 18 m and are included amongst surface biofilm data (crosshatched data; n = 2). Surface carbonates from one representative surface transect from 21, 26, and 27 m at each site (complete data detailed in Figure 2.7) are included here.

2.3.2 Isotopic composition of surface carbonates – nodules and biofilm

2.3.2a Measured surface carbonates across sites and depths

Surface biofilm and nodule carbonate $\delta^{13}C$ values are shown in Figure 2.7. At Three Poles, the measured $\delta^{13}C$ values from green nodules ranged from +1.9 to +3.2‰ (mean +2.6 ± 0.5‰, n=6) and in purple nodules +1.9 to +2.9‰ (mean 2.3 ± 0.5‰, n=4). Based on the limited variation observed at TP, subsequent measurements of nodule carbonate at other sites were analyzed in duplicates (n=2). Nodule carbonates from SBE and SBW were similarly enriched as TP (+1.6 to +3.5‰), indicating consistent biosignature presence throughout the nodules. Data from one representative surface transect at each site from 21, 26, and 27 m is included in Figure

2.7 (complete data detailed in Figure 2.8). Comparing all nodule and surface biofilm carbonates identified an observable decrease in $\delta^{13}C_{carb}$ that correlated with depth. At 10 and 18 m, surface carbonates were generally enriched above the predicted range of values for equilibrium precipitation (one exception at 18 m with biofilm from TP). Surface transect carbonates from 21 m showed a transition of $\delta^{13}C_{carb}$ values from above to within the predicted range of values for equilibrium precipitation. At 26 and 27 m, almost all transect carbonates recorded within the predicted equilibrium range (one exception at 26 m from TP).

2.3.2b Measured surface carbonates across individual microbialite surfaces

$\delta^{13}C$ data of biofilm carbonates from surface microbialite transects on structures from 21, 26, and 27 m are represented in Figure 2.8. No differences in $\delta^{13}C$ were recorded when transects crossed the SWI. Instead, $\delta^{13}C$ of biofilm carbonates generally decreased with distance from the apex of the structure. At SBE, enrichments above the equilibrium range were identified for the entirety of Transect 2, while the other two transects had transitions into the predicted range of values for equilibrium precipitation occurring between 6 and 8 cm from the apex. At TP, enrichment was strictly limited to the apex for Transects 1 and 3 before transitioning into the equilibrium range at ~ 2 cm, while Transect 2 maintained enrichment until ~ 9 cm below the apex. The sample from SBW at 27 m failed to indicate any enrichment at the apex or anywhere else on the structure; all $\delta^{13}C$ values recorded within the predicted range of values for equilibrium precipitation.

2.3.3 Isotopic composition of internal microbialite carbonate

$\delta^{13}C$ values of carbonates from intra-microbialite transects are displayed in Figure 2.9. At 10 m, an enrichment of ~ 3‰ is recorded in both green and purple nodules at the interface where the base of nodules meet the microbialite surface. Beneath this area, $\delta^{13}C$ values decrease

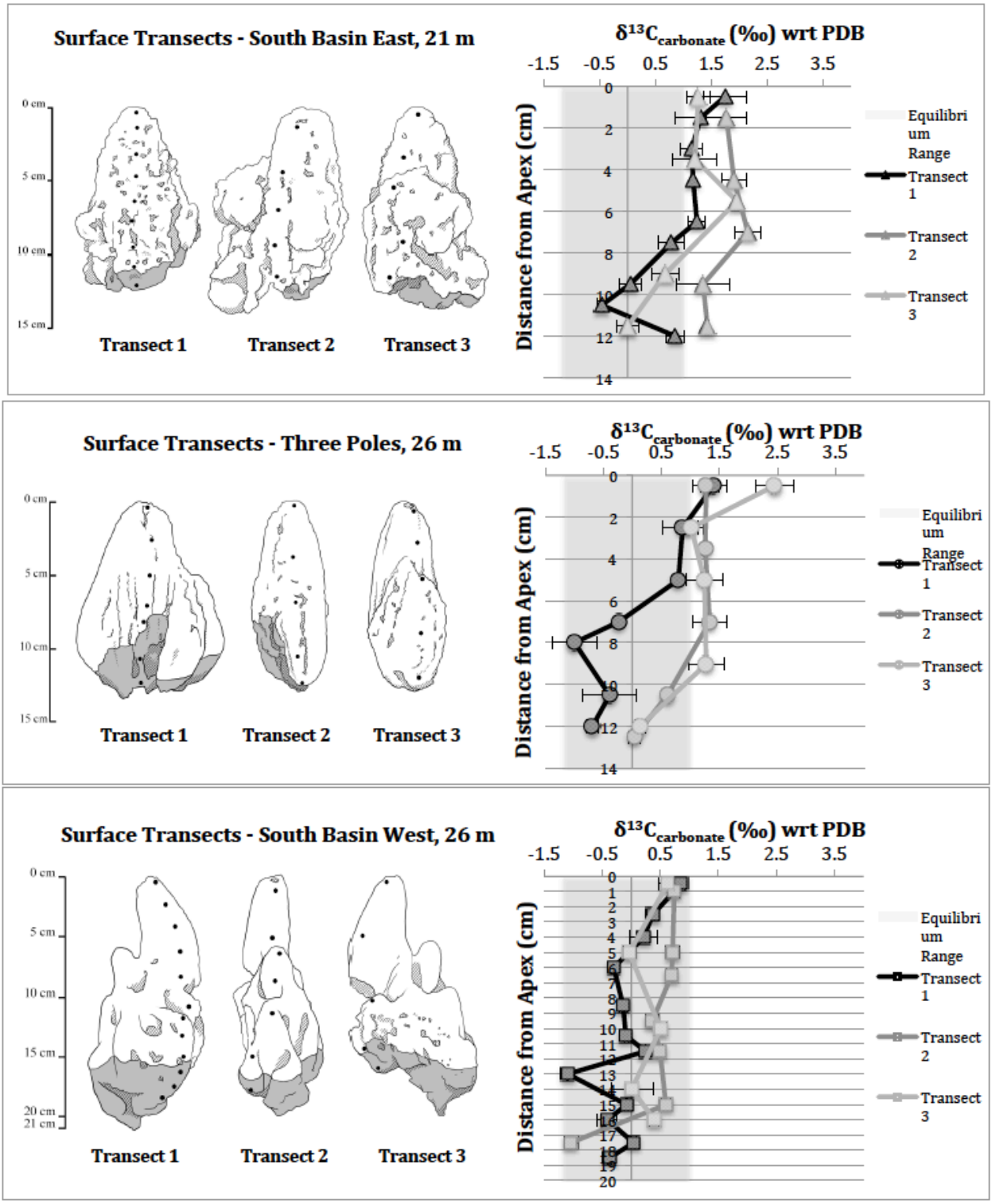

Figure 2.8: Surface carbonate transects (taken from Figure 2.5) and corresponding $\delta^{13}C$ data at each site.

