

The age of Rubisco: the evolution of oxygenic photosynthesis

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ABSTRACT

The evolutionary history of oxygenesis is controversial. Form I of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) in oxygen-tolerant organisms both enables them to carry out oxygenic extraction of carbon from air and enables the competitive process of photorespiration. Carbon isotopic evidence is presented from ~2.9 Ga stromatolites from Steep Rock, Ontario, Canada, ~2.9 Ga stromatolites from Mushandike, Zimbabwe, and ~2.7 Ga stromatolites in the Belingwe belt, Zimbabwe. The data imply that in all three localities the reef-building autotrophs included organisms using Form I Rubisco. This inference, though not conclusive, is supported by other geochemical evidence that these stromatolites formed in oxic conditions. Collectively, the implication is that oxygenic photosynthesizers first appeared ~2.9 Ga ago, and were abundant 2.7–2.65 Ga ago.

Rubisco specificity (its preference for CO₂ over O₂) and compensation constraints (the limits on carbon fixation) may explain the paradox that despite the inferred evolution of oxygenesis 2.9 Ga ago, the Late Archaean air was anoxic. The atmospheric CO₂:O₂ ratio, and hence greenhouse warming, may reflect Form I Rubisco's specificity for CO₂ over O₂. The system may be bistable under the warming Sun, with liquid oceans occurring in either anoxic (H₂O with abundant CH₄ plus CO₂) or oxic (H₂O with more abundant CO₂, but little CH₄) greenhouse states. Transition between the two states would involve catastrophic remaking of the biosphere. Build-up of a very high atmospheric inventory of CO₂ in the 2.3 Ga glaciation may have allowed the atmosphere to move up the CO₂ compensation line to reach stability in an oxygen-rich system. Since then, Form I Rubisco specificity and consequent compensation limits may have maintained the long-term atmospheric disproportion between O₂ and CO₂, which is now close to both CO₂ and O₂ compensation barriers.

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INTRODUCTION

The history of the air is one of the Precambrian's great puzzles (Macgregor, 1927). How has air composition changed? Did oxygenic photosynthesis begin late, around 2.3 Ga ago (Kopp *et al.*, 2005)? Or was oxygen liberated by life much earlier, perhaps even as early as 3.8 Ga ago (Rosing & Frei, 2004)? Or is the answer to the puzzle 'somewhen' between these extremes?

The air is a biological construction. Of the major gases, dioxygen and CO₂ are cycled by oxygenic photosynthesis and respiration. The dinitrogen burden, which is responsible for the bulk of air pressure, is jointly managed by nitrifying and denitrifying bacteria and anammox planctomycetes, as well as by archaea (Capone & Knapp, 2007; Deutsch *et al.*, 2007). Nitrous oxide is also part of the biological nitrogen cycle.

Methane is produced by methanogenic archaea and (since the late Palaeozoic) by biomass burning. It is consumed by biological methane oxidation or destroyed by atmospheric OH. Of the major components, only volcanic carbon dioxide, volcanic nitrogen (offset by lightning fixation), geothermal methane (recycling organic debris), and the argon are abiotic. Water vapour is dependent on the biologically managed greenhouse gases (CO₂, CH₄ and N₂O).

The key catalyst, the Maxwell's demon of the atmosphere, is ribulose bisphosphate carboxylase/oxygenase, commonly termed Rubisco. The specificity of Rubisco (its preference for CO₂ over O₂) enables life to capture carbon from CO₂ in the environment. There are four major forms of Rubisco (Fig. 1) (e.g. see Hanson & Tabita, 2001; Ashida *et al.*, 2003, 2005). Form I Rubisco, the focus of this study, is today the main interlocutor between carbon in the air and carbon in organic matter. It is used by cyanobacteria: some cyanobacteria use

[†]deceased

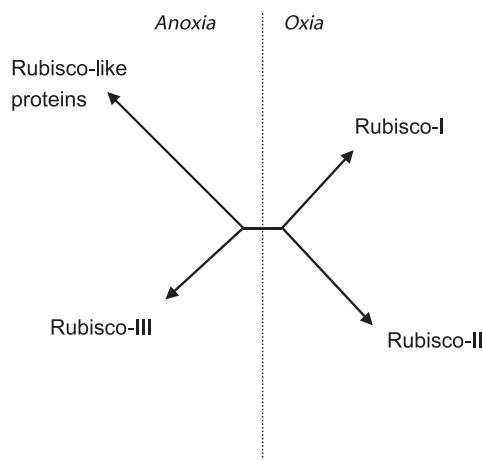


Fig. 1 The forms of Rubisco. Rubisco stems from an unknown root, diverging to four forms. Forms I and II are found in aerobic organisms (e.g. cyanobacteria, plants, eukaryotic algae, and purple bacteria), while Form III and Rubisco-like proteins (Form IV) are found in anaerobic prokaryotes.

Form I A Rubisco, while other cyanobacteria use Form 1B, as do green algae and higher plants; some proteobacteria use Form 1C, while other algae use Form 1D (e.g. see Badger & Price, 2003; Badger *et al.*, 2005). Cyanobacteria are very diverse. For example, *Prochlorococcus marinus*, the dominant photosynthetic organism in the modern ocean, occurs in two main forms, a strain adapted to high light levels in the upper part of the water column, which is typically nutrient-poor from competitive cell growth, and a strain living in the deeper (80–200 m) low-light zone, which is richer in nutrients (Dufresne *et al.*, 2003). This clade of cyanobacteria probably evolved in the Phanerozoic, but its diversity of habitats was probably occupied long previously by earlier cyanobacteria. Form I is also used by algae, plant chloroplasts, and many aerobic autotrophic bacteria, including anoxygenic photosynthetic proteobacteria that can grow aerobically in the dark but only grow photosynthetically in the light in the absence of oxygen (Badger & Price, 2003).

Form II Rubisco is present in many proteobacteria, both aerobic and anaerobic, though some aerobic anoxygenic bacteria grow faster in the light in the presence of oxygen but do not have Rubisco (Swingley *et al.*, 2007). Form II also occurs in peridinin-containing dinoflagellates (Morse *et al.*, 1995; see also Morden & Sherwood, 2002). The methanogenic archaea sequenced to date contain Form III Rubisco. They are anaerobes. However, it should be noted that Rubisco does not appear to play a major role in carbon fixation in methanogens. Form IV Rubisco-like-proteins (RLP) are mainly found in anaerobes, such as *Bacillus* and green sulphur bacteria. RLP are not strictly Rubisco, as they are not proven to have carboxylase and oxygenase functions.

Tcherkez *et al.* (2006) showed that in modern oxygenic photosynthetic organisms Form I Rubisco's specificity may be (in their words) 'nearly perfectly optimised' to this task

(Gutteridge & Pierce, 2006). This optimization may be very ancient. While the fine-tuning of the enzyme's specificity has almost certainly improved over the long aeons of geological time, even in a few million years (a geological snapshot) there are potentially billions of generations of cyanobacteria: natural selection pressure would suggest that evolutionary optimization may have occurred early. Thus, the uniformitarian assumption that the optimization of Form I Rubisco, and hence also its carbon isotopic signature, has not greatly changed over time, is defensible, though without evidence either way.

The history of Form I Rubisco must be tightly linked to the evolution of oxygenic photosynthesis, which it enables, and presumably followed a prior anoxygenic history (from which Form III Rubisco and RLP may have diverged) that may have been rooted in chemolithotrophs. The geological record of atmospheric oxygen is controversial. Although it is possible that local oxygen-rich conditions existed as early as >3.7 Ga ago (Rosing & Frei, 2004), and that hydrogen loss to space would have been a source of oxidation power (Catling *et al.*, 2001), there is strong evidence from the mass-independent fractionation (MIF) of sulphur isotopes that suggests the early Archaean atmosphere was anoxic (Farquhar *et al.*, 2000; Farquhar & Wing, 2003). MIF is created in the stratosphere (e.g. by the photolysis of SO₂) and can only survive if sulphur is not rehomogenized prior to entering the sedimentary record. For example, S₈ aerosol falling unoxidized to the seabed would preserve the MIF record. This means that MIF is unlikely to occur in an oxygen-rich atmosphere. However, special cases occur. Near the poles, the troposphere is thin and very cold, and there is evidence for MIF in modern volcanic-derived sulphates in polar ice, despite their descent to the ice cap through modern oxic air (Savarino *et al.*, 2003; Baroni *et al.*, 2006).

The MIF record suggests anoxia was maintained until about 3 Ga ago. Between 3 Ga and 2.45 Ga ago, the MIF record (Kasting & Ono, 2006) includes samples in which $\Delta^{33}\text{S}$ is near 0‰. Ohmoto *et al.* (2006) report the absence of MIF in samples from both 2.76 Ga and 2.92 Ga sediments. This suggests that these Late Archaean rocks preserve a hint of oxic conditions, at least locally, in which re-homogenization of MIF took place. One possible explanation (among several) is that a 'Yo-Yo' ocean existed (see discussion in Kasting, 2006; Knauth, 2006; Ohmoto *et al.*, 2006, and between Holland, 1999 and Ohmoto, 1997), in which the ocean/atmosphere system flipped between oxic and anoxic states during the Late Archaean. However, the wider record is of anoxic air until 2.45 Ga ago. The MIF record suggests the transition to oxygen-rich air was complete by 2 Ga ago (Farquhar & Wing, 2003).

These questions are more generally put as 'how did the oxidation state of the atmosphere evolve?' To answer the puzzle, evidence is available in Archaean geology, mainly from the geochemistry of sediments (Canfield, 2005). As instrumentation improves, new tools emerge. Just as the measurement of ³³S, mentioned above, brought deep new insight into the history of atmospheric oxygen (Farquhar & Wing, 2003), the study

of transition metal isotopes (Mo, Fe) offers new insight into the sedimentary record. In addition to the geological evidence, biomolecular palaeontology and molecular phylogeny both support sharp new attacks into the evolution of metabolism. The phylogenetic results need calibration against geological records, but may eventually allow Archaean ecologies to be mapped out.

Archaean sedimentary environments are the best places to search for answers (e.g. see discussion in Westall, 2005). Much attention has been given to investigation of organo-sedimentary deposits in the Archaean (Nisbet, 1987). Anoxygenic photosynthesis dates back prior to 3.4 Ga ago (Tice & Lowe, 2004; Grassineau *et al.*, 2006; Westall *et al.*, 2006). Among many other lines of evidence, the study of 3.2-Ga-old iron-rich carbonates in palaeosols shows that the mid-Archaean air had much more CO₂ than today (Hessler *et al.*, 2004). However, modelling implies a key factor in greenhouse warming was almost certainly methane (Kharecha *et al.*, 2005), and surface conditions may have been warm.

