Examining Archean Methanotrophy

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Abstract

The carbon isotope ratios preserved in sedimentary rocks can be used to fingerprint ancient metabolisms. Organic carbon in Late Archean samples stands out from that of other intervals with unusually low $\delta^{13}\text{C}$ values (~ -45 to -60 ‰). It was hypothesized that these light compositions record ecosystem-wide methane cycling and methanotrophy, either of the aerobic or anaerobic variety. To test this idea, we studied the petrography and carbon and oxygen isotope systematics of well-known and spectacular occurrences of shallow water stromatolites from the 2.72 Ga Tumbiana Formation of Western Australia. We examined the carbonate cements and kerogen produced within the stromatolites, because methanotrophy is expected to leave an isotopic fingerprint in these carbon reservoirs. Mathematical modeling of Archean carbonate chemistry further reveals that methanotrophy should still have a discernible signature preserved in the isotopic record, somewhat diminished from those observed in Phanerozoic sedimentary basins due to higher dissolved inorganic carbon concentrations. These stromatolites contain kerogen with $\delta^{13}\text{C}_{\text{org}}$ values of ~ -50 ‰. By microsampling different regions and textures within the stromatolites, we determined that the isotopic compositions of the authigenic calcite cements show a low degree of variation and are nearly identical to values estimated for seawater at this time; the lack of low and variable $\delta^{13}\text{C}_{\text{carb}}$ values implies that methanotrophy does not explain the low $\delta^{13}\text{C}_{\text{org}}$ seen in the coeval kerogen. These observations do not support a methanotrophy hypothesis, but instead hint that the Late Archean may constitute an interval wherein autotrophs employed markedly different biochemical processes of energy conservation and carbon fixation.

Keywords

Stromatolite, C isotopes, clumped isotopes, anaerobic oxidation of methane, reductive acetyl-CoA pathway

1. Introduction

It is widely recognized that organic matter contained in sedimentary successions of Late Archean age (circa 2.5 to 2.9 Ga) has unusually ^{13}C -depleted isotopic compositions ($\delta^{13}\text{C}_{\text{org}} \sim -45$ to -60‰ PDB) compared to younger intervals (Phanerozoic $\delta^{13}\text{C}_{\text{org}} \sim -22$ to -32‰ PDB) (Eigenbrode and Freeman, 2006; Fischer et al., 2009; Schoell and Wellmer, 1981; Thomazo et al., 2009). From its discovery in ultra-mature Late Archean kerogens from the Superior Province and the Tumbiana Formation of the Fortescue Group, Western Australia, it was hypothesized that this isotopic signal reflects the enhanced paleoenvironmental cycling of methane (Hayes, 1994; Schoell and Wellmer, 1981).

Oxidation and assimilation of methane by methanotrophic organisms yields exceptionally ^{13}C -depleted biomass. Recognizing this, Hayes (1994) introduced the concept of a Late Archean “Age of Global Methanotrophy”, to explain the $\delta^{13}\text{C}$ values seen in Archean organic matter. This was originally envisioned as aerobic methanotrophy (Hayes, 1994) but later based on two discoveries—microbial consortia capable of anaerobic oxidation of methane (AOM) in modern anoxic environments (Boetius et al., 2000) and the widespread mass independent fractionation of sulfur isotopes in Archean basins (Farquhar et al., 2000), Hinrichs (2002) updated the idea to include AOM—expanding on a point made similarly by Schoell and Wellmer (1981) on the basis of modern sedimentary pore fluid biogeochemical profiles. By comparing accumulation rates of methane oxidation from modern methane seeps, anoxic coastal sediment, and the Tumbiana Formation, Hinrichs (2002) showed that AOM processes could reasonably explain the Archean isotopic signal, possibly even under the condition of lower seawater sulfate concentrations. Subsequent studies developed biogeochemical variations on this theme again involving oxygenic photosynthesis or anoxygenic photosynthetic pathways, but these hypotheses all draw on abundant ecosystem methanotrophy in large part to explain the ^{13}C -depleted kerogen, with the disappearance of this isotopic signal in younger Precambrian strata due to either a decline in atmospheric methane or changes in the relative abundance of electron acceptors for methanotrophy (Coffey et al., 2013; Eigenbrode and Freeman, 2006; Stüeken et al., 2015; Thomazo et al., 2009; Yoshiya et al., 2012).