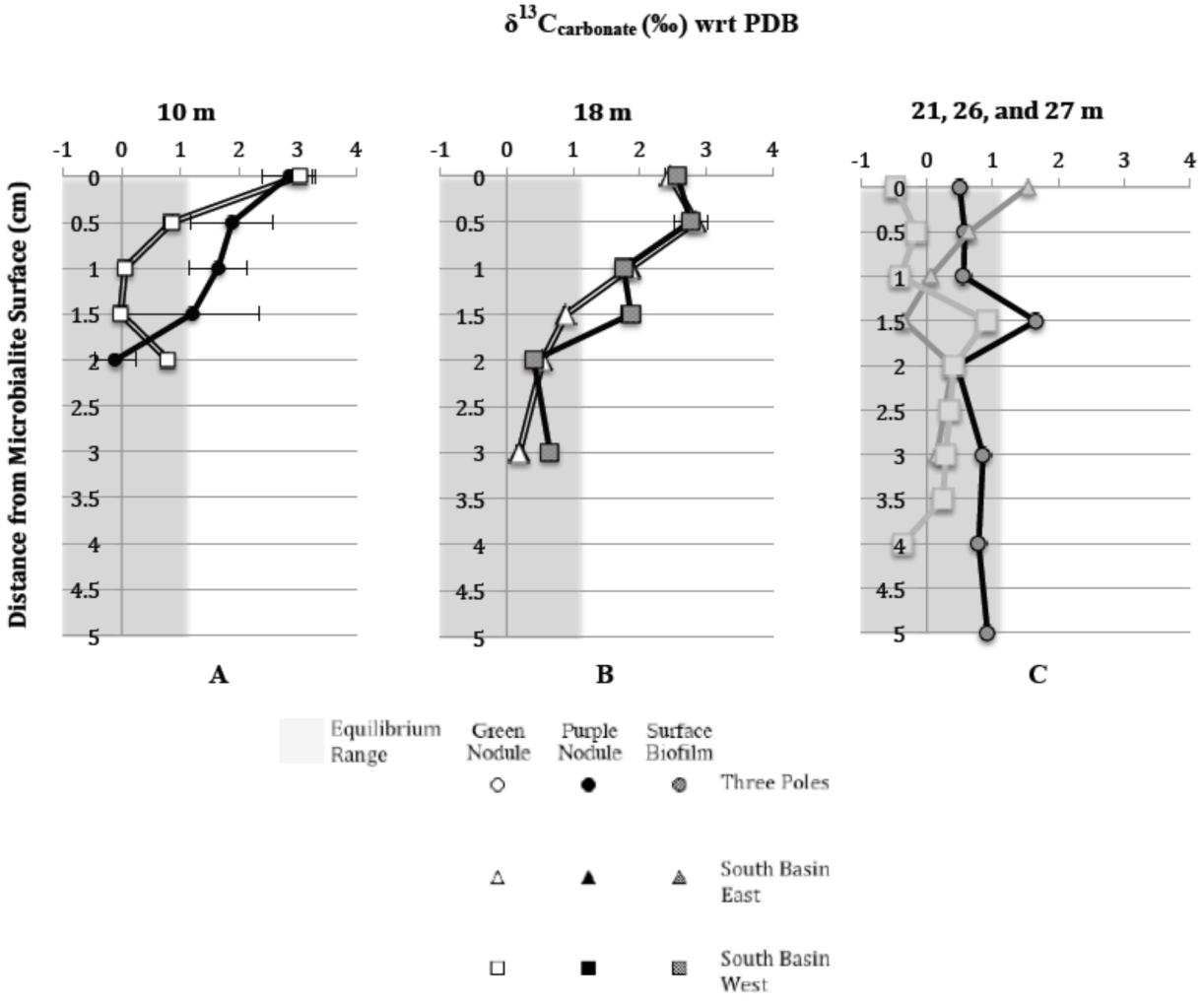

Figure 2.9: Isotopic composition of interior carbonate from 10 (A), 18 (B), and 21/26/27 m (C). Trends are representative examples from each site that best demonstrated downtrends in $\delta^{13}C$ from enrichment to equilibrium.

along the sampling transect until they transition into the predicted range of values for equilibrium precipitation at ~ 0.5 and ~ 1.5 cm (Figure 2.9A). Intra- microbialite transects from 18 m demonstrated a similar downtrend beneath the surface, with surface enrichments of ~ 2.5‰ decreasing into the equilibrium range between 1.5 and 2 cm (Figure 2.9B). Surface carbonates at 21 m record a smaller enrichment of ~ 1.5‰ but quickly decrease into the equilibrium range before 0.5 cm (Figure 2.9C). At deeper depths, carbonates from 26 m all recorded values within the predicted range of values for equilibrium precipitation, except for one enriched value from TP

~ 1.5 cm below the surface.