Carbon isotopic compositions in particular provide persistent signatures of life (Schidlowski, 1988; Hayes, 2004). In many cases Archaean carbon isotopic results have been taken to demonstrate that the material is the product of an oxygenic biosphere (e.g. Buick, 1992; Schidlowski & Aharon, 1992; Schidlowski, 2002). Isotopic evidence has generally been interpreted as suggesting a stepwise history of metabolic evolution, with oxygenesis reshaping the previously anoxic global environment during the Late Archaean and Proterozoic (Des Marais *et al.*, 1992; Canfield, 2005). Possible direct evidence for oxygenic photosynthesis is present in the later Archaean record (2.7–2.5 Ga), with biomolecular traces in bitumens from shales in the Pilbara, Western Australia, being interpreted as cyanobacterial (Brocks *et al.*, 2003).

Kopp *et al.* (2005) sharply challenged the consensus that oxygenic photosynthesis evolved in the Archaean, proposing instead the hypothesis that it began in the Palaeoproterozoic, triggering the glaciation at that time. In particular, they disputed the conclusions drawn from biomolecular evidence. To summarize their views briefly, they reject all identification of oxygenic cyanobacteria prior to 2.3 Ga, broadly dismissing the biomarker evidence on varied grounds (e.g. as contaminants, or as products of other microbial reactions). They prefer to date the first oxygenic photosynthesis at 2.3 Ga, associated with massive Mn deposits in South Africa and global glaciation. They argue that the older C isotopic evidence represents products of other metabolic pathways, not oxygenic photosynthesis.

The interpretation of the data is complex and controversial (see discussion by Canfield, 2005). Did oxygenesis only begin in the early Proterozoic (2.3 Ga ago) as inferred by Kopp *et al.* (2005)? Or were there organisms producing oxygen in the Archaean, but without increasing the atmospheric oxygen burden beyond trace levels? After 2 Ga ago there is agreement the atmosphere contained O₂. There is therefore general consensus that oxygenic photosynthesis occurred after 2.3 Ga,



Fig. 2 2.65 Ga stromatolites, Cheshire Fm., Belingwe, Zimbabwe. (Thanks to M.J. Bickle)

but the date when the first oxygenesis began remains hotly contested.

To address the dispute about the date of the first oxygenic photosynthesis (i.e. mediated by Photosystem II in conjunction with Form I Rubisco), we here return to detailed carbon isotopic studies in Archaean organosedimentary rocks. This work addresses these problems by searching for evidence of Form I Rubisco in unusually well-preserved late Archaean reefs. These chosen rocks (Figs 2 and 3) are of remarkably low metamorphic grade. Both organic carbon and carbonate are used to seek out fingerprints of Rubisco.

The first part of this study presents results from Late Archaean sedimentary successions. The most likely (though not unique) interpretation of our C isotopic results is that they are evidence for Rubisco 1 at ~2.9 Ga and ~2.7 Ga ago. If this is accepted, our results support the view that oxygenic photosynthesis operated in the Late Archaean. If in turn this conclusion is accepted, then the next puzzle is to explain the MIF and sedimentological evidence that suggests the air remained anoxic until the 2.3 Ga Great Oxidation Event in the Proterozoic (e.g. see Holland, 1999) even though oxygen had been produced for 600 Ma previously?

The second part of this study addresses the question of understanding the relationship between CH₄, CO₂ and O₂ in the ancient air. In general, the problem of understanding the history of atmospheric CO₂ and O₂ mixing ratios has mainly been seen in terms of tectonics and inorganic geochemistry (e.g. Lowe & Tice, 2007). We put forward the complementary hypothesis that the *bio*-chemistry of Form I Rubisco, including its CO₂: O₂ specificity, may also have played an important role in atmospheric evolution and, by managing the greenhouse and thereby stabilizing liquid oceans, may have influenced the geological evolution of the planet as a whole.

This study links biochemistry with geochemistry: to introduce biochemical readers to geochemical arguments, and vice versa, explanatory discussions are included.

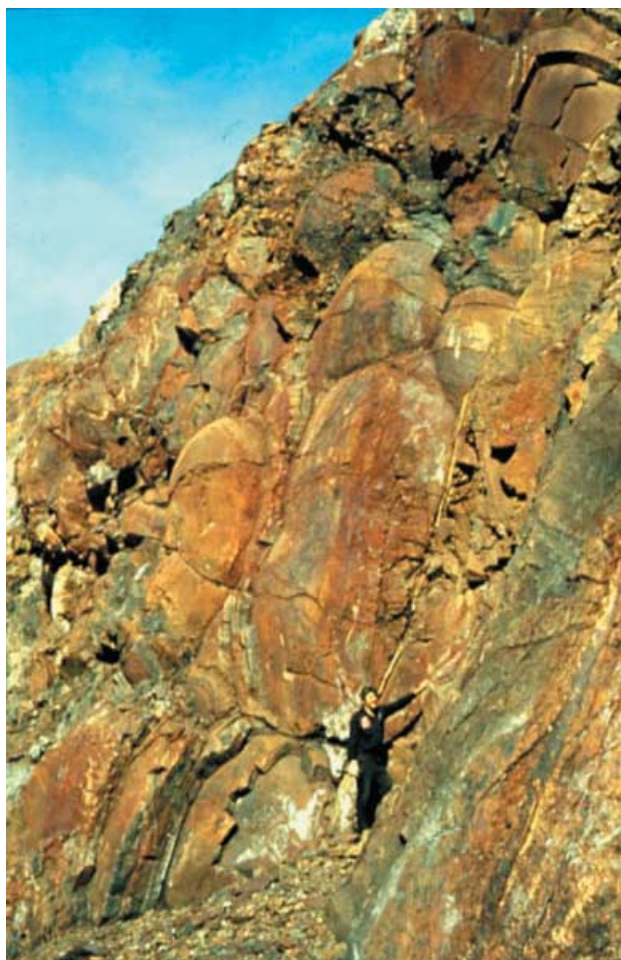


Fig. 3 ~2.9 Ga, stromatolites, Steep Rock, north-western Ontario, Canada.

Carbon capture by microbial organisms

Geologically, organic carbon that is preserved in the rock record consists of remnant debris, once biomass and oils, which is then (even in the best preserved material) further reprocessed by diagenesis (including biological activity) and burial metamorphism. Deciphering the isotopic record of the carbon is thus complex and depends on the history of the carbon.

Carbon from the mantle enters the surface carbon cycle by degassing from both subaerial and submarine volcanoes. CO_2 enters the atmosphere or is transported by ocean currents. Atmospheric CO_2 equilibrates with the ocean surface. Compared to geological timescales, this external CO_2 inventory is rapidly moved across the planet. In the water, carbon is present as bicarbonate and carbonate ions, dissolved CO_2 , and carbonic acid. In effect, from the viewpoint of sedimentary geochemistry, the CO_2 in both air and ocean acts as a single fairly well-mixed system.

The transfer of CO_2 from air to ocean is efficient (high yielding) as only a small isotopic fractionation is imposed. The carbonic anhydrase (CA) family of metalloenzymes, which

catalyse the reversible hydration of CO_2 to bicarbonate, may be significant in speeding this process, thereby permitting large blooms of phytoplankton. Carbonic anhydrase is extremely efficient and the CO_2 to HCO_3^- step has only a small isotopic effect on C. *In vitro*, the catalysed conversion of dissolved CO_2 to HCO_3^- has an α (rate constant with $^{12}\text{CO}_2$ /rate constant with $^{13}\text{CO}_2$) of 1.0001 (Raven, 1998). It is possible that modern plankton secrete CA into the uppermost surface of the ocean (Nimer *et al.*, 1999). This would enhance CO_2 intake, from the air by the water surface, and then next from the water to the cell, and finally facilitate the delivery of CO_2 to Rubisco.

Within the seawater, carbon has various fates. Some carbon is recaptured by alteration of lavas in the oceanic crust (Sleep & Zahnle, 2001; Bjerrum & Canfield, 2004) and thence subduction to the mantle. In the Archaean, this process may have been a significant part of the global carbon budget. In inter-pillow spaces in 3.5 Ga Pilbara basalts, hydrothermal carbonate occurs with $\delta^{13}\text{C}$ 0‰ (Nakamura & Kato, 2004), suggesting that between mid-Archaean surface water and deep ocean the carbon isotopic compositional gradient was small (Kharecha *et al.*, 2005) compared to the modern ocean with its zooplankton and faecal pellets.

Dissolved CO_2 in seawater is isotopically depleted in ^{13}C relative to dissolved inorganic carbon (DIC) by 8.2‰ at 30 °C and by 11.6‰ at 0 °C (Hayes *et al.*, 1999). The temperature effect is significant (around 0.12‰/°C). Organisms deriving C from DIC thus tap a source that is enriched in ^{13}C compared to seawater CO_2 . Conditions may have been very different in the Archaean, but the isotopic composition of atmospheric and hence seawater CO_2 ultimately depends on volcanic degassing from the mantle and then the sequestration of C into carbon and carbonate. Mantle evolution is slow and isotopic ratios in degassed CO_2 in the late Archaean may have been broadly similar to today. However, if the Archaean ocean temperature were very different from today, this would have had a strong isotopic effect on the DIC.

Both the capture of carbon from seawater into cyanobacterial cells and its progress within cells are typically mediated by CA. CA, which is one of the most widely distributed enzymes in nature, appears to be very ancient. It has several distinct forms that may independent evolutionary origins, and is found both in aerobic bacteria (e.g. So *et al.*, 2004) and in anaerobic archaea (Tripp *et al.*, 2004). Modern CAs use Zn^{2+} as the metal ion (accessible in oxic water), but Fe^{2+} is used in an anaerobic archaeon, in what may be a very old branching of CA (Tripp *et al.*, 2004) dating back to an early Archaean anaerobic ocean.

In the modern biosphere, most carbon capture by life is carried out with the involvement of one of the forms of Rubisco, mainly Form I Rubisco in oxygenic aerobes. But the transport of carbon from the ambient environment to the Rubisco is complex. In typical cyanobacteria, there are extremely effective carbon concentrating mechanisms, including both bicarbonate transporters and CO_2 uptake systems (Badger & Price, 2003; Badger *et al.*, 2005; Giordano *et al.*, 2005). These CA-dependent

systems introduce HCO_3^- to the cytosol of the cell, and then from there transfer it to the carboxysome, a microcompartment containing Rubisco in which elevated CO_2 is sequestered. Here, the carbon is fixed.