Despite its attractiveness, there are several outstanding issues with the global methanotrophy hypothesis that could benefit from further testing. 1) While substantially more

¹³C-depleted than expected by carboxylation from the Calvin cycle, the isotopic composition of Late Archean organic matter is not unique for methanotrophs and is consistent with other biochemical pathways (Blaser et al., 2013; Fischer et al., 2009; Gelwicks et al., 1989). 2) Sulfate levels may have been sufficiently low as to greatly limit the extent of sulfate reduction-driven AOM in Late Archean marine basins (Crowe et al., 2014), though methane oxidation might have been instead accomplished anaerobically via ferric iron phases (Beal et al., 2009) or high-valent nitrogen-bearing compounds (Haroon et al., 2013). Finally, 3) existing data from Archean carbonates with exceptionally low $\delta^{13}\text{C}_{\text{org}}$ values do not host isotopic and textural features one might expect of carbonates produced in environments of prevalent methanotrophy.

Fortunately, environments in which methanotrophy is a principal part of the carbon mass flux leave isotopic signatures not just in the organic phases present, but also in the carbonate phases produced; this forms the logic for an approach to test the global methanotrophy hypothesis. Both aerobic methanotrophy and AOM increase dissolved inorganic carbon (DIC) in the environment and strongly alter the carbon isotope ratios by adding isotopically light DIC. Furthermore, AOM also generates a strong flux of alkalinity and thus promotes the precipitation of carbonates as described by the following net reaction: $\text{CH}_4 + \text{SO}_4^{-2} + \text{Ca}^{+2} \rightarrow \text{CaCO}_3 + \text{H}_2\text{S} + \text{H}_2\text{O}$ (Michaelis et al., 2002). Authigenic carbonates formed by methanotrophic processes have been widely observed in modern marine sediments (e.g. Luff and Wallmann, 2003; Marlow et al., 2014) and can be readily preserved in the geological record where they have been recognized on the basis of isotopic and textural features in Phanerozoic strata, and perhaps as old as 635 Ma (Bristow and Grotzinger, 2013; Peckmann and Thiel, 2004). AOM creates both carbonate and organic matter with remarkably low $\delta^{13}\text{C}$ values (Bristow and Grotzinger, 2013)(Table S1). We compiled a comprehensive C isotope dataset from AOM sites preserved in Phanerozoic basins (Table S1); the data illustrate that $\delta^{13}\text{C}_{\text{carb}}$ values can be down to -59‰ and $\delta^{13}\text{C}_{\text{org}}$ values can be as low as -133‰, with typical $\delta^{13}\text{C}$ values both substantially more variable and much lower overall than Archean values.

To test the methanotrophy hypothesis, we closely examined the petrographic textures and isotopic compositions of kerogen and authigenic carbonate cements in stromatolites collected from the Late Archean Tumbiana Formation—an archive that has become an effective stratotype for ¹³C-depleted Archean organic matter (Eigenbrode and Freeman, 2006; Hayes, 1994; Thomazo et al., 2009). These rocks are well suited for this analysis because they contain both

kerogen and authigenic calcite cements preserved in stromatolitic laminations. We measured the carbonate cements that precipitated in the stromatolites at a finer texture-specific scale than has been done previously to look for any evidence of AOM, and combined traditional C and O isotope ratio measurements on the carbonate cements with clumped isotope analyses to evaluate the impacts of post-depositional processes on these materials. Finally we interpret the data in the context of a model of Archean carbonate chemistry, and discuss other potential metabolisms that might better explain the observations, such as the possibility that the ^{13}C -depleted Archean kerogens reflect an entirely different mode of biochemical carboxylation.