2.4 DISCUSSION

2.4.1 Light controls on surface carbonate biosignatures with depth

Results of this study demonstrate that a photosynthetic biosignature in surface biofilm and nodules is common at all study sites above a depth of 21 m. The $\delta^{13}C_{carb}$ enrichment of +2.1 to +2.8‰ observed in nodular carbonates at all sites is consistent with previous findings of the photosynthetic biosignature at TP by Brady *et al.* (2010). This study also examined surface biofilm samples and found that they were similarly isotopically enriched (+1.6 to +2.6‰) above 21 m with the exception of 1 sample. The observation of enrichment in the surface biofilm implies that the isotopic influence of photosynthesis on DIC is sufficient in the smaller volume of the surface biofilm to produce a biosignature. Thus, the difference in biomass thickness between nodules and surface biofilm does not appear to limit biosignature production. The fact that nodules and surface biofilms were enriched in SBW where monthly sunlight exposure is reduced by ~ 30% indicates that variation in surface light at different lake locations also has little effect on the generation of the biosignature. Instead, $\delta^{13}C_{carb}$ variability observed in correlation with increasing depths at each study site supports that light exposure is the primary control on biosignature formation. PAR levels at ~18 – 20 m decrease to ~10% of the total surface value (Brady *et al.*, 2014), suggesting that the loss of the photosynthetic biosignature at 21 m may be the result of reduced photosynthetic activity failing to induce sufficient enrichments to DIC. In this situation, the rate of exchange with bulk water outpaces the rate of preferential uptake of ^{12}C by microbial photosynthetic activity, recording $\delta^{13}C$ values that fall within the predicted range of values for equilibrium precipitation. Alternatively, photosynthetic effects to DIC may be consistent across depths, but increased relative heterotrophic activity at deeper depths (Russell *et*

al., 2014) may result in larger outputs of ^{12}C masking photosynthetic enrichments. The decreasing trend of $\delta^{13}C_{carb}$ values can potentially reflect the extent to which DIC becomes increasingly depleted by heterotrophic activity. Therefore, depths of 21 m where $\delta^{13}C$ values begin to decrease from enrichment into the predicted equilibrium range suggest an interface where autotrophic and heterotrophic processes balance. At depths below 21 m, $\delta^{13}C_{carb}$ values recording within the equilibrium range are expected due to DIC becoming increasingly ^{13}C-depleted by heterotrophic respiration. At 27 m, sufficient heterotrophic activity results in large shifts that consistently produce $\delta^{13}C_{carb}$ values in the predicted equilibrium range. Absence of $\delta^{13}C_{carb}$ values that record below the predicted range of values for equilibrium precipitation suggests that biosignatures of heterotrophy are not producible due to consistent net balance of opposing microbial metabolisms recording average $\delta^{13}C_{carb}$ values that do not deviate from equilibrium. At 27 m depths, dominant microbial activities, whether they are autotrophic or heterotrophic, are therefore insufficient to generate a robust biosignature of either process.

2.4.2 Spatial distribution of $\delta^{13}C$ on deep structures

Vertical profiling of $\delta^{13}C_{carb}$ along structures from 21 and 26 m indicated photosynthetic biosignatures were present at the apex. This supports that the apex is the site of greatest light exposure and where phototrophic organisms are most likely to localize, precipitate, and record biosignatures. It also supports previously hypotheses that deep microbialites exhibit phototaxic orientation as a result of accretionary buildup from concentrated abundance of phototrophic precipitation at the apex. Interfaces of biosignature loss, where $\delta^{13}C_{carb}$ transitioned from enrichments towards predicted equilibrium values, were observed along the lateral regions of the microbialite structure at variable distances from the apex. Transect 1 on the SBE structure from 21 m demonstrated one such interface approximately 7 – 8 cm from the apex. At 26 m depths

from TP, Transect 1 showed similar transitions at ~ 2 cm from the apex. At 27 m depths from SBW, all $\delta^{13}C_{carb}$ values recorded within the predicted range of values for equilibrium precipitation. With deeper depths, the distance of the biosignature interface from the apex essentially decreases. This suggests that the expanse of photosynthetic activity producing biosignatures is also decreasing as a result of limited sunlight reaching these surfaces. Multiple sampling transects on individual microbialite surfaces support this since biosignature presence is variable. For instance, Transect 2 from the SBE structure from 21 m demonstrated continuation of the photosynthetic biosignature entirely along the lateral region of the structure towards the SWI. Even at 26 m depths, photosynthetic enrichments at TP were maintained on Transects 2 and 3 approximately 9 – 10 cm along the lateral regions of the structure. Enrichments seen below the apex and along the lateral regions of the microbialite may therefore demonstrate areas of the microbialite surface that have increased photosynthetic activity due to better phototaxic orientation. Regional variability on the microbialite surface where biosignatures are lost is likely attributed to poor light exposure gradually reducing the magnitude of photosynthetic shifts to DIC. A loose interpretation of the spatial distribution of phototrophs inducing $\delta^{13}C_{carb}$ enrichments at deeper microbialite structures is proposed in Figure 2.10.

Despite sub-sampling across colour zones, interfaces where $\delta^{13}C_{carb}$ transitioned from enrichment towards equilibrium did not correlate with transitions in biofilm colour. Instead, transitions were seen within the green portion of the biofilm, where autotrophic abundance is greater (Brady *et al.*, 2014). This supports that decreased relative photosynthesis is likely the dominant mechanism limiting biosignature presence along the lateral regions of the structure. However, given that increased heterotrophy relative to autotrophy exists within biofilm closer to the SWI, net balance of autotrophic and heterotrophic effects to DIC may also be partially responsible for $\delta^{13}C_{carb}$ values recording within the predicted equilibrium range. Although