Following Laws *et al.* (1995), when carbon enters a cell, the isotopic discrimination factor of the cell is given by

$$\varepsilon_p = A + (B - A)C_i/C_e$$

where ε_p denotes the isotopic effect associated with primary production, C_i and C_e are, respectively, the internal (C_i) and external (C_e) concentrations of total CO_2 (i.e. $^{12}\text{CO}_2$ plus $^{13}\text{CO}_2$), A is the discrimination factor associated with diffusion of CO_2 into the cell and B the isotopic factor associated with CO_2 fixation within the cell, and ε_p is the isotopic discrimination factor of the cell defined by

$$\varepsilon_p = 1000 (\delta_e - \delta_p)/(1000 + \delta_p)$$

where δ_e is the $\delta^{13}\text{C}$ of the external CO_2 and δ_p that of the cell carbon. Assume that the growth rate μ of the cell is related to C_i and C_e by a relationship of the form

$$\mu = K_1 C_e - K_2 C_i$$

This implies that the gross transport of CO_2 into and out of the cell is proportional to the external and internal concentrations, and the growth rate is proportional to the net CO_2 transport into the cell. Then (after Laws *et al.*, 1995), substituting $(K_1 C_e - \mu)/K_2$ gives

$$\varepsilon_p = A + (B - A) (K_1 - \mu/C_e)/K_2$$

If the isotopic discrimination effects of respiration are small, then this implies that ε_p is in effect nearly a linear function of μ/C_e . Furthermore, in the limiting case if K_1 is nearly the same as K_2 , then as μ/C_e tends to zero (e.g. either high external CO_2 or low growth rate in a population of cells that are limited by other nutrients, or both), ε_p approaches B , the combined carboxylation fractionation of Rubisco. Thus in the limiting case, the key factor is B , which is mainly the Rubisco signature: this limiting case may be what is seen in the Archaean geological record if CO_2 were high and oxygen release by oxygenesis removed key metal ions by precipitation from previously anoxic water. If A is a more minor factor than B , then the geological record of carbon isotopes is to some extent independent of the evolutionary history of carbon concentrating mechanisms bringing CO_2 into the cell.

Identification of carbon isotopic fractionation by Form I Rubisco is central to understanding the isotopic record of oxygenesis. In conditions of abundant CO_2 , carbon isotopic fractionation by Form I Rubisco appears to be linearly correlated with CO_2/O_2 specificity (Tcherkez *et al.*, 2006). Organisms with highly CO_2 -specific Rubiscos show larger fractionation.

If the specificity (i.e. ratio in which CO_2 is selected over O_2) is over 150, then the carbon isotopic effect is around 30%. However, if specificity is below 10, then the effect drops towards 20%. This is at 25 °C. Specificity itself has an Arrhenius-type temperature dependency, with high CO_2 specificity at low temperatures (say 5 °C) and low specificity at, for example, 60 °C (Tcherkez *et al.*, 2006). Thus, isotopic fractionation and specificity are fine-tuned together by evolution, in association with delivery of CO_2 to the enzyme via CA.

Each CO_2 concentrating step has isotopic consequences. In seeking out traces of Archaean life by drawing parallels with modern equivalents, a valid approach is to search backwards in time for isotopic signatures comparable to those seen today, on the uniformitarian assumption that what is now taking place is similar to what was then occurring: that the present is at least an analogy with the past. Going backwards in time, there must have been a moment of change 'somewhen' before which the modern system did not operate, either because the Rubisco functioned differently in the past, or because the carbon-concentrating system was less evolved (e.g. with one or more of the steps being less efficient or absent), or from some combination of many factors that led to an evolutionary step-change. At that past moment, there should have been a discontinuity in the isotopic record. Finding that point, or at least constraining its time, is the purpose of this search.

Isotopic signatures of Rubisco forms in organic matter in aerobic and anaerobic settings

The uniformitarian approach depends on comparison with modern organisms that fix carbon. These have very variable selectivity for ^{12}C , depending on the biosynthetic pathway used, with consequently wide variation in overall isotopic effect ε , given by $\delta_{\text{product}'} = \delta_{\text{reactant}'} - \varepsilon$, where $\delta_{\text{product}'}$ and $\delta_{\text{reactant}'}$ are the instantaneous isotopic compositions of product and reactant, and ε is the isotopic effect associated with the reaction. This follows the approximation of Hayes (2001) – more precisely, $\varepsilon = 10^3(\alpha - 1)$, where $\alpha = [(\delta_{\text{reactant}'} + 1000)/(\delta_{\text{product}'} + 1000)]$. Rubisco in modern cells typically shows a strong isotopic discrimination in the range from –35‰ to –17‰ (Guy *et al.*, 1993; Goericke *et al.*, 1994; Robinson & Cavanaugh, 1995; Erez *et al.*, 1998), depending on conditions. Typical ranges between substrate and 'first stable product that incorporates the fixed carbon' have been tabulated according to enzymatic catalyst by Hayes (2001).

Form I Rubisco

Organisms that fix carbon by Form I Rubisco (e.g. cyanobacteria and chloroplasts) have strong selectivity for ^{12}C . Studying purified enzyme from spinach, which is probably widely representative for plants, Roeske & O'Leary (1984, 1985) and Guy *et al.* (1993) found ε roughly 30‰ at pH around 8 and 25 °C. For enzyme from a freshwater cyanobacterium (*Synechococcus* sp.), ε is ~22‰.

Biomass values are somewhat different from those in pure enzyme. As a broad generalization, ϵ for total organic carbon in phytoplankton using Form I Rubisco is about 20–25‰, with a maximum value of 25‰ (Hayes *et al.*, 1999; see for example Scott *et al.*, 2004). Modern oxygenic phytoplankton that use Form I Rubisco (cyanobacteria and eukaryotic algae) do so in aerobic conditions with abundant ambient CO₂. Dissolved CO₂ typically has δ CO₂ about -7‰ (see discussions below). This gives a maximum value of δ_{organic} of about -32‰. Note that the transfer of CO₂ from atmosphere to ocean or from ocean through the cell wall to HCO₃⁻ in the cell, assisted by CA, has only a small isotopic discrimination. Thus in the transfer of carbon from atmospheric CO₂ to the ocean as dissolved CO₂, and thence into the cell mass, the main isotopic effect is imposed in the cell by the Rubisco (factor B above), rather than selection during uptake and entry.

More generally, modern marine phytoplankton show a very wide range of $\delta^{13}\text{C}$ (Johnston & Kennedy, 1998). In closed system experiments in which CO_{2(aqueous)} is varied, $\delta^{13}\text{C}$ becomes lighter as CO₂ increases: presumably in situations where the CO₂ is more easily available, then organisms can be more selective and $\delta^{13}\text{C}$ tends to -28‰ at high CO_{2(aqueous)} (Johnston & Kennedy, 1998). However, availability of CO₂ will reflect competing biological activity in the water, not the atmospheric supply. Although the mid-Archaean atmosphere may have had high atmospheric CO₂, in the water CO_{2(aqueous)} would be taken up proximally to the organisms in bacterial blooms, so that the water would rapidly become depleted to the biological limit. Growth occurs to this local limit, whatever the distant supply of CO₂, and the carbon isotopes in the cells would reflect limiting conditions. Perhaps under CO₂-rich air, other nutrients such as P and accessible N were the main limiting factors.

Form II Rubisco

The light-capture mechanisms of anoxygenic photosynthetic organisms using Form II Rubisco (e.g. *Rhodospirillum rubrum*) may have evolved prior to oxygenic Form I Rubisco cells (Nisbet *et al.*, 1995). Thus if evidence for Form I of Rubisco is present in the Archaean record, evidence for Form II may also be found.

For those cells using Form II of Rubisco, the typical overall fractionation associated with fixation of inorganic carbon during autotrophic growth is probably lower than for Form I Rubiscos (Raven, 1998; Hayes, 2001), though there is wide variation and the database is small. The fractionation associated with Form II carbon fixation remains poorly known. In experiments using purified enzyme, Roeske & O'Leary (1985), using Form II Rubisco from the photosynthetic α -Proteobacterium *R. rubrum*, found a carbon isotopic shift of about 18‰ at 25 °C. This was supported by Guy *et al.* (1993), who reported a carbon isotopic discrimination of between -23 to -20‰ for *R. rubrum* rubisco. Experiments were at 25 °C with pH around 8.

In organisms, Form II Rubisco does indeed appear to be less selective for ¹²C than Form I, as the experiments imply. In chemoautotrophic symbioses in which the bacterial partner provided an internal source of carbon for the host, collected from the modern seabed (i.e. growing from modern ambient C sources, for example DIC between 0‰ and 5‰), Robinson & Cavanaugh (1995) recognized a group expressing Form I Rubisco in which $\delta^{13}\text{C}$ ranged from -35 to -27‰ (their '-30‰ group') and a likely Form II group (Robinson *et al.*, 1998) with $\delta^{13}\text{C}$ from -16‰ to -9‰ (their '-11‰ group') (Robinson & Cavanaugh, 1995). In Form II enzyme from vestimentarian tubeworm symbionts, Robinson *et al.* 2003 found ϵ to be 19.5‰ at 30 °C and pH ~7. However, the biomass of the vestimentarian had $\delta^{13}\text{C}$ between -16‰ and -9‰, substantially richer in ¹³C than would be expected. In part this enrichment may record the isotopic fingerprint of the Rubisco reaction when CO₂ exchange is slow and the accessible CO₂ is depleted: the organism becomes less selective.

The evidence presented above suggests that, generally, bacteria using Form II Rubisco may have a smaller carbon isotopic effect than Form I, but the data are very limited. The Form II group (-11‰) identified by Robinson *et al.*, 1998, 2003, and Robinson & Cavanaugh (1995) may reflect local conditions of CO₂ supply. However, the vestimentarian example is useful in the study of Archaean sediments laid down below the photic zone near proximal hydrothermal systems. In the deeper parts of microbial mats and the deeper oceanic photic zone, limitation of CO₂ supply occurs and a smaller carbon isotopic effect may in part occur because of prior competition by overlying Form I Rubisco cells. Within microbial mats, overlying cyanobacteria have first access to CO₂ drawn from the air-water interface. Isotopic fractionation is a consequence of partial uptake yield. If usable carbon is freely available, then the thermodynamically favoured isotope is selected. However, in conditions of shortage of usable CO₂, then a higher proportion of the accessible carbon is taken and the fractionation is consequently less. Hunger drives appetite: in conditions where supply is limited, cells may be less selective for the 'tasty' (thermodynamically advantageous) carbon. Thus, a shortage of available carbon (e.g. bicarbonate), which may particularly affect Form II Rubisco users deeper in a microbial mat, may force these bacteria to be less selective against the thermodynamically unfavoured ¹³C, which in turn will raise biomass $\delta^{13}\text{C}_{\text{reduced}}$.