2. Geological background and methods

The Tumbiana Formation was deposited at 2721 ± 4 Ma during an interval of rifting and subsidence, and onlaps the Pilbara craton (Blake et al., 2004). It ranges in thickness from a few meters up to 320 m, and is lithologically diverse, consisting of conglomerates, shale, mudstones, siltstones, sandstones, breccias, basalts, tuff, and limestone (Thorne and Trendall, 2001). It is notably laterally variable containing many different facies that have been proposed to represent either lacustrine (Awramik and Buchheim, 2009; Buick, 1992; Coffey et al., 2013) or shallow marine settings (Sakurai et al., 2005; Thorne and Trendall, 2001); notably however, these facies all reflect shallow water paleoenvironments. This contrasts with typical methanotrophic environments both today and in Phanerozoic basins (typically slope/deep marine or within sedimentary pore fluids, (Campbell et al., 2002; Campbell, 2006; Marlow et al., 2014; Michaelis et al., 2002) making the highly ^{13}C -depleted kerogens in the Tumbiana Formation intriguing and unique.

Samples were collected from outcrops of the Meentheena Member of the Tumbiana Formation at the Redmont/Knossos Area, Western Australia ($22^{\circ}02'44''$ S, $118^{\circ}59'34''$ E) (Fig. 1). The Meentheena Member constitutes the uppermost sequence of the Tumbiana Formation, and is composed of a 30 to 50 m thick unit of interbedded carbonate (mainly limestone) and immature siliciclastic and volcanoclastic lithologies. The unit also contains spectacular occurrences of a diversity of well-preserved stromatolites (Awramik and Buchheim, 2009; Coffey et al., 2013; Flannery and Walter, 2012)—the lithologies targeted in this study. In the sampling locality, the exposure of the Meentheena Member outcrop is 11 m thick (Fig. S1). Here the Meentheena Member contains trough cross-stratified and wave-rippled sandstones and grainstones from coarse to very fine-grained, the latter of which become interbedded with stromatolites up section. Associated with mudcracks, these stromatolites developed in an

intertidal environment that deepens to dominantly subtidal facies upsection (Sakurai et al., 2005).

Outcrop samples were cut into slabs and polished to reveal textures and provide fresh surfaces for analysis. Petrographic thin sections were made from book-matched surfaces of the slabs sampled for isotopic analysis, and imaged using light and electron microscopy. Scanning electron microscopy (SEM) and X-ray dispersive spectroscopy (EDS) were performed using a combined ZEISS 1550VP Field Emission SEM and Oxford INCA Energy 300 X-ray dispersive spectrometer at Caltech.

Powders for carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopic analysis were collected using a micro-rotary drill with a 2 mm bit. Diverse textures (stromatolite with abundant grains, stromatolite with abundant cement, intercolumn sediment, across areas of differential recrystallization) were sampled in order to create a detailed cm-scale isotope ratio map of textures within these ancient microbial structures, and uncover the effects of diagenesis. Samples for organic $\delta^{13}\text{C}$ measurements were made on 5 to 10 g bulk stromatolite samples targeting organic-rich laminae, and excluding intercolumn sedimentary fill, cut and then powdered using a mortar and pestle.

Inorganic $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopic measurements were performed at the University of Michigan Stable Isotope Laboratory. Carbonate sample powders were placed in stainless steel combustion boats and baked under vacuum at 200°C to remove volatiles and water. Samples were then placed in borosilicate reaction vessels and reacted at 77°C with 4 drops of anhydrous phosphoric acid for 8 minutes in a Finnigan MAT Kiel IV preparation device coupled directly to the inlet of a Finnigan MAT 253 isotope ratio mass spectrometer. For 19 of the 50 samples, replicate splits were measured to assess accuracy and precision. Two NBS standards (NBS 18 and NBS 19) were run with the samples. Oxygen isotope ratio data were corrected for acid fractionation and source mixing by calibration to a best-fit regression line defined by standards. Analytical uncertainty is better than 0.1‰ for both carbon and oxygen isotopes. Data are reported in delta notation relative to VPDB standard.