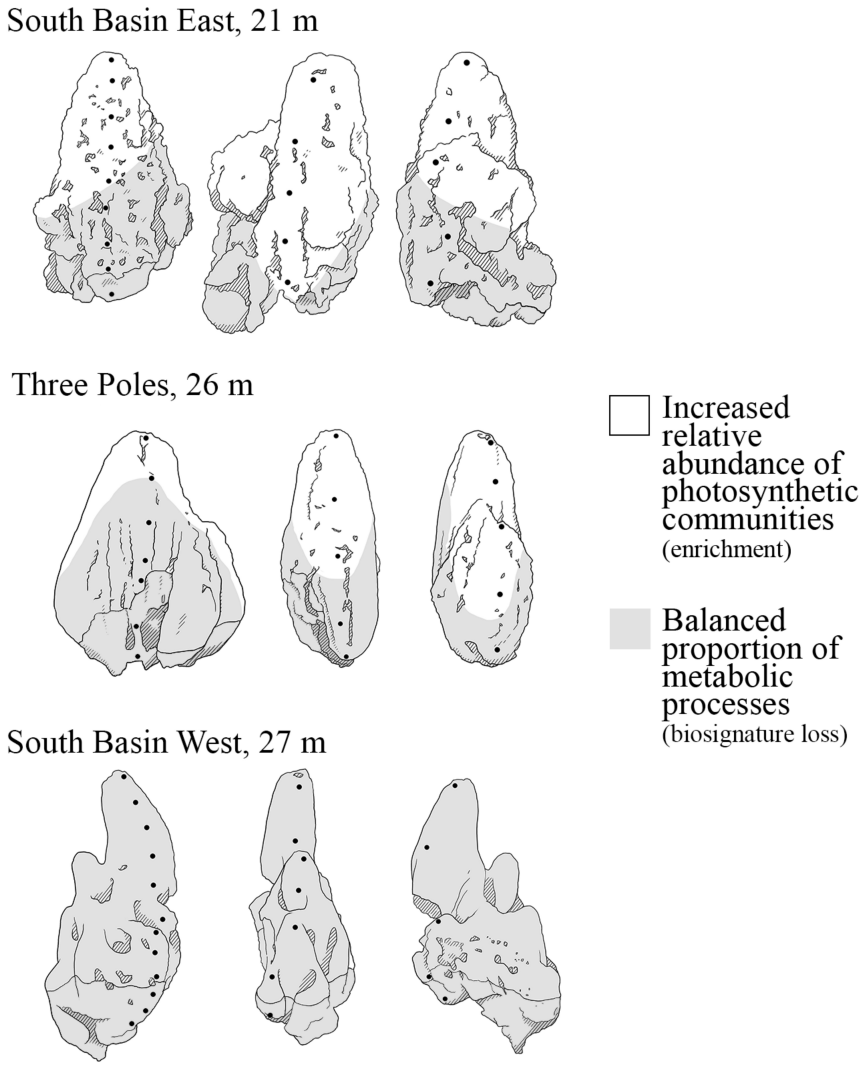

Figure 2.10: Possible spatial arrangement of ^{13}C-enriched carbonates on deep microbialite structures.

vertical sub-sampling towards the SWI did not reveal trends towards lower $\delta^{13}C_{carb}$ values that could be attributed to a heterotrophic biosignature, regions of the microbialite below interfaces of biosignature loss consistently recorded $\delta^{13}C_{carb}$ values within the predicted equilibrium range. This suggests that the mechanism of autotrophic and heterotrophic effects to DIC balancing to produce average $\delta^{13}C$ values is likely limiting biosignature formation along the lateral regions of microbialites. Therefore, neither phototrophic nor heterotrophic processes are sufficient enough

to produce spatial arrangement of biosignatures along individual microbialite surfaces at 21, 26, or 27 m depths.

2.4.3 Loss of enrichment within microbialite interior and obstacles to preservation

Results indicated that $\delta^{13}C_{carb}$ preservation within the microbialite interior is variable. Beneath the microbialite surface, carbonates were enriched up to depths of ~0.5 to ~2 cm, after which point $\delta^{13}C_{carb}$ generally recorded values within the predicted equilibrium range. This suggests an interface where secondary infilling begins to overprint photosynthetic biosignatures with isotopically lighter carbonate. Heterotrophic decay beneath the surface is known to degrade preserved microbial filaments to create vacant pores within the carbonate matrix that are susceptible to carbonate infilling (Harwood Theisen *et al.*, 2015). Void spaces in between and around photosynthetically enriched carbonates can be filled with ^{13}C-depleted carbonates brought about by heterotrophic effects on DIC. The result is a carbonate matrix that is comprised of varying isotope compositions reflecting both phototrophic and heterotrophic processes. Depending on the DIC composition and extent of precipitation within these pore spaces, enriched $\delta^{13}C$ values can become overprinted over time with increased infilling from ^{13}C-depleted DIC. The subsequent net result can therefore produce average $\delta^{13}C_{carb}$ values that do not deviate substantially from equilibrium, or, if overprinting is sufficient, may produce heterotrophic biosignatures. Previous ^{14}C investigations of microbialite carbonate in Pavilion Lake estimated microbialite growth rates of the past 1000 years to be approximately 0.050 mm $year^{-1}$ (Brady *et al.*, 2009), suggesting that photosynthetic enrichments can become overprinted across the ~0.5 to ~2 cm interface in 100 – 400 years. However, Figure 2.11A demonstrates that $\delta^{13}C_{carb}$ values can greatly fluctuate within this interface, and that photosynthetic enrichments at ~1 to ~2 cm can be preserved after initial transitions suggests they are lost. The variability of biosignature

preservation over this time period is proposed to result from factors limiting sufficient overprinting, such as changing precipitation rates and/or DIC composition. Variation in precipitation rates is known at Pavilion Lake. Previous investigations of microbialite growth rates have indicated that base rates of 0.025 to 0.050 mm $year^{-1}$ should expect to yield microbialites between 25 and 50 cm in height since Pavilion's last glaciation ~10 000 years ago (Laval *et al.*, 2000; Brady *et al.*, 2009). However, numerous microbialites in Pavilion that significantly exceed these heights suggest that precipitation rates in the past must have been faster. Subsequent estimates of microbialite growth rate at Pavilion range between 0.012 mm to 0.025 mm $year^{-1}$ (Brady; unpublished data). While it is unknown precisely how precipitation rates may have fluctuated or slowed down throughout the lake's history, this variability supports differences in the length of time required to overprint $\delta^{13}C_{carb}$ enrichments in microbialites.