In the natural record, though the -30‰ (Form I) and -11‰ (Form II) groups recognized in chemoautotrophs by Robinson & Cavanaugh (1995) are merely illustrative, they may typify what is preserved in the rocks. However, these groupings are broad generalizations that depend on ambient conditions. In detail, the pattern is complex. The -30‰ signature of modern cyanobacteria is also only illustrative: there is much variation. Moreover, as discussed above, these broad values relate to ambient modern conditions, and modern temperatures.

Forms III and IV

Among anaerobes that use Form III Rubisco or Form IV Rubisco-like-protein (note that in these the function of the Rubisco is poorly understood), the isotopic effect of carbon capture may vary widely. For organisms using the acetyl-CoA pathway and the enzyme formate dehydrogenase, ϵ is typically 15–36‰, while if carbon monoxide dehydrogenase is involved, ϵ is very large, around 52‰. Form III methanogenic archaea (acetyl Co-A pathway) sharply fractionate carbon (Hayes, 2004) and the methane emitted is characteristically very depleted in ^{13}C . This is especially marked if there is repeated carbon cycling in the biological community, so that methanogenesis further selects from already fractionated organic carbon. Thus, Archaean and Proterozoic organic carbon with C isotopes lighter (more negative) than $\delta^{13}\text{C}_{\text{reduced}} - 35\text{‰}$ is most probably interpreted as organic debris that incorporates at least some carbon from methane cycling (e.g. Hayes, 1994). However, it should be remembered that carbon in lipids from anammox planctomycetes can have $\delta^{13}\text{C}_{\text{lipids}} - 47\text{‰}$ (Schouten *et al.*, 2004).

In contrast, some bacteria (e.g. aquificales and desulphobacters) can have very low selectivity for carbon isotopes (Londry & Des Marais, 2003; Londry *et al.*, 2004). For cells fixing carbon by the reductive or reverse tricarboxylic acid (TCA) path ϵ is 4–13‰ (Hayes, 2001). Cells using the 3-hydroxypropionate cycle (e.g. *Chloroflexus aurantiacus*, a green nonsulphur bacterium) have $\epsilon \sim 0\text{‰}$ (but lipids may be highly fractionated).

The isotopic signature of Form I Rubisco is indirectly expressed in carbonate minerals

The isotopic relationship between dissolved CO_2 , DIC and carbonate mineral is discussed by Hayes *et al.* (1999). The isotopic depletion of dissolved CO_2 relative to sedimentary carbonate can be expressed in terms of $\Delta_{\text{carbonate}}$. This has two components: (i) the isotopic difference between CO_2 and DIC (discussed above: – DIC is about 8.2‰ richer in ^{13}C than CO_2 at 30 °C, a value that is temperature dependent, increasing by +0.12‰ per °C temperature increase), and (ii) the difference between DIC and carbonate minerals (which is relatively small and around 1.2‰, with carbonate more depleted than DIC). In total, $8.2 - 1.2 = 7\text{‰}$, for water at 30 °C, $\Delta_{\text{carbonate}}$ (i.e. $\delta^{13}\text{C}_{\text{carb}}$ around 0‰ minus $\delta^{13}\text{C}_{\text{CO}_2}$ around –7‰) is thus about 7‰.

The average isotopic fractionation between organic matter in sediment and coexisting carbonate mineral (Hayes *et al.*, 1999) can be expressed as ϵ_{TOC} (this is approximately $\delta^{13}\text{C}_{\text{carb}} - \delta^{13}\text{C}_{\text{reduced organic matter}}$), where $\epsilon_{\text{TOC}} = \Delta_{\text{carbonate}} + \epsilon_p - \Delta_2$.

Here the term ϵ_p denotes the isotopic effect associated with primary production (discussed above). The term Δ_2 is the isotopic shift associated with secondary biological processes. Where the shift is associated with respiratory remineralization, Δ_2 is typically small and positive, around 1.5‰ (Hayes *et al.*,

1999). In cases where secondary inputs occur from nonphotosynthetic organisms, Δ_2 can be negative by several ‰, as in the modern Black Sea deposits (see discussion in Hayes *et al.*, 1999): this anoxic parallel with significant chemoautotrophic input is clearly relevant in considering Archaean deposits.

On the modern (i.e. late Tertiary and Quaternary) Earth and throughout the Phanerozoic record, the association of carbonate (especially calcite) with $\delta^{13}\text{C}_{\text{carb}}$ around 0‰ and organic matter with $\delta^{13}\text{C}_{\text{reduced}}$ in the range from –30 to –25‰, when deposited in generally oxic conditions, is interpreted as the unambiguous record of an atmosphere/ocean system in which biological productivity is dominated by oxygenic photosynthesis (Form I Rubisco). Form I Rubisco's carbon isotopic effect then selectively extracts ^{12}C into organic matter with $\delta^{13}\text{C}_{\text{reduced}}$ around –30 to –25‰ in the modern biosphere. Carbonate precipitated in the same system is thus correspondingly enriched, more positive.

For the bulk of the Earth's limestones, throughout geological time, $\delta^{13}\text{C}_{\text{carb}}$ is roughly around 0‰ (Schidlowski, 1988). At first sight, this simply records that $\Delta_{\text{carbonate}}$ is $\sim 7\text{‰}$ (i.e. $\delta^{13}\text{C}_{\text{carb}}$ around 0‰ from $\delta^{13}\text{C}_{\text{CO}_2\text{seawater}}$ around –7‰) and hence is an artefact of $\delta^{13}\text{C}_{\text{CO}_2\text{seawater}}$, which in turn is a consequence of the $\delta^{13}\text{C}_{\text{CO}_2\text{atmosphere}}$ being around –8‰. However, the atmosphere/ocean $\delta^{13}\text{C}$ is itself determined by two factors – the volcanic carbon-gas input and the organic extraction of carbon. Life, by actively exploiting the kinetics of thermodynamic disequilibrium, takes precedence over passive inorganic extraction.

The bulk ratio of the carbon isotope inventory of the earth's mantle is unlikely to have changed markedly since the Late Archaean, and the operation of seafloor hydrothermal systems, including carbonate deposition, is also likely to have been steady. If so, the isotopic signature of the physical processes in the carbon cycle may have been broadly steady since that time. This assumption (which can be disputed, and is undoubtedly untrue in detail) then leaves the organic extraction of carbon and ambient temperature as the only major variables. The remnant inorganic carbonate is correspondingly enriched in ^{13}C (i.e. less negative $\delta^{13}\text{C}_{\text{reduced}}$) (Schidlowski, 1988, 2002). The organic extraction of carbon is balanced by the inorganic precipitation so that

$$\Phi_{\text{reduced}} \delta^{13}\text{C}_{\text{reduced}} + \Phi_{\text{carbonate}} \delta^{13}\text{C}_{\text{carbonate}} = \delta^{13}\text{C}_{\text{CO}_2\text{seawater}} \sim -7\text{‰}$$

(see Bjerrum & Canfield, 2004 for a more sophisticated treatment of this relationship), and Φ refers to fractional fates of the carbon supply.

If $\Delta_{\text{carbonate}}$ is $\sim 7\text{‰}$, then, from Laws *et al.* (1995),

$$\epsilon_{\text{TOC}} = \Delta_{\text{carbonate}} + \epsilon_p - \Delta_2 = 7 + \epsilon_p - 1.5 = \epsilon_p + 5.5 = \sim 30\text{‰}$$

assuming that ϵ_p is very roughly 25‰ (Goericke & Fry, 1994) for Rubisco-supported oxygenesis.

The two signatures of Form I Rubisco-supported autotrophy on a global scale capable of setting atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ are thus: (i) a difference between reduced carbon and carbonate minerals, $\epsilon_{\text{TOC}} \sim 30\%$, and (ii) $\delta^{13}\text{C}_{\text{carbonate}} \sim 0\%$, because $\Delta_{\text{carbonate}}$ is held around $\sim 7\%$, and $\delta^{13}\text{C}_{\text{CO}_2\text{seawater}} \sim -7\%$.

The second signature is not as robust as the first, as it includes far more assumption. Moreover, using this present parallel as a key to the interpretation of past Archaean material makes assumptions about productivity: only in a highly productive biosphere is the cycling of organic carbon on a global scale that is able to control C-isotopes in air and hence the bulk of carbon precipitating as carbonate. Nevertheless, the two isotopic signatures together provide a clear target to search for, while hunting for early evidence of oxygenesis.

Many modern and Phanerozoic stromatolitic strata, whose sedimentology implies they were laid down in broadly oxic (or at least nonreducing) settings, contain the association of C-isotopically heavy carbonates and C-isotopically light organic matter (with roughly 25–30% difference between $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{reduced}}$). The uniformitarian interpretation of such rocks is thus that they are powerfully indicative of a global system in which oxygenic photosynthesis (Form I Rubisco aerobes) dominates the partitioning of carbon. As far back in time that the double signatures can be found, the simplest interpretation is that they imply global-scale oxygenesis and temperatures broadly similar to the Phanerozoic planet. In uniformitarian logic, this should be the null hypothesis. However, in applying this interpretation to the Archaean, in the absence of plants, the burden of proof is higher.

This analysis is uniformitarian as it depends on the assumption of the coexistence of organic carbon and carbonate. Going back in time, 'somewhen' uniformity must break. Prior to the moment of the first occurrence of carbonate and hence the double signature, oxygenic life may or may not have existed, but if it did flourish, either it handled C differently (in the CO_2 concentration mechanism or in the specificity of the Rubisco, or atmospheric CO_2), or temperature was different.

THE GEOLOGICAL EVIDENCE

Sediments in the 2.7 Ga Ngezi Group, Belingwe belt, Zimbabwe

To identify autotrophic processes, we study the entire C isotopic facies: organic carbon, carbonate carbon, and sedimentological environment, placing the interpretation in the context of the depositional setting of the host rock.

The samples used here are chosen for their excellent preservation (Figs 2 and 3). In comparison to other rocks of similar age, they have experienced exceptionally low thermal and structural histories since deposition. They are from Belingwe, Zimbabwe (~ 2.65 to ~ 2.9 Ga) (Martin *et al.*, 1980; Abell *et al.*, 1985a; Grassineau *et al.*, 2001, 2002, 2006), Mushandike, Zimbabwe

(~ 2.9 Ga) (Abell *et al.*, 1985b; Moorbath *et al.*, 1987) and from Steep Rock, Ontario, Canada (~ 2.9 Ga) (Davis & Jackson, 1985; Wilks & Nisbet, 1985, 1988; Nisbet & Wilks, 1989; Stone, 2004). Parallel studies of S isotopes on much of this material have already been published (Grassineau *et al.*, 2001, 2002, 2006) or are in preparation.