For clumped isotope analysis at Caltech, sample powders were dried at 50°C. Five to ten milligrams of sample powder were dissolved in 90°C phosphoric acid for twenty minutes. The CO_2 was purified by gas chromatography in the method developed by Passey et al. (2010), and different isotopologues of carbon dioxide (cardinal masses 44 through 49) were measured on a modified Finnigan MAT253 gas-source mass spectrometer. Sample unknowns were analyzed for eight acquisitions in seven cycles, with each cycle bracketed by measurements of reference gas.

Raw values for Δ^{47} were calculated relative to the reference gas, and then converted to the absolute reference frame by Ghosh et al. (2006) for low-temperature samples (<55°C) and the absolute reference frame by Eiler et al. (2009) for higher temperature samples using the transfer function as in Dennis et al. (2011). From clumped isotope temperatures and oxygen isotope ratio data, we estimated the $\delta^{18}\text{O}$ of the water from which the carbonates precipitated using the relationship from Kim and O'Neil (1997).

For organic carbon analyses, sample powders were placed in tin combustion boats and decarbonated with hydrochloric acid. $\delta^{13}\text{C}_{\text{org}}$ values were measured at UC-Davis using a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer. The samples were combusted at 1020°C with chromium oxide and silvered cobaltous/cobaltic oxide catalysts. Following combustion, the helium carrier and analytes flowed through a water trap. Sample unknowns were calibrated against NIST standard reference materials and analytical uncertainty was better than 0.10‰. Data are reported in delta notation relative to VPDB.

3. Results

The Tumbiana stromatolites analyzed here are columnar laminated structures ranging in height from 4 cm to 18 cm. They are erect, non-branching with wide-intercolumn fill areas occurring in small clumps or within bioherms consistent with previous descriptions by Coffey et al. (2013) and Awramik and Buchheim (2009). Biological influence in the formation of these structures is suggested by laminae that systematically vary in thickness over the stromatolite apices, a criterion for biogenicity (Buick et al., 1981) (Fig. 2A). Additionally, at their edges, these laminae bend beyond the angle of repose and imply trapping and binding by some cohesive agent, such as microbial filaments (Frantz et al., 2015), with subsequent cementation. The laminae are composed of micritic calcite cements, varying in thickness from < 200 μm to 2 mm, alternating with thinner dark siliceous layers, with small amounts of residual kerogen. While some of the carbonate appears to have been recrystallized, this recrystallization varies in coarseness between layers (Fig. 2B, Fig. S2). Microscopy reveals a component of detrital material within the carbonate laminae, often in detrital layers filled with aluminosilicates, apatite, chlorite, and quartz (Fig. 2D, Fig. S2). The fill material deposited between stromatolites varies in composition between hand samples, but is predominantly siliciclastic sediment and carbonate

cement with more rare detrital carbonate grains (Fig. 2CD, Fig. S2). It is important to note that petrographic textures of calcite cements and kerogen in laminations both support the interpretation that these phases had authigenic and coeval origins in the stromatolites (Grotzinger and Knoll, 1999; Lepot et al., 2008). Additionally, the detailed textures of the Tumbiana stromatolites are distinct from Phanerozoic AOM seep carbonates, which are dominated by homogeneous and clotted microcrystalline carbonates, and rarer stromatolitic fabrics with complex and variable carbonate accretion orientations, botryoidal cements, irregular filled cavities, and *in situ* brecciation (Peckmann and Thiel, 2004). In modern methanic carbonates precipitated in anoxic bottom waters, large extensive structures of slabs, pavements, and upward growing chimneys and mounds are observed, but their internal structure is thrombolytic/clotted and distinct from the textures seen in Tumbiana stromatolites (Michaelis et al., 2002; Peckmann et al., 2001).