Alternatively, it must be noted that variation in the 100 – 400 year interface where biosignatures are lost can be ascribed to insufficient infilling from different DIC compositions over time. DIC has the potential to be altered via a variety of different methods, such as surface water and groundwater inputs of CO_2, pCO_2 variability, water outflow, and evasions of CO_2 (Bade *et al.*, 2004). These changes over time can produce ranges of equilibrium DIC values that, subsequent to photosynthetic enrichment or heterotrophic depletion, produce $\delta^{13}C_{carb}$ values that differ greatly from present day measurements. For example, assuming that sufficient infilling occurs, detection of an elevated $\delta^{13}C_{carb}$ value may reflect an isotopically heavier equilibrium DIC composition of the past and not a microbial photosynthetic biosignature in the present. False interpretations of microbial effects in $\delta^{13}C_{carb}$ are therefore possible. Constraining this variability within Pavilion microbialites is difficult since it is currently unknown whether significant changes to DIC have occurred over time. However, these DIC effects are often regulated by significant environmental and climate change within a particular region (Curtis *et al.*, 1999; Guo

et al., 2006). In the past 400 years, it is unlikely that massive change to Pavilion's weather dynamics occurred to produce large variations to DIC that are significantly incongruent with present day values. Therefore, while changing DIC composition over time suggests an additional mechanism potentially contributing to observed enrichment beyond the intra-microbialite interface of biosignature loss, it is not considered a large factor.

Using an isotopic mass balance, predicting the amount of carbonate mass needed to precipitate from either equilibrium or heterotrophically-depleted DIC to overprint surface photosynthetic biosignatures is possible. The formula used for calculating isotopic mass balance is shown in Equation (2.1).

$$\delta^{13}C_{measured} = F \bullet (\delta^{13}Cenriched) + (1\text{-}F) \bullet (\delta^{13}C_{abiotic\ or\ heterotrophic}) \quad (2.1)$$

F represents the minimum fraction of total carbonate needed to precipitate from either DIC composition. $\delta^{13}C_{measured}$ represents the upper threshold of the predicted equilibrium range (+1.1‰) where photosynthetic enrichments are lost. $\delta^{13}C_{abiotic\ or\ heterotrophic}$ values are based on minimums of predicted $\delta^{13}C_{carb}$ values that precipitate from either equilibrium (abiotic) or heterotrophically-depleted DIC. Based on the predicted range of carbonate values for equilibrium precipitation, -1.5‰ represents the lowest end member value for equilibrium DIC composition. Conversely, -25‰ representing expected $\delta^{13}C_{carb}$ from heterotrophically-depleted DIC is based on previous investigations of $\delta^{13}C_{organic\ matter}$. These findings indicated that organic matter associated with surface nodules in Pavilion were depleted by ~ -28‰ (Brady *et al.*, 2010). Minimum fractionation of this carbon source on the order of ~3.4‰ during heterotrophic respiration (Blair *et al.*, 1985) would therefore suggest expected $\delta^{13}C_{carb}$ values of ~ 25‰. The minimum fraction of total carbonate required to precipitate from either DIC composition and

overprint surface biosignatures are shown in Table 2.

Table 2
Calculated proportions of carbonate required to precipitate from either equilibrium and/or heterotrophic-altered DIC to overprint surface enrichments into the predicted range of values for equilibrium. Photosynthetic enrichments are general values based on the level of enrichment characterized in Pavilion Lake.

	Fraction of Total Carbonate Mass (%) Required to Overprint Enrichments	
Photosynthetic Enrichment (‰ PDB)	**Equilibrium DIC**	**Heterotrophically Depleted DIC**
3.5	46	14
3.0	40	13
2.5	32	12
2.0	21	10
1.5	17	8

Intra-microbialite transects indicated maximum levels of enrichment on the order of 3‰ (Figure 2.8). Based on this maximum, ~ 40% of total measured carbonate must precipitate from equilibrium DIC to overprint 3‰ enrichments to + 1.1‰. Conversely, only ~ 13% of total carbonate is required to precipitate from heterotrophically-depleted DIC to achieve the same result. Since smaller proportions of total carbonate mass are required to precipitate from heterotrophically-depleted DIC in order to mask surface enrichments, heterotrophic infilling presents a viable mechanism to support the loss of enrichments within the carbonate matrix.

An additional consideration for why $\delta^{13}C_{carb}$ may become increasingly enriched beneath the surface is due to the challenge associated with constraining the direction of accretionary microbialite growth. At deeper depths, limited light exposure is believed to influence the phototaxic configuration of microbialites. The vertical nature of these structures results from localized accretionary buildup at the apex, making historical investigations to their development an easier task as the growth direction can be more confidently assessed. At shallower depths, however, more sunlight suggests biomass production outpaces carbonate

precipitation (Laval *et al.* 2000; Omelon *et al.*, 2013), producing loose and porous structures. Unlike deeper depths, excessive light availability is thought to influence extensive microbial mat coverage and precipitation, leading to randomized and multi-directional growth of microbialites that forms additional and/or larger surfaces. Figure 2.11B displays an example of an additional microbialite surface at ~ 1.5 cm below the originally purported surface. If this surface has sufficient light exposure, the expected autotrophic abundance on this surface would suggest active precipitation and support the reappearance of photosynthetic enrichments at ~ 1.5 cm along the sampling transect. Thus, fluctuations in $\delta^{13}C_{carb}$ beneath the ~ 0.5 to ~ 2 cm interface may result from inaccurate sampling along multiple surfaces where photosynthetic biosignatures can be produced. Unidirectional sampling intended to correlate with accretionary development over time may require radial or multi-directional assessment to accurately reflect biosignature preservation in these samples.