Sediments in the Ngezi Group in the Belingwe belt were chosen to search for signatures of Rubisco. Shallow-water coastal sands, silts, shales, laminated limestones, and stromatolites occur in both the 2.7 Ga Manjeri Formation, and in the overlying ~ 2.65 -Ga-old (Bolhar *et al.*, 2002) Cheshire Fm. (Martin *et al.*, 1980; Grassineau *et al.*, 2002, 2006). In the Manjeri Fm. the stromatolites are a minor part of the succession, limited to a few small reefs a few tens of metres long and a few metres thick. The stromatolites are associated with rocks of shallow-water facies. Some thin (few m) carbonate beds with planar lamination of probable microbial origin also occur. In the Cheshire Fm. in contrast, the luxuriant stromatolites are superbly preserved in several locations as thick reefs. Reefs are hundreds of metres long and tens of metres thick, and extensive laminated carbonate beds also occur. The facies is shallow-water and at times evaporative (Martin *et al.*, 1980). Associated with the stromatolites are mud-cracked silts. The rocks studied from the Cheshire Fm. (Fig. 2) are unstrained and of very low metamorphic grade (Martin *et al.*, 1980; Abell *et al.*, 1985a,b). There is no evidence of input of fluids bearing organic chemicals processed by high temperature Fischer-Tropsch processes.

Rubisco's isotopic preference depends on temperature and pH (Roeske & O'Leary, 1984, 1985; Tcherkez *et al.*, 2006). Archaean sea temperature is controversial (e.g. debate between Shields & Kasting, 2007; Robert & Chausidon, 2006, 2007). Very warm seas (50 – 70 °C) would have very large hypercane storms. Though rip-up clasts and tempestites do occur in the Manjeri Fm. ironstones, they could have been the result of hurricanes associated with volcanic eruptions. Ambient Belingwe sedimentary environments may have been but were not necessarily stormier than in modern tropical seas. Considering the conditions needed for limestone precipitation (Grotzinger & Kasting, 1993), it is possible that seawater pH or alkalinity or both were likely not very greatly different from today's ocean. Thus, there are no reasons in the Belingwe sediments to expect conditions that would radically have altered Rubisco's isotopic preference.

Results of measurements are shown in Fig. 4 and Table 1.

In the Manjeri Fm. stromatolites, $\delta^{13}\text{C}_{\text{carb}}$ is $\sim 0\%$ and $\delta^{13}\text{C}_{\text{reduced}}$ has a wide isotopic spread with a marked abundance in samples with $\delta^{13}\text{C}_{\text{reduced}}$ around -23% (Fig. 4). 'Light' carbon isotopic values also occur in the Manjeri Fm. shales. Here a broadly bimodal distribution is visible, with long tails. The first population has $\delta^{13}\text{C}$ around -20% , the second peak, which is more numerous, is around -35% . In the shales, authigenic carbonate (formed within the mud) has $\delta^{13}\text{C}_{\text{carb}}$ around -15% to -5% .

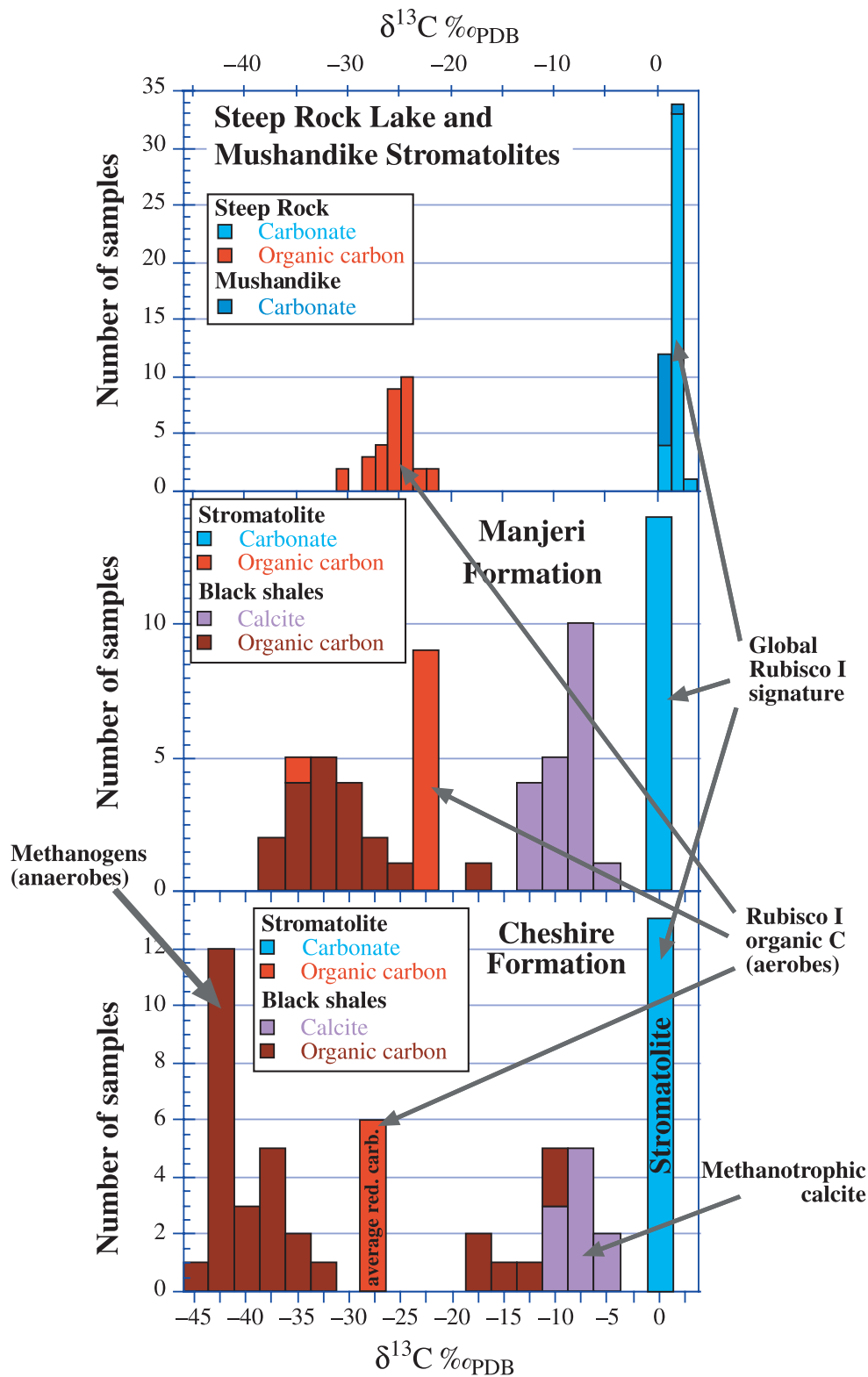


Fig. 4 C isotope measurements from the Steep Rock Lake stromatolites and from the Manjeri and Cheshire Fms. in the Belingwe greenstone belt. Data from Abell *et al.*, 1985a,b, Grassineau *et al.*, 2001, 2002, 2006 and NG unpublished. Organic carbon at -30% to -25% may show the signature of Form I Rubisco aerobic conditions with abundant CO_2 , while $\delta^{13}\text{C}$ -45% to -35% suggests carbon processed by methanogens. The $\delta^{13}\text{C}$ 0% stromatolitic carbonates, reflecting atmospheric input via ocean/air CO_2 exchange and extraction of a light carbon fraction, are interpreted as indirect evidence for oxygenic photosynthesis. One explanation of calcite $\delta^{13}\text{C}$ between -5% and -10% is that it derives from authigenic CO_2 released by methanotrophy.

Table 1 Stromatolitic successions: ages, $\delta^{13}\text{C}$, interpretation

Geological unit	Age of strata	$\delta^{13}\text{C}_{\text{carbonate}}$	$\delta^{13}\text{C}_{\text{reduced}}$	Possible interpretation
Pongola Supergroup (from Eglington <i>et al.</i> , 2003 and Ono <i>et al.</i> , 2006)	>2.84 Ga	Range +1.2 to +2.1‰, best estimate of original value +1‰	Two populations: -26‰ and -32‰	Calvin cycle
Steep Rock stromatolites	>2.83 Ga	+0.1 to +2.9‰ original value near 0‰?	-30.6 to -21.6‰	Calvin cycle
Mushandike stromatolites	>2.83 Ga	+0.1 to +1.3‰ original value near 0‰?		Calvin cycle
Manjeri Fm. stromatolites	2.7 Ga	-0‰	~-23‰	Calvin cycle
Manjeri Fm. shales	2.7 Ga	-15‰ to -5‰	-38‰ to -17‰ bimodal	Methanogens and ?methanotrophs
Cheshire Fm. stromatolites	2.65 Ga	+0.2 \pm 0.3‰	-28.6 \pm 3.3‰	Form I Rubisco
Cheshire shales	2.65 Ga	-15‰ to -5‰	-45‰ to -32‰	Methanogens and ?methanotrophs

In stromatolite samples from the Cheshire Fm. (Fig. 2), $\delta^{13}\text{C}_{\text{carb}}$ values are tightly clustered around $+0.2 \pm 0.3\text{‰}$ and $\delta^{13}\text{C}_{\text{reduced}}$ $-28.6 \pm 3.3\text{‰}$ (Fig. 4). The rocks show no sign of significant postdepositional isotopic resetting and the metamorphic grade is extremely low (Martin *et al.*, 1980; Abell *et al.*, 1985a). In shales of the Cheshire Fm., $\delta^{13}\text{C}_{\text{reduced}}$ ranges from -45‰ to -32‰ (Grassineau *et al.*, 2002, 2006) and many $\delta^{13}\text{C}_{\text{reduced}}$ values are lighter than -40‰ . In addition, a significant subpopulation of $\delta^{13}\text{C}_{\text{reduced}}$ ranges between -15‰ to -10‰ . Calcite in these shales has $\delta^{13}\text{C}_{\text{carb}}$ from -15‰ to -5‰ (Grassineau *et al.*, 2002, 2006).

2.9 Ga Mushandike (Zimbabwe) and Steep Rock (Canada) successions

Among the oldest large-scale still-carbonate stromatolite reefs extant in the geological record are those at Steep Rock NW Ontario, Canada (Wilks & Nisbet, 1985, 1988; Nisbet & Wilks, 1989) (Fig. 3), at Mushandike, Zimbabwe (Abell *et al.*, 1985b), and in strata of similar age in the Pongola belt, South Africa (von Brunn & Hobday, 1976; Gutzmer *et al.*, 1999; Eglington *et al.*, 2003). The Steep Rock and Mushandike stromatolites were sampled in this study but the Pongola material was not analysed in this work. All three sequences are >2.83 Ga old: the rounding of ~ 2.9 Ga, approximating the likely age, is used here for convenience.