$\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{18}\text{O}$ measurements of the micritic cements reveal a narrow range of values, from -0.93‰ to 1.18‰ (PDB) and -19.00‰ to -14.20‰ (PDB), respectively (Table S2). The data are distributed tightly with a mean and standard deviation of $\delta^{13}\text{C}_{\text{carb}}$ $0.57 \pm 0.38\text{‰}$ (1σ) and $\delta^{18}\text{O}_{\text{carb}}$ $-18.40 \pm 0.89\text{‰}$ (Fig. 3A). Clumped isotope measurements reveal the impacts of both burial diagenetic and near surface weathering processes. Temperature estimates near the centroid of the $\delta^{13}\text{C}_{\text{carb}}$ distribution are $114 \pm 9^\circ\text{C}$. We interpret this as a diagenetic temperature, which is supported by the very low $\delta^{18}\text{O}$ values observed. The $\delta^{18}\text{O}$ composition of the water in equilibrium with the carbonates at these temperatures is $\sim -3\text{‰}$ VSMOW, consistent with recrystallization in the presence of ice-free marine and to a lesser degree relatively heavy meteoric pore fluids. In areas of the samples where near surface weathering has taken place (marked clearly by the oxidation of ferrous iron in pyrite and carbonate and resulting in the precipitation of secondary oxides, Fig. S3), the isotopic data show a trend with clumped temperature, causing $\delta^{18}\text{O}$ to increase and $\delta^{13}\text{C}_{\text{carb}}$ to drop (albeit very slightly $\sim 1\text{‰}$). From this we interpret that infiltration and exchange with heavier meteoric waters raised the $\delta^{18}\text{O}$ values, as average precipitation in Western Australia has $\delta^{18}\text{O}$ values from -4.07‰ to -6.44‰ VSMOW (Liu et al., 2010) and a mean annual temperature *ca.* 30°C . Meteoric waters infiltrating the surface rock or near subsurface would be close to ambient air temperature and thus both lower the temperature and increase the $\delta^{18}\text{O}$ values of the carbonate.

Our measured $\delta^{13}\text{C}_{\text{carb}}$ values conform with other carbonate isotopic measurements on the Tumbiana Formation throughout the basin in a variety of carbonate facies (e.g. Awramik and Buchheim, 2009; Coffey et al., 2013; Eigenbrode and Freeman, 2006; Thomazo et al., 2009; Yoshiya et al., 2012), and from Late Archean carbonate platforms more broadly (Fischer et al., 2009). When the $\delta^{13}\text{C}$ data are mapped back onto the sampled stromatolitic surfaces, little variation and no texture-specific pattern is visible among the values aside from the alteration trend discussed above due to surface weathering (Fig. 3). Notably no differences were observed between stromatolitic cements and detrital, intercolumn-filling carbonate. This lack of variation is highly distinct from expectations of carbonates precipitated in methanic environments, which show distinct isotopic signatures for different textures as well as wider variation (5-10‰) within textures at similar length scales (e.g. Campbell et al., 2002; Peckmann et al., 2001). The results show $\delta^{13}\text{C}_{\text{carb}}$ values with an isotopic composition very similar to that estimated for dissolved inorganic carbon in Late Archean seawater, not the variable and ^{13}C -depleted values observed in modern and ancient AOM environments (Table S1). While it remains possible that an unwavering mixture of isotopically distinct DIC pools could result in the same near seawater value, this scenario is unlikely based on the low degree of variation between fabrics, paleoenvironments (facies), and global comparisons.