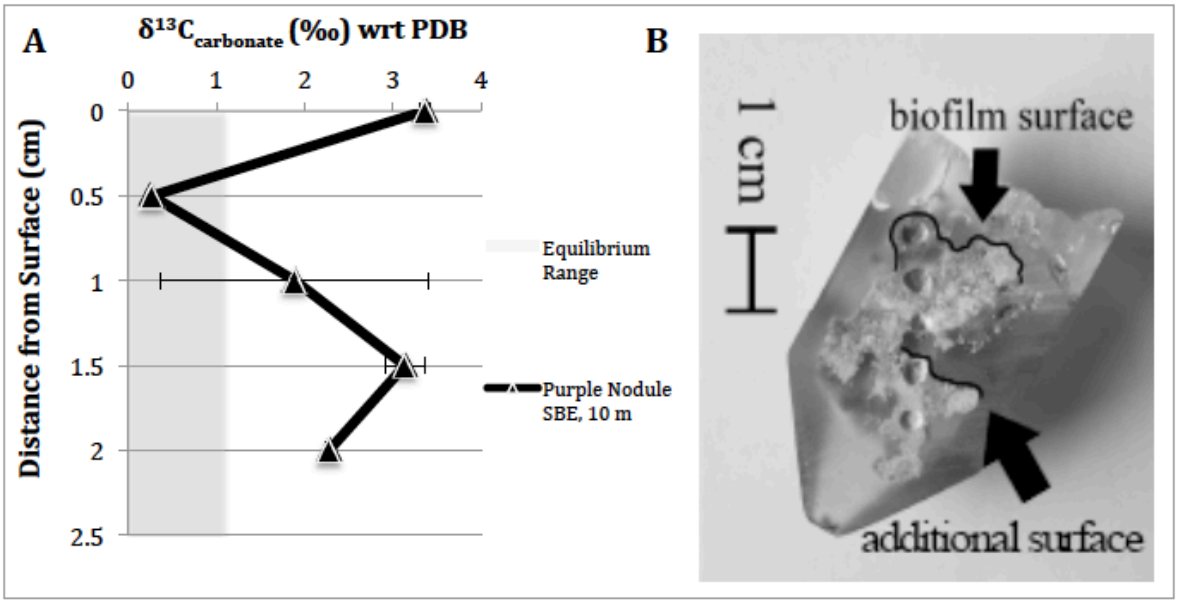

Figure 2.11: Representative transect sampling carbonate beneath purple nodule shows enrichments occurring beneath equilibrium-recorded carbonates (**A**). Changing surface topography partway along the transect indicates opportunities for additional surfaces (**B**) to record enrichments well beneath what was originally purported to be the surface.

CONCLUDING REMARKS

At Pavilion, nodule and surface biofilm carbonates from areas of the lake where surface sunlight was variable demonstrated $\delta^{13}C$ enrichments representing photosynthetic biosignatures. However, decreasing light levels correlated with lake depth supported a decreasing $\delta^{13}C_{carb}$ trend, to the extent where photosynthetic biosignatures were no longer existent. The transition of $\delta^{13}C_{carb}$ from enrichment to the predicted range of equilibrium values at 21 m is believed to represent an interface where reduced photosynthesis, increased relative heterotrophy, or a combination of both start to limit biosignature formation. At depths of 21, 26, and 27 m, heterotrophic biosignatures were not identified, but decreasing $\delta^{13}C_{carb}$ trends along vertical transects from the surface apex towards the SWI indicated interfaces where biosignature loss is also potentially mediated by changing metabolic effects. On multiple sides of individual microbialites, variability in the distances of these interfaces from the apex suggests that light exposure on these structures influences the extent of photosynthetic activity forming biosignatures. Therefore, biosignature loss across lake depth and microbialite surface interfaces suggests that light attenuation throughout Pavilion's water column is the primary control on proportions of metabolic communities influencing biosignature formation.

Beneath the microbialite surface, carbonates were enriched up to depths of ~ 0.5 to ~ 2 cm, after which point $\delta^{13}C_{carb}$ generally fluctuated within the predicted equilibrium range. This suggested biosignatures may be preserved on the order of 100 – 400 years.

Loss of the biosignature is thought to result from isotopically lighter carbonate from secondary heterotrophic infilling overprinting photosynthetic enrichments. However, photosynthetic enrichments identified beneath the interface where biosignatures were lost suggested inconsistencies to biosignature preservation over time. Fluctuations in $\delta^{13}C_{carb}$ may be a result of insufficient overprinting brought about by inconsistent precipitation rates and changing DIC

composition over time. An isotopic mass balance indicated that fractions of total measured carbonate precipitating from equilibrium and heterotrophically-depleted DIC compositions overprint maximum $\delta^{13}C_{carb}$ enrichments of ~ 3‰ by ~ 40% and ~ 13%, respectively. Smaller inputs of heterotrophic infilling required to overprint photosynthetic enrichments supports this mechanism as limiting biosignature preservation in Pavilion. Finally, accurate intra-microbialite transects correlated with growth direction should be better constrained since the potential to cross multiple surfaces and observe $\delta^{13}C_{carb}$ fluctuations is possible if past microbialite accretion was irregular.

These findings represent further characterization of the photosynthetic biosignature within Pavilion Lake. Results demonstrated that light variability within the lake is a primary control on biosignature development, which is potentially mediated by variable proportions of autotrophic and heterotrophic metabolisms within the lake. The 100 – 400 year lifespan of photosynthetic biosignatures identified within microbialite structures suggests that isotopic biosignatures in modern microbialite systems are geologically short-lived and potentially invaluable as an ancient or astrobiological tool for interpreting life. The lack of heterotrophic biosignatures, too, suggests that constraining life within the geologic record can be difficult if competing biological isotope effects average to abiogenic standards. Further investigations at Pavilion Lake should consider improved correlation between genetic, light, and isotopic data to better illustrate the dynamic nature of the photosynthetic biosignature's formation and disappearance.

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SUPPORTING INFORMATION

Supporting information for this study can be found in the Appendix.

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CHAPTER 3
CONCLUDING REMARKS AND FUTURE RESEARCH

Resolving the influences that limit the generation and preservation of isotopic biosignatures in analog microbialite systems is important for accurate interpretations of biogenicity within the geologic record. This knowledge helps to affirm the viability of biosignatures that are found within the geologic record and their potential to interpreting life in unknown, and even perhaps extraterrestrial, geological systems. In order to understand the influences affecting biosignature generation and preservation, this thesis employed stable isotope analyses on microbialite carbonate from three locations and depths within Pavilion Lake to better characterize $\delta^{13}C_{carb}$ outcome in changing light regimes. In addition, the preservation of surface photosynthetic biosignatures within microbialite structures was investigated to constrain a lifespan of their viability. The primary hypotheses on which these studies were based include: 1) photosynthetic biosignatures are controlled by light level and differ across sites where surface light is variable, 2) phototrophic biosignatures deplete with increasing depth and record biosignatures of heterotrophy below 25 m, 3) individual microbialite surfaces from >25 m indicate decreasing trends of $\delta^{13}C_{carb}$ towards heterotrophy, and 4) $\delta^{13}C_{carb}$ enrichments identified at the microbialite surface are lost within the microbialite interior and record biosignatures of heterotrophy. In testing these hypotheses, the results of this research have helped to better characterize the nature of the photosynthetic biosignature amongst different light regimes, and provide insights to how autotrophic and heterotrophic processes influence the formation and preservation of this biosignature.