Although there is substantial evidence for localized deposition of earlier (pre-3 Ga) sedimentary carbonates, they are typically lacustrine (Allwood *et al.*, 2007), silicified or otherwise replaced by secondary minerals. Unsilicified shallow-water calcic carbonates are largely absent in the pre-3 Ga geological record. Although pre-3 Ga carbonate with $\delta^{13}\text{C} \sim 0\text{‰}$ does occur (Schopf, 2006), it is extremely rare (see later below) and may occur in hydrothermal settings (Nakamura & Kato, 2004).

Mushandike succession

The Mushandike stromatolites are at least 2.83 Ga old (Moorbath *et al.*, 1987), and were probably laid down about 2.9 Ga ago. The Mushandike stromatolites are geographically not far from the Belingwe belt. They may be roughly contemporary with the

lower, ~ 2.9 Ga Mtshingwe Group succession in the Belingwe belt, which contains abundant diamictites (poorly sorted sediments with coarse clasts and fine matrix), including a major and well-developed boulder bed, and also coarse mass-flow breccias (Nisbet *et al.*, 1993).

Isotopic results from hand-held drill cores into the Mushandike carbonates (Fig. 4) (Abell *et al.*, 1985b) show that $\delta^{13}\text{C}_{\text{carb}}$ ranges from $+0.1\text{‰}$ to $+1.3\text{‰}$. The Mushandike calcites have undergone metamorphism and recrystallization, which probably shifted $\delta^{13}\text{C}_{\text{carb}}$ by 1‰ to 2‰ to heavier (more positive) ratios (Abell *et al.*, 1985b). This implies that original values were around 0‰ and similar to those in the Cheshire stromatolites (Abell *et al.*, 1985b).

Steep Rock succession

The Mosher Carbonates in the Steep Rock succession include a reef of widely varied stromatolitic dolomites and limestones (Fig. 3) (Wilks & Nisbet, 1985, 1988) which rests with a well-exposed unconformity on 3 Ga basement. The Mosher Carbonates are over 10 km long and in places hundreds of metres thick.

Coincidentally with the Mushandike stromatolites, and the Pongola sequence (below), the Steep Rock stromatolites are also at least 2.83 Ga old (Davis & Jackson, 1985; Stone, 2004). The Steep Rock stromatolites pass upwards into a unit known as the 'Manganiferous Paint Rock' (Wilks & Nisbet, 1988), which includes iron ores. The Paint Rock is typically about 4 wt% Mn (but locally up to 19 wt% Mn). However, the present oxidation state of the Paint Rock rock is postdepositional and ferruginous lateritic pisolites probably record post-Cretaceous termite-mediated alteration and bauxite formation (Wilks & Nisbet, 1988).

Isotopic results from the Steep Rock samples collected by the late PIA and EGN are shown in Fig. 4. Organic carbon has $\delta^{13}\text{C}_{\text{reduced}}$ averaging -25.4‰ , varying from -30.6 to -21.6‰ . Carbonate is slightly heavier than 0‰ , with $\delta^{13}\text{C}_{\text{carb}}$ ranging from $+0.1$ to $+2.9\text{‰}$ (Grassineau *et al.*, 2006). The carbonates were subject to low-grade metamorphism at 2.55 Ga (M. Regelous, unpublished). $\delta^{13}\text{C}_{\text{carb}}$ was likely slightly shifted in this event, as in the Mushandike rocks, and original $\delta^{13}\text{C}_{\text{carb}}$ values may have been close to 0‰ .

Pongola succession

Eglinton *et al.* (2003) and Veizer *et al.* (1990) both report carbon isotopes on carbonate samples (aragonite, calcite, ankerite, and dolomite) from the Pongola Supergroup, which is >2.84 Ga old (Gutzmer *et al.*, 1999). These rocks have been subject to some postdepositional decarbonation, shifting C isotopic ratios. In five samples studied by Eglinton *et al.* (2003) $\delta^{13}\text{C}_{\text{carb}}$ ranges from +1.2 to +2.1‰. They infer a best estimate of $\delta^{13}\text{C}_{\text{carb}}$ about 1‰ for marine carbonate at the time of deposition. Ono *et al.* (2006) studied organic matter in the Mozaan group and found two populations with $\delta^{13}\text{C}_{\text{reduced}}$ of -26‰ and -32‰. As in Steep Rock, some strata in the succession described by Eglinton *et al.* (2003) are Mn rich. Evidence for glaciation in the Pongola rocks is discussed below.

INTERPRETATION OF THE GEOLOGICAL EVIDENCE: DATING THE ORIGIN OF OXYGENESIS

2.7 Ga Ngezi Group: Manjeri and Cheshire Formations: carbon isotopes

The obvious explanation for the C isotopic results from the ~2.7 Ga Ngezi Group is that carbon was captured by photosynthesis, and then recycled by methanogens. This hypothesis is supported by several distinct lines of evidence.

1. Much of the carbon has $\delta^{13}\text{C}_{\text{reduced}}$ in the range from -30‰ to -20‰. This is the range likely to be produced by autotrophic capture by photosynthetic organisms using either Form I Rubisco or Form II Rubisco.
2. There is a separate distinct reduced facies, containing sulphides and clumps of organic carbon, that has $\delta^{13}\text{C}_{\text{reduced}}$ in the range from -40‰ to -30‰. This is most likely the signature of anaerobic methanogens.
3. In the stromatolites, there is a bimodal distribution of carbon, with $\delta^{13}\text{C}_{\text{carbonate}}$ around 0‰ and reduced carbon in the range $\delta^{13}\text{C}_{\text{reduced}}$ from -30‰ to -25‰.

The $\delta^{13}\text{C}$ contrast between carbonate and the coexisting organic matter is most simply interpreted as a record of carbon isotopic selection by organisms using the Calvin-Benson-Bassham cycle or the acetyl coA pathway. If it is assumed that in the Late Archaean the CO_2 degassed from volcanoes was similar to that degassed today, and $\delta^{13}\text{C}$ in CO_2 in ambient seawater was around -7‰, then the $\delta^{13}\text{C}_{\text{carb}}$ isotopic record is consistent with capture by life of say 15–25% of the well-mixed global inventory dissolved carbon in the water (but see also Bjerrum & Canfield, 2004).

The finding of a major peak in carbonate $\delta^{13}\text{C}_{\text{carb}}$ ~0‰ coexisting in the rock with an abundance of organic matter with $\delta^{13}\text{C}_{\text{reduced}}$ about -30 to -25‰ is, assuming uniformity, most simply interpreted as evidence for carbon capture by cyanobacteria using Form I Rubisco. These may have lived in association with anoxygenic Form II Rubisco proteobacteria in the aerobic or transiently aerobic upper part of microbial mats.

Deeper within microbial consortia, in the anoxic organic-rich bases of stromatolite-forming microbial mats, some very 'light' $\delta^{13}\text{C}_{\text{reduced}}$ values suggest that methanogens were probably also present (i.e. nonphotosynthetic Form III Rubisco organisms) (recall that Rubisco does not appear to play a major role in C fixation in methanogens). This interpretation depends on the validity of the uniformitarian parallel with the present-day analogy: though this assumption is unsupported, equally, there are no grounds to reject uniformity.

In the shales of the Cheshire Fm., the most likely explanation of the very depleted $\delta^{13}\text{C}_{\text{reduced}}$ (lighter than -40‰) is that carbon cycling occurred via methanogenesis (Hayes, 1994), further implying methanogenic archaea (Form III Rubisco) were present in the mud, which was rich in organic debris. It is possible that the isotopically light authigenic carbonate in the Cheshire shales formed from CO_2 produced within the sediment by oxidation of methane as it rose in the mud (i.e. methanotrophic precipitation), or from inorganic carbon released during the oxidation of organic matter produced by nonmethanogenic autotrophs.

In the Cheshire stromatolite reefs (Martin *et al.*, 1980) the absence of significant clastic content implies that reef-forming took place quickly, so that clastic input such as dust from unvegetated land or mud from floods was only a small part of the record. The size and purity of the reefs thus suggest the reef-building organisms were probably highly productive. This inferred fast growth is weak further support for the hypothesis that oxygenic photosynthesis was occurring, because it can be argued that only this process could have sustained the productivity needed. Other metabolic pathways such as anoxygenic photosynthesis would have been limited in the absence of oxygenesis, in a setting not directly proximal to active volcanism and with sulphate-poor ocean surface waters (Canfield *et al.*, 2000).

The proximity of oxic (stromatolites) and anoxic (mud) facies inferred in this interpretation is not improbable: in comparable Phanerozoic and modern lagoons and ponds, anoxic mud often occurs close to vigorous oxygenesis.

Independent evidence for oxygenesis in the ~2.7 Ga Ngezi Group rocks

The inference of oxygen-rich conditions in the Ngezi Group rocks is powerfully supported by independent geochemical evidence that the conditions were oxic (Siebert *et al.*, 2005). Mo concentrations in the Belingwe sediments vary but range up to 6 ppm. In the same material, $\delta^{98/95}\text{Mo}$ values are between -2.1‰ and -1.4‰. These values, more typical of post-Archaean to modern rocks than Archaean strata, indicate that Mo was present in solution. The presence of oxic water is typically necessary for soluble Mo. This in turn strongly implies that the air was sufficiently oxidizing to mobilize Mo during terrestrial weathering and that water conditions were oxidizing (at least locally in Belingwe lagoons) (Siebert *et al.*, 2005). Given that

nitrogenase is an FeMo metalloprotein and thus needs an Mo supply, this raises the interesting speculation that nitrogen fixation may have evolved by this time, contributing to the enhanced productivity (Zerkle *et al.*, 2006) (though paradoxically, nitrogenase is very oxygen sensitive and needs biological compartmentation).

There is a caveat to this inference of oxic conditions from the Mo record: the evidence is not absolute as there are other ways to mobilize reduced Mo in putative Archaean oceans. However, Fe isotopic results also support the presence of oxygen-rich surface waters in the Ngezi Group Rocks (Archer & Vance, 2006). Taken collectively, the C, Mo and Fe isotopic case for oxic water becomes very strong.

The Cheshire Fm. stromatolites are similar in age to the Carawine Dolomite in Australia. In these ~2.6 Ga rocks, Ono *et al.* (2003) found a small $\Delta^{33}\text{S}$ signal, ranging from -2.5% to -1.1% , consistent either with some oxygen emission to air at this time, or with local conditions (e.g. oxygen-rich water) that rehomogenized sulphur (such as S_8 particles) before it was incorporated in the sediment. In contrast, the smaller, less abundant Manjeri Fm. stromatolites may be of similar age to the Jeerinah Fm. reported by Ono *et al.* (2003), in which $\Delta^{33}\text{S}$ ranges from -0.1% to $+8.1\%$, suggesting oscillation between oxic and anoxic conditions. Ohmoto *et al.* (2006) similarly reported the absence of MIF in 2.76 Ga lake sediments in the Pilbara, Australia.