$\delta^{13}\text{C}_{\text{org}}$ measurements show that the kerogen in the stromatolitic laminae is notably ^{13}C -depleted with values from -49.91‰ to -53.2‰ (PDB), matching well with other organic measurements made on Tumbiana Formation carbonate outcrops in the Redmont area (Coffey et al., 2013; Eigenbrode and Freeman, 2006; Stüeken et al., 2015; Yoshiya et al., 2012). It is important to note that at a bulk-scale sedimentary organic matter can integrate biological sources with distinct isotopic compositions (Freeman et al., 1990); bulk values need to be considered as possible mixtures accordingly. *In situ* ion microprobe measurements of kerogen from the Tumbiana Formation uncovered two populations of $\delta^{13}\text{C}_{\text{org}}$ values around -52.3‰ and -34.4‰, the former was interpreted as a signature of methanotrophy while the latter a signal of photoautotrophy (Williford et al., 2015). That our measured bulk $\delta^{13}\text{C}_{\text{org}}$ values reflect one of these populations suggests that these unusual ^{13}C -depleted values accurately describe the composition of a microbial community which makes up the majority of the kerogen in the sedimentary rock, and are not spurious values resulting from a mixture of biomass from different populations with isotopic compositions substantially different than bulk observations.

Importantly, these community values are substantially lower than those expected from carboxylation using Rubisco and the Calvin cycle (e.g. Guy et al., 1993), but not commonly as low as those observed in known methanotrophic biomass.

4. Results in the context of Archean carbonate chemistry

Although the $\delta^{13}\text{C}_{\text{carb}}$ values suggest methanotrophy was not a dominant metabolic process occurring in the Tumbiana stromatolites, it is important to consider this data in the context of the differing seawater carbonate chemistry that characterized the early Earth (Fischer et al., 2009; Grotzinger and Kasting, 1993; Hotinski et al., 2004). To alleviate the faint young sun paradox, it is thought that the early atmosphere contained a higher concentration of greenhouse gases, specifically CO_2 (Owen et al., 1979; Sagan and Mullen, 1972). The amount of CO_2 that existed in the Late Archean atmosphere is not well-constrained and model dependent; most efforts to quantify this found a lower limit of partial pressure of CO_2 at 0.03 atm with an upper limit at 5 atm (Grotzinger and Kasting, 1993). Seawater in equilibrium with the early atmosphere would have had higher amounts of dissolved inorganic carbonate species (DIC), and would tend to mute the metabolic signal of AOM in authigenic carbonates, all else kept equal (e.g. Bristow and Grotzinger, 2013).

To test this, we constructed a simple mathematical model that describes the carbonate chemistry and carbon isotope systematics of possible AOM environments for any concentration of atmospheric CO_2 building on similar logic to previous approaches (Bristow and Grotzinger, 2013; Fischer et al., 2009). To solve the carbonate system we used a range of pCO_2 and pH values and the Matlab solver developed by Zeebe and Wolf-Gladrow (2001). pH of Late Archean seawater is not known with certainty, but can be constrained to within limits. From basin analysis of carbonate platforms and carbon cycle theory, it appears that the fluid Earth throughout Precambrian time produced carbonates to alleviate fluxes of carbon and alkalinity in a fashion similar to today (Grotzinger and James, 2000; Higgins et al., 2009). Leveraging this carbonate mineral saturation and higher pCO_2 , it is likely that the pH of seawater was lower (Grotzinger and Kasting, 1993); a more recent model calculates for a pCO_2 of $10^5 \mu\text{atm}$, a pH of 6.9 (Halevy et al., 2010). For the Meentheena Member of the Tumbiana Formation, the basin history is not understood sufficiently well to solve the carbonate chemistry with a high degree of confidence—it could be marine (e.g. Sakurai et al., 2005) or lacustrine (e.g. Awramik and

Buchheim, 2009) in origin. However from lithological constraints, one can reasonably assume that CaCO_3 saturation states in this body of water were greater than unity, but were probably less than ~10 (Bergmann et al., 2013; Higgins et al., 2009). Because carbonate production was not tied to sources of alkalinity at the mouths of rivers that fed the Fortescue basin, it is also reasonable to assume calcium concentrations were in excess of carbonate ion concentrations (e.g. Higgins et al., 2009). This provides coarse constraints on the more likely solution space for DIC content of the basin waters, and a description of the order-of-magnitude uncertainty in the reconstruction (Fig. 4, Fig. S4).