3.1 DISSERTATION SUMMARY

In order to elucidate if light was controlling biosignature formation, carbonates from both green and purple nodules, as well as surface carbonates associated with general biofilm, were

analyzed in changing light regimes. These changes in light availability were associated with several locations within the lake, particularly at the surface on opposite sides of the South basin, as well as with attenuation of sunlight throughout the water column. $\delta^{13}C_{carb}$ values were compared between surface biofilm and nodules at all three sites: Three Poles, South Basin East, and South Basin West. Findings showed that nodules were found at ~ 20 m depths at all study sites. $\delta^{13}C_{carb}$ values from surface biofilm and nodules indicated that photosynthetic biosignatures attributed to carbonates enriched in ^{13}C were present at all locations ($\delta^{13}C_{carb}$ = +1.9 to +3.2‰), but decreased with depth. At 21 m, carbonates began to record $\delta^{13}C_{carb}$ values both above and within the predicted range of values for equilibrium precipitation ($\delta^{13}C_{carb}$ = - 0.2 ± 1.3‰), suggesting a depth interface where biosignatures are lost. At this interface, decreases in $\delta^{13}C_{carb}$ are suggested to result from either: i) reduced photosynthetic activity failing to induce sufficient enrichments to DIC, or ii) increased relative heterotrophy increasing ^{12}C content in the local DIC pool to precipitate average $\delta^{13}C_{carb}$ values. At depths below 21 m, almost all $\delta^{13}C_{carb}$ values recorded within the predicted equilibrium range. However, vertical profiling of microbialite surfaces at these depths indicated that $\delta^{13}C_{carb}$ variability was spatially arranged. Photosynthetic enrichments were found at variable distances from the surface apex of structures from 21 and 26 m, suggesting that surface light incidence is variable and may be affecting the extent of photosynthetic activity producing biosignatures. No trends below the predicted range of equilibrium were identified, and even at SBW at 27 m no heterotrophic biosignatures were identified. This suggested that dominating microbial metabolisms at these depths, whether phototrophic or heterotrophic, were unable to record biosignatures of either process.

Within microbialite structures, surface enrichments were preserved between ~ 0.5 and ~ 2 cm beneath the microbialite surface. Beyond this point, intra-microbialite carbonates recorded $\delta^{13}C_{carb}$ values within the predicted equilibrium range. This distance from the surface in which

biosignatures are lost suggested that this photosynthetic biosignature has a lifespan of approximately 100 – 400 years, based on previous estimates of microbialite growth rate (0.05 mm year-1) in Pavilion Lake (Brady *et al.*, 2009). Mechanisms contributing to this loss over time are suggested to result from heterotrophic infilling of void spaces left behind in the carbonate fabric by degraded cyanobacterial filaments (Harwood Theisen *et al.*, 2015). However, fluctuations of $\delta^{13}C_{carb}$ beneath the surface indicated that preservation over time is variable. Photosynthetic enrichments that reappear beneath equilibrium-recorded carbonates are thought to exist due to insufficient infilling from differences in precipitation rates and DIC composition over time. Known variation in carbonate precipitation rates within Pavilion suggested that enrichments may be preserved due to accretionary precipitation outpacing infilling processes required to overprint photosynthetic enrichments. Alternatively, changes to DIC composition over time, potentially brought about by groundwater inputs, changing pCO_2, CO_2 evasions, etc., may produce enrichments or depletions that incongruent with present day interpretations of $\delta^{13}C_{carb}$. In other words, measured enrichments below equilibrium-recorded carbonates in the present may indicate an isotopically heavier equilibrium DIC of the past. The extent to how much variable DIC compositions overprint photosynthetic enrichments also affects $\delta^{13}C_{carb}$ preservation. Although Pavilion doesn't indicate any evidence of changing DIC composition over time, an isotopic mass balance comparing equilibrium and heterotrophically-depleted DIC indicated differences in the amount of carbonate required to sufficiently overprint enrichments. $\delta^{13}C_{carb}$ enrichments on the order of ~ 3‰ can be overprinted into the upper threshold of the predicted equilibrium range (+1.1‰) if ~ 40% of total carbonate mass precipitates from equilibrium DIC (-1.5‰). Alternatively, only ~ 13% of total carbonate mass must precipitate from heterotrophically-depleted DIC (-25‰) in order to sufficiently overprint photosynthetic enrichments. The smaller amount of carbonate required from heterotrophically-depleted DIC

suggested that heterotrophic infilling is a viable mechanism contributing to the loss of biosignatures within Pavilion. Finally, poor constraint of growth direction in shallow samples suggested that measured enrichments below equilibrium-recorded carbonates were a result of crossing over multiple surfaces. Shallow samples indicated multiple surfaces for photosynthetic communities to produce biosignatures due to excessive light influencing the expanse of microbial mat coverage and precipitation. Enrichments may be detected if sampling transects are not appropriately correlated to the historical accretions of carbonate in irregular (i.e. not unidirectional) growing structures.

These findings ultimately indicated that photosynthetic biosignatures within Pavilion Lake are potentially poor tools for interpreting life within the geologic record. This thesis demonstrated that dominating autotrophic and heterotrophic processes contribute to the creation and erasure of the photosynthetic biosignature. While the short- lived 100 – 400 year lifespan of the biosignature suggests poor preservation potential, the fact that biological processes can mediate biosignature loss complicates the distinction between abiogenic and biogenic formations of carbonate. This does not confidently suggest that biosignature applications are useful for investigations spanning geological timescales. However, preservation may still be possible in scenarios where biological processes are significantly reduced or absent. For example, if Pavilion were to undergo rapid evaporation and subsequent sedimentation, microbial mat communities would die and biological activity would cease. Surface enrichments in this scenario can be expected to preserve well over time since overprinting processes are also absent. Therefore, even though Pavilion does not support the extended preservation of the photosynthetic biosignature, other dynamics associated with preservation may still make isotopic biosignatures valuable to geological and astrobiological research. Further research can continue to explore and improve understanding on biosignature dynamics and preservation potential.