Interpretation of the $\Delta^{33}\text{S}$ signal comes with a caveat: explanations of MIF other than anoxia are possible. The period around 2.7 Ga ago was a time of major global volcanic activity, attested by the thick sequences of lavas worldwide (including those in the Ngezi Group). On the modern Earth, despite 21% O_2 in the air, MIF is observed in volcanic ash deposited in the polar ice (Savarino *et al.*, 2003; Baroni *et al.*, 2006). It is thus possible that some MIF signature seen in 2.6–2.7 Ga strata similarly record volcanic sulphide deposited after massive volcanic eruptions that put a global S load into the stratosphere. While the hypothesis of a ‘Yo-Yo’ record of oscillation between oxic and anoxic conditions is attractive, the small $\Delta^{33}\text{S}$ signal that is contemporary with the stromatolites may not record atmospheric oscillation. It may simply be a record of massive volcanic events that pierced the stratosphere of a uniformly oxic atmosphere and then fell out in quantities so large that the re-homogenization processes were overwhelmed. That said, the MIF record at around 2.7 Ga is – to use the weak positive support of a double negative – ‘not inconsistent’ with the hypothesis that oxygenation had evolved.

If oxygen were present in the water, sulphate would also have been formed, though sulphate abundance would likely be kept low by abundant sulphate-reducers. Grassineau *et al.* (2002, 2006, unpublished) found very wide ranges in $\delta^{34}\text{S}$ in adjacent pyrite samples in the Manjeri Fm. In one Manjeri Fm. drill-core sample from relatively shallow facies, $\delta^{34}\text{S}$ ranged within a few centimetres from -18.3% to -3% , while in a deep-water sapropel sample, in a highly anaerobic facies from the same forma-

tion, $\delta^{34}\text{S}$ ranged from $+7.4\%$ to $+16.9\%$ within millimetres. Overall in the Ngezi Group, $\delta^{34}\text{S}$ ranges by 40‰, from -21% to $+17\%$ (Grassineau *et al.*, 2002, 2006).

A small part of this $\delta^{34}\text{S}$ signal may come from MIF processes, via disproportionation in water of the S_8 allotrope of sulphur, after it had fallen from the air as a settling aerosol. Applying Ono *et al.*'s (2003) formulation $(\Delta^{33}\text{S} - 0.51)/0.64 \sim \delta^{34}\text{S}$ to the $\Delta^{33}\text{S}$ range from the Carawine Dolomite gives a range of impact on $\delta^{34}\text{S}$ of between about 4‰ and 1‰, small in relation to the 40‰ range observed. The bulk of the $\delta^{34}\text{S}$ signal is likely to be from microbial processes (Ono *et al.*, 2003; for discussion of older sulphur record see Ohmoto *et al.*, 1993, 2001). In the Belingwe rocks, most probably the large $\delta^{34}\text{S}$ signal records biological cycling by sulphurate in shallow water.

Grassineau *et al.* (2001, 2002, 2006) inferred the presence in shallow water of proteobacteria, probably including both aerobes and photosynthetic anaerobes (Form I or Form II Rubisco). In stringers of highly anoxic facies, sulphides associated with carbon clumps in shallow anoxic facies may record recycling by anoxygenic photosynthesis (e.g. anaerobic green S bacteria using Form IV Rubisco-like protein). Reduction of S species must have been widespread in both shallow- and deep-water facies, especially in highly anoxic deeper-water facies in close proximity to highly depleted carbon clumps. The highly positive $\delta^{34}\text{S}$ in sulphides in deep-water anoxic facies may record anaerobic methane oxidation by consortia of archaea and sulphate-reducing bacteria, exploiting oxidation power provided by fluids fluxing from underlying oxidized iron-containing clastics of the upper Manjeri Fm., as in the modern Black Sea (Jorgensen *et al.*, 2004). If so, methanogens were present in the deeper parts of the debris pile.

Thus, the uniformitarian interpretation of the Ngezi Group sedimentary facies and C isotopic signatures (Fig. 4), with their markedly distinct organic and carbonate $\delta^{13}\text{C}$, and abundant other evidence of aerobic conditions (e.g. Mo, Fe isotopes), is that they are a record of ~2.7 Ga microbial consortia that founded on autotrophic productivity by oxygenic cells using Form I Rubisco.

2.9 Ga evidence for oxygenesis

The Steep Rock, Mushandike and Pongola stromatolites, all coincidentally dated as >2.83 or >2.84 Ga, are among Earth's oldest large-scale carbonate reefs.

In the three successions, results show that $\delta^{13}\text{C}_{\text{reduced}}$ is around -25% , and $\delta^{13}\text{C}_{\text{carb}}$ around $+1.5\%$. In all three, the carbonates may have been subject to a slight postdepositional metamorphic resetting (Abell *et al.*, 1985b; Eglington *et al.*, 2003) shifting $\delta^{13}\text{C}$ by a few ‰. As with the younger Ngezi Group samples, the results, together with the characteristic difference, 27‰, between $\delta^{13}\text{C}$ in organic matter and carbonate suggest, though do not prove, that processes controlling carbon isotopic partitioning were oxygenic.

The carbon isotopic evidence (Fig. 4; Table 1) and the close similarity of the sedimentary facies to the Belingwe material mean that if oxygenesis is accepted for the Cheshire reef, then the same uniformitarian criteria also imply that the Steep Rock and Mushandike reefs were built by organisms that used Form I Rubisco – in other words, an oxygenic ecology based on cyanobacteria.

As with the Cheshire Fm stromatolites, the hypothesis of oxygenic photosynthesis at >2.83 Ga is consistent with the kilometre-scale and thickness of the Steep Rock carbonate reef, which is poor in clastic material despite resting unconformably on granitic craton (Wilks & Nisbet, 1985, 1988) and thus deposited proximally to a dusty landmass. The size, thickness and facies of the reef imply a highly productive ecology, unless the deposition period was very prolonged and winds soft.

Supporting evidence for ~2.9 Ga oxygenesis: (a) glaciation and (b) $\Delta^{33}\text{S}$ MIF record

Glaciation

In the ~2.9 Ga Mozaan Group, the upper part of the Pongola Supergroup, South Africa, Young *et al.* (1998) reported diamictites, with convincing evidence for glaciation. Rocks of possible glacial origin are also found in the 2.9 Ga Witwatersrand succession (Harland, 1981). In the Belingwe belt, the ~2.9 Ga Mtshingwe Group contains very extensive coarse mass flow breccias and a large boulder bed (Nisbet *et al.*, 1993). These are of uncertain origin – either coastal in a region of active tectonics, or glacial, or both (Nisbet *et al.*, 1993). Although *not* our preferred hypothesis in the absence of observation of dropstones, these Mtshingwe Group diamictites may indeed record ~2.9 Ga glacial environments.

Evidence for glaciation in ~2.9 Ga rocks is interesting in the context of the hypothesis that oxygenic photosynthesis began then. When oxygenic cyanobacteria first evolved, the release of free oxygen would have challenged the methane budget. Within the sediment, the increase in sulphate supply would promote anaerobic methane oxidation by consortia of archaea and sulphate-reducing bacteria, and the increase in oxidative capacity would also promote methane uptake by sediment-hosted methanotrophic bacteria. In the air, the new oxygen would reduce the atmospheric methane inventory by increasing the overall oxidative capacity of the atmosphere. Abundant newly released oxygen would have shortened the atmospheric lifetime of emitted methane, speeding conversion to CO_2 .

Methane is a potent greenhouse gas, destroyed by atmospheric OH (which is produced by water photolysis). The reduction in greenhouse warming would have induced climate cooling. However, note that the global productivity of the biosphere would have increased sharply when oxygenesis evolved, increasing the amount of reduced organic debris in sediment and thus increasing *in situ* methane production (Catling *et al.*, 2001; Kharecha *et al.*, 2005). Methane produced in sediment is not necessarily immediately emitted:

much may collect and be stored for tens or hundreds of millions of years in sediment-hosted gas and clathrate. The trade-off between the evolution of oxygenesis and methane production (much of which may have been buried in sediment), methane emission, methane atmospheric lifetime, and atmospheric burden is complex and not easily constrained; the impact on greenhouse warming is even more uncertain.

The evidence for ~2.9 Ga glaciation does suggest that the global greenhouse was challenged at this time. Though many factors can induce global cooling, the likeliest challenge to the greenhouse would be elimination of the atmospheric methane burden to trace levels by the combined impact of increased anaerobic methane oxidation, methanotrophy, and increased oxidative capacity of the atmosphere, following the evolution of oxygenic photosynthesis.

$\Delta^{33}\text{S}$ MIF record

Direct supporting MIF isotopic evidence for oxic geochemistry in the ~2.9 Ga rocks at Steep Rock and Mushandike is not available. However, though the Steep Rock Mn-rich deposits may have been reworked in the late Phanerozoic, original Mn deposition was probably in the Archaean, consistent with local presence of upwellings of anoxic water into oxygenated photic regions.

In Pongola sulphides, $\Delta^{33}\text{S}$ is near 0‰, suggesting weak or no mass-independent fractionation of S isotopes (Ono *et al.*, 2006). The MIF record from the Mozaan group supports the notion that oxygen was being produced in large enough amounts to effect global S isotope homogenization. The $\Delta^{33}\text{S}$ values of nine samples range from -0.5‰ to +0.4‰ (Ono *et al.*, 2006). This range is much smaller than measured in much of the other Archaean record, though it is outside the narrow Phanerozoic and modern range. Ono *et al.* (2006) concluded that the atmosphere above the Mozaan Group sediments at the time they were laid down was slightly oxidized, with oxygen levels above 10^{-5} but below 10^{-2} of present atmospheric level. From this evidence Ono *et al.* (2006) inferred the existence of oxygenic photosynthesis at 2.9 Ga. Siebert *et al.* (2005), in a ~2.95 Ga sample from the West Rand Group of the Witwatersrand, also found 'a hint of an incipient, possibly transient rise in oxygen levels'. This interpretation is also supported by the finding by Ohmoto *et al.* (2006) that MIF is absent in 2.92 Ga marine shales in the Pilbara, Australia.