To calculate the expected isotopic composition of authigenic carbonate, we used a closed box model that depends on a simple isotope mass balance between two pools: seawater DIC ($\delta^{13}\text{C} = 0\text{‰}$) and ^{13}C -depleted DIC metabolically derived from the oxidation of methane ($\delta^{13}\text{C} = -60\text{‰}$). This simple mixing relationship is observed in authigenic structures above the sediment in the modern anoxic environment of the Black Sea (Michaelis et al., 2002; Peckmann et al., 2001), as well as increasingly with depth in sediment pore-fluid AOM environments (Bristow and Grotzinger, 2013). Bracketing the ranges of $\delta^{13}\text{C}$ from modern AOM environments and assuming a similar relative metabolic fluxes of methane oxidation to organic matter production in Archean environments (Bristow and Grotzinger, 2013), calculations show that an Archean AOM environments should have $\delta^{13}\text{C}$ values between -2‰ and -30‰ (Fig. 4). This calculation assumes a degree of coupling between methane oxidation and DIC and organic carbon production. Although lower global productivity may have characterized the Archean biosphere, the assumption of similar metabolic fluxes only considers rates of AOM needed to produce enough biomass to leave the depleted $\delta^{13}\text{C}_{\text{org}}$ signature (Hinrichs, 2002); due to poor energetics, anaerobic methanotrophy has characteristically low growth yields and must oxidize substantial amounts of methane to obtain sufficient energy from this process for growth (~99:1; Thauer et al., 2008). Altogether, we observe that the typically ^{13}C -depleted AOM carbonate values should have been only somewhat muted by higher DIC (Fig. 4). Supporting the results of this calculation, we note that low and variable $\delta^{13}\text{C}$ values have been observed in authigenic carbonates in Archean strata. These are captured by early diagenetic siderite preserved in iron formation and ferruginous shales, and have been widely interpreted as the products of anaerobic microbial metabolism, likely dissimilatory iron reduction (Fischer et al., 2009; Johnson et al., 2013).

Thus despite their highly ^{13}C -depleted kerogens, Tumbiana stromatolites do not record $\delta^{13}\text{C}_{\text{carb}}$ values expected for AOM carbonates precipitated under conditions of Archean carbonate chemistry. This result suggests that AOM was not especially prevalent in this paleoenvironment, and that the microbial mats present during the development of these sedimentary structures were not comprised of large proportion of methanotrophs. While it remains possible that either or both aerobic and anaerobic methanotrophy were present as important metabolisms on the early Earth, it appears that neither was responsible for the striking isotopic signature seen in Tumbiana kerogens.