3.2 DIRECTIONS FOR FUTURE RESEARCH

Although microbialite research in the Pavilion Lake area has been on going for many years, questions still remain about the nature of microbialite and biosignature development. Additional investigations into biosignature preservation can potentially resolve evidence of heterotrophic biosignatures. Kelly Lake is a nearby lake that also contains microbialite structures. Investigating and comparing preservation dynamics between Pavilion and Kelly can better ascertain if similar factors affect biosignature formation and loss. Further characterization of biosignatures in modern microbialite systems can provide additional insights to their viability as astrobiological tools.

3.2.1 Supplementary light and genetic data

A further focus to this study to support observed trends in $\delta^{13}C_{carb}$ should include concurrent light and genetic data. Supplementary measurements of light level and PAR coordinated to microbialite surfaces where stable analyses were made can support the extent to which light is lost in the water column. Likewise, comparisons across study sites can quantify the magnitude of difference in light availability that is potentially affecting biosignature formation in different areas of the lake, especially at depths of 27 m on the western side where no photosynthetic biosignatures were detected. Correlating $\delta^{13}C_{carb}$ data with light measurements can better suggest a precedent of light availability that is sufficient for photosynthetic activity to shift DIC and create biosignatures. This would better support the suggested interface at 21 m depths where biosignatures are limited by balancing proportions of autotrophic and heterotrophic processes influencing biosignature formation. In addition, at depths below 21 m, variability in the spatial interface where biosignature were lost suggested that variable light incidence is influencing the extent of phototrophic abundance on these structures. Corresponding light data to

biosignature loss would indicate whether this is possible. While some genetic characterization has been performed in Pavilion Lake, collection of genetic data contemporaneous with carbonate sampling would also support population abundances in association with these $\delta^{13}C_{carb}$ transitions along microbialite surfaces.

3.2.2 Additional investigations into heterotrophic biosignatures

This thesis was the first undertaking that investigated the preservation potential of isotopic biosignatures in Pavilion Lake. While biosignatures of autotrophy were shown to disappear by hypothesized heterotrophic infilling below the ~ 0.5 to ~ 2 cm interface beneath the microbialite surface, the lack of a robust heterotrophic biosignature up to 5 cm beneath the surface may be attributed to insufficient heterotrophic activity depleting ^{13}C. Further research can consider characterizing $\delta^{13}C_{carb}$ values further down along the sampling transect in structures from deeper depths, where microbialite size tends to be much larger and heterotrophic activity is hypothesized to be more abundant. Sampling beyond 5 cm from the microbialite surface may represent $\delta^{13}C_{carb}$ values that have sufficiently been depleted by overprinting to indicate heterotrophic biosignatures. In addition, sampling transects that better correlate to the accretionary growth of microbialites can improve accuracy in interpreting $\delta^{13}C_{carb}$ beneath the microbialites surface. Minimizing the chance for enrichments to interfere with interpreting biosignature preservation can improve the accuracy in which trends of $\delta^{13}C_{carb}$ are expected to decrease. Further improvement on the experimental limitations outlined in this study may increase the likelihood of identifying a heterotrophic biosignature.

An additional microbialite morphotype at deeper depths that was not investigated in this study could also potentially source biosignatures of heterotrophy. At depths of ~ 55 m, microbialites similar to variants at ~ 30 m have been found to contain extremely dark-coloured

biofilms that exhibit large concentrations of manganese (Chan *et al.*, 2014; PLRP unpublished data). Although manganese cycling is currently unknown in Pavilion Lake, the added depth and decrease in PAR (Lim *et al.*, 2009; Brady *et al.*, 2014) may present an opportunity to investigate biosignatures of heterotrophy potentially associated with these microbialites.

3.2.3 Parallel investigations at nearby Kelly Lake

Nearby Kelly Lake is another example of a modern, analog microbialite system. Although smaller in size (1.6 km x 0.5 km, max depth 40 m), Kelly Lake is chemically similar to Pavilion in several aspects. It is a dimictic, ultra-oligotrophic freshwater lake with a mean pH of ~ 8.3 (Lim *et al.*, 2009) and is slightly supersaturated with respect o calcium carbonate (PLRP, unpublished data). Sedimentation rates are far greater in Kelly Lake, but $CaCO_3$ is a negligible constituent of the particulate material (Lim *et al.*, 2009). The microbialites in Kelly Lake are believed to have similar growth rates relative to Pavilion and there is evidence for new microbialite formation over the past ~ 450 years (Brady *et al.*, 2013). Isotopic enrichments indicating photosynthetic activity have been similarly identified in Kelly's microbialite carbonates (Ferris *et al.*, 1997; Soles, 2013), however no investigations into their preservation over time have been performed. Connecting the two lake systems would be useful in understanding the factors influencing microbialite growth within this region, and support whether similar controls for biosignature production and preservation in Pavilion are consistent in similar environments.

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APPENDIX

Table S1
Inorganic isotope compositions of surficial ("top") and basal ("bottom") components from green and purple nodules in Pavilion Lake.

10 m	THREE POLES				SOUTH BASIN EAST				SOUTH BASIN WEST			
	Green Nodule		Purple Nodule		Green Nodule		Purple Nodule		Green Nodule		Purple Nodule	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
δ^{13}C‰ PDB					3.7	3.1	2.4	3.2	2.8	3.6	3.7	3.4
δ^{18}O‰ PDB					-10.0	-9.5	-9.7	-9.4	-9.4	-8.7	-9.1	-9.5
18 m	Green Nodule		Purple Nodule		Green Nodule		Purple Nodule		Green Nodule		Purple Nodule	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
δ^{13}C‰ PDB	2.6	1.2	/	1.8	3.5	2.1	2.7	2.2	2.7	2.1	1.7	2.5
δ^{18}O‰ PDB	-9.4	-9.9	/	-9.6	-9.3	-9.3	-9.0	-9.3	-9.7	-9.3	-9.4	-9.5