Thus the ~2.9 Ga MIF data and evidence for glacial sediments, implying significant atmospheric oxygen and thus an attack on the global methane burden, are supportive of the hypothesis that this was the time oxygenesis began. Zahnle *et al.* (2006) showed that the disappearance of a strong MIF signal is more connected with the collapse of atmospheric methane than directly with the failure of volcanism or rise of oxygen. They attribute the decline in methane to the growth in the oceanic sulphate pool. Atmospheric systems are strongly nonlinear, and have great inheritance: a major preexisting atmospheric methane inventory would be difficult to challenge by new oxygenesis, especially as that oxygenesis would

equally increase productivity and hence the supply of moles of reduced organic matter to methanogens.

Absence of evidence for large-scale carbonates in older (pre-2.9 Ga) rocks

Very few pre-2.9 Ga carbonates occur in the record, and those that do are on an extremely small scale. Though stromatolites older than 3 Ga do occur in Barberton and the Pilbara (e.g. Buick *et al.*, 1981; see more recent discussion of evidence in Allwood *et al.*, 2006, 2007, also see Schopf, 2006), carbonates are typically silicified. When carbonate is present, it is characteristically ankeritic or iron rich. Limestones are now absent although some deposits, now dolomitized, may originally have been aragonitic. In contrast to the >10-km-long Steep Rock reef, the older stromatolites prior to 2.9 Ga are on a much smaller scale. Whether these older stromatolites are of organic origin is controversial (Lowe, 1994; Allwood *et al.*, 2006, 2007).

The carbonates in the 3.45 Ga Strelley Pool Chert, Pilbara, Australia (Allwood *et al.*, 2006, 2007), are among the best developed pre-3 Ga examples. The outcrop is extensive – this was a widespread, long-lived stromatolite reef including significant carbonate. They were probably lacustrine (Allwood *et al.*, 2007; see also Buick, 1992). The setting was anoxic (Van Kranendonk *et al.*, 2003). In part the deposits may record special local hydrothermal settings, but some sediments have little hydrothermal input. Silicified stromatolites and carbonates deposited between 3.4 Ga and 3.2 Ga ago also occur in the Barberton belt (Byerly *et al.*, 1986; Toulkerides *et al.*, 1998; see also Westall *et al.*, 2006).

It has been strongly argued that the Strelley Pool stromatolites are indeed biogenic (see Allwood *et al.*, 2007, and Van Kranendonk *et al.*, 2003 and references cited therein for discussion of this point), raising the possibility that the biocalcification may have been cyanobacterial (Kazmierczak & Altermann, 2002). In local high pH circumstances, even though the global ocean may have been acid under a CO₂-rich atmosphere, biogenic carbonate deposition may have been made possible by the local alkalinity: possibly, the Strelley Pool setting was like this. One possibility thus is that the 3.4 Ga stromatolites were formed in anoxic alkaline lake waters by anoxygenic photosynthesizers. These may have included anoxygenic cyanobacteria.

Isotopically, the pre-2.9 Ga carbon record is thin. Schopf (2006) summarizes the evidence (his fig. 6) from pre-3 Ga rocks in the Pilbara and Barberton. Typically, $\delta^{13}\text{C}_{\text{reduced}}$ is $-25 \pm 10\%$, similar to the measurements in the later rocks and perhaps recording either or both oxygenic (Form I Rubisco) or anoxygenic (Form II Rubisco) photosynthesis. The carbonate also has a value comparable to the later rocks: $\delta^{13}\text{C}_{\text{carbonate}}$ is around 0‰ (e.g. Nakamura & Kato, 2004). Note the singular word 'value' – the sample size is typically one. Preserved carbonate is very rare, small scale, not typically primary syn-sedimentary aragonite or calcite, and can be characteristically of hydrothermal association. The rocks are not from extant large limestone reefs.

The absence of large pre-2.9 Ga marine limestones is consistent with evidence from iron-rich carbonates (Hessler *et al.*, 2004) that implies high CO₂ partial pressure. Before biosphere productivity increased with the advent of oxygenic photosynthesis, seawater pH may have been so low that large-scale calcite precipitation was inhibited, except in rare lacustrine locations (e.g. near hydrothermal systems) of unusual alkalinity and high pH (model of Grotzinger & Kasting, 1993). Both the Pilbara and the Barberton have abundant komatiitic lavas, which would have dominated the terrain around the sedimentary deposits that are now silicified carbonates. The Strelley Pool Chert's lacustrine and evaporative (hypersaline) facies occurs in a regional belt rich in magnesian lavas, some of which would have been exposed at the time of the Strelley Pool palaeo-lake. This characteristic setting may be significant and bears comparison with modern stromatolites in present-day lakes fed by streams draining large ultramafic and mafic massifs. Streams flowing into the Strelley Pool lake may have been alkaline.

That there is no record of large reefs of limestone before ~2.9 Ga ago may simply be an accident of preservation – that rocks formed in appropriate shallow-water settings, protected from clastic debris, have not survived. Although the record is sparse, shallow-marine sequences of this age exist. Yet large, extant, unsilicified marine carbonate reefs older than ~2.9 Ga are puzzlingly absent. Does this absence carry message? The simplest explanation of the start of the Ca-carbonate record is the evolution of oxygenic photosynthesis and consequent draw-down of atmospheric CO₂.

Do the Steep Rock, Mushandike and Pongola limestones record the first appearance of Form I Rubisco?

To recapitulate: anoxic liquid oceans probably existed long prior to the evolution of Form I Rubisco and oxygenic photosynthesis (Van Kranendonk *et al.*, 2003; Hessler *et al.*, 2004). These oceans were probably sustained as liquids by a greenhouse warming from both high methane and high CO₂ inventories under a faint young sun (Kasting, 2001; Kharecha *et al.*, 2005; Kasting & Ono, 2006).

At ~2.9 Ga ago, the first known now-extant large-scale carbonate reef formed at Steep Rock, and similar rocks occur in the Mushandike and Pongola bodies. In each of these there is C isotopic evidence most easily (though not uniquely) interpreted by the hypothesis that Form I Rubisco was active. Moreover, to reiterate, there is also possible evidence of glaciation, and of $\Delta^{33}\text{S}$ close to 0‰ in the Pongola sequence.

Cyanobacteria flourish under air that is rich in CO₂. If cyanobacteria capable of oxygenic photosynthesis (i.e. Form I Rubisco) first appeared in the shallow photic zone at this time (presumably evolving from a prior anoxygenic biota), the immediate productivity burst (Kharecha *et al.*, 2005) would take up dissolved CO₂. A possible scenario is that with the presence of highly productive oxygenic photosynthesizers

(Schidlowski, 1988) (e.g. cyanobacterial mats in a lagoon), ambient CO₂ in water would be depleted. Carbonic anhydrase, some forms of which appear to be very ancient, may already have evolved, and pronounced diurnal cycles may have been comparable to those in modern microbial communities. This would create a local environment of rising pH in quiet shallow waters, permitting precipitation of carbonate reefs with $\delta^{13}\text{C}_{\text{carb}} \sim 0\%$, as oxygen was released. The high Mn content of some of the associated sediments (e.g. the Mn Paint Rock) implies precipitation where incursions of anoxic waters entered local oxic lagoons. This context lends interest to the hypothesis that oxygenic photosynthesis began with Mn-bicarbonate solutions (Dismukes *et al.*, 2001).

The Steep Rock, Mushandike and Pongola results, the first strong appearance of the twinned characteristic isotopic signatures of organic matter with $\delta^{13}\text{C}_{\text{reduced}}$ around -30 to -20% , in association with residual contemporary $\delta^{13}\text{C}_{\text{carbonate}}$ around 0% in sedimentary carbonates drawn from the atmosphere/ocean inventory, are consistent with the evolution of cyanobacteria around 2.9 Ga ago. Taken collectively, the Steep Rock/Mushandike/Pongola evidence is thus most simply interpreted as the first records of highly productive oxygenic photosynthesis that drew down CO₂ in the water and permitted the onset of carbonate deposition in local reefs. This conclusion is wholly independent of biomarker and biomolecular evidence (e.g. Summons *et al.*, 1999).

Most modern cyanobacterial cells live among the oceanic picoplankton (Dufresne *et al.*, 2003). Though modern cyanobacterial picoplankton clades may only date back a few hundred million years (to previous climate catastrophes), the step from mat-dwelling cell to free-floating cell is comparatively small. If oxygenic cyanobacteria existed in reef mats 2.9 Ga ago, then it is possible to speculate that picoplankton also evolved at this time – indeed, the free floating forms may have evolved first.

After oxygenesis began, because of the selection of ¹²C by Form I Rubisco organisms, the remaining environmental CO₂ destined for inorganic precipitation would be correspondingly enriched in ¹³C. Though diurnal draw-down cycles are strong, CO₂ in shallow water eventually equilibrates with ambient air and air is well-mixed globally. In the late Archaean the fractions of carbon degassed from volcanoes that entered each sink are not well known and remain controversial. For the fraction captured by organisms Bjerrum & Canfield (2004) infer 0–10%, though the later record (e.g. see discussion in Kharecha *et al.*, 2005) suggests values around 20%. The large Steep Rock reef with $\delta^{13}\text{C}_{\text{carb}}$ of 0% suggests that global biological productivity driven by oxygenic photosynthesis was capable of reducing atmospheric CO₂ from this time (as distinct from earlier transient depletion of CO₂ dissolved in protected lake waters by anoxygenic photosynthesizers).

Global environmental change on the onset of oxygenesis

On the onset of oxygenesis, the pre-existing global atmospheric CO₂ inventory (perhaps around 1000 ppm – Kasting & Ono,

2006, see also Catling *et al.*, 2001 for qualitative comment) would have set a mole limit on total global oxygen release by a sudden bloom of newly evolved cyanobacteria. Even if capture of most of the prior atmospheric CO₂ inventory took place quickly, with release of mole-equivalent oxygen directly to air, without destruction by atmospheric chemistry, the initial inventory of atmospheric oxygen would have only risen to a maximum of the order of a thousandth of a bar.

A good, if inverse, parallel case is the change that took place in the Holocene Black Sea since sulphate was introduced from the Mediterranean around 8200 BP. This example shows that enhanced surface productivity produces more reduced debris that sinks to the bottom. In the Black Sea (Euxine sea) the deeper water below about 50–100 m is strongly anoxic (euxinic), even though it is overlain by strongly oxic waters and these in turn by the modern oxic atmosphere. The bottom has become more, not less anoxic.

A second modern parallel is Lake Kivu, in Africa, which contains 50 km³ of methane (at STP) in a total lake volume of 580 km³ (Jannasch, 1975). The uppermost 35 m of the lake are strongly oxic, while below 50 m the water is anoxic and has a very high methane content. In the interface zone, the rate of methane oxidation is of the same order of magnitude as the rate of methane production in the lake (Jannasch, 1975).

In the Archaean