5. Alternative Carbon Fixation Pathways

If methanotrophy was not responsible for the notably low $\delta^{13}\text{C}_{\text{org}}$ values in the Tumbiana Formation, what are the alternatives? Under the somewhat delicate assumption that carbon isotope fractionations of biosynthetic pathways have not significantly changed since their inception, one reasonable and attractive possibility is the reductive acetyl Coenzyme A (CoA) pathway (sometimes called the Wood-Ljungdahl pathway), which a number of anaerobic microbes (including acetogens, methanogens, and sulfate reducers) use to synthesize acetyl-CoA from carbon dioxide (Fischer et al., 2009). This is the only known carbon fixation pathway that does carboxylation and at the same time generates ATP (Fuchs 2011), using a clever biochemical energy conservation scheme termed electron bifurcation (Martin, 2012; Nitschke and Russell, 2012; Thauer et al., 2008). Autotrophic acetogens using the reductive acetyl-CoA pathway produce acetate with large fractionations ($\epsilon = -38\text{‰}$ to -68‰) from substrate DIC, with a large kinetic isotope effect likely associated with the key enzyme of that pathway: carbon-monoxide dehydrogenase (Blaser et al., 2013; Gelwicks et al., 1989). The produced acetate can further fuel heterotrophic metabolisms and propagate this light isotopic signal in the ecosystem. Thus we hypothesize that the low $\delta^{13}\text{C}$ values of Tumbiana Formation kerogens—and perhaps those observed in Archean strata elsewhere (e.g. Fischer et al., 2009)—reflect the greater predominance of reductive acetyl-CoA pathway as an entry point for CO_2 into the biosphere. Interestingly, from theory it has been argued that the reductive acetyl-CoA pathway represents the most primitive form of carbon fixation known (Fuchs, 2011; Martin, 2012; Nitschke and Russell, 2012); our observations provide some geological support for this idea.

Finally, the average standard redox potentials for reactions in the reductive acetyl-CoA pathway are more reducing than all other known carboxylation pathways, and the key enzymes involved make extensive use of cofactors that are highly O₂-sensitive (Fuchs, 2011). Consequently this pathway was never evolutionarily adapted for aerobic metabolisms. If it is correct that the unique isotopic signature seen in Tumbiana Formation organic matter reflects carboxylation via this pathway, it suggests that O₂ levels in these shallow current-swept and well-lit paleoenvironments were sufficiently low to support significant populations of these anaerobic microorganisms.

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Figures

Fig. 1. General geological map of Tumbiana Formation exposures modified from Sakurai et al. (2005). The Redmont sampling locality is marked.

Fig. 2. (A) Image of TB2f stromatolite. Note the laminae thicken and thin across stromatolite apices. (B) Photomicrograph in transmitted cross-polarized light shows differential coarseness of recrystallization between laminae in TA1b. (C) Scanning electron image of the contact between stromatolite versus fill material in TA1b. (D) Scanning electron image of a layer of detrital sediment within intercolumn fill material in TB2b. Abbreviations for minerals identified using X-ray dispersive spectroscopy: Ab = Albite, Ap = Apatite, AS = Fe, Mg aluminosilicate, Cal = Calcite, Chl = Chlorite, Py = Pyrite, Qz = Quartz. Arrow shows up direction if image is rotated.

Fig. 3. Carbon, oxygen, and clumped isotope data from Tumbiana stromatolites. (A) Crossplot of carbon and oxygen isotope ratios from micromilled carbonate textures. Clumped isotope temperature estimates are shown in filled symbols, and record a trend with C and O isotopes in regions of near-surface weathering. Locations of analytical spots and mapped $\delta^{13}\text{C}_{\text{carb}}$ values for (B) TA1f, (C) TB2f, and (D) TB2b. Colorbar of isotopic values shown in (B) is the same for all samples. White stars indicate locations of clumped isotope spots sampled ~ 5 mm below the surface. Dashed white lines show area affected by meteoric water infiltration, delimited by presence of secondary iron oxides (Fig. S3).

Fig. 4. Estimates of the C isotopic composition of hypothetical AOM carbonate under conditions of Archean seawater carbonate chemistry. (A) Calculation of DIC in mmol/kg as a function of atmospheric CO₂ and seawater pH. Horizontal black line marks a lower limit of pCO₂ from Grotzinger and Kasting (1993). For reference, diagonal black curves mark calculated saturation states ($\Omega = 1$ and $\Omega = 10$) given modern [Ca²⁺]. Grey star shows modern seawater, and black star is a reasonable Archean seawater estimate using constraints from Halevy et al. (2010). (B) AOM carbonate signature with modern AOM region noted based off DIC and $\delta^{13}\text{C}_{\text{carb}}$ values and hypothetical Archean AOM carbonates using DIC estimates from (A).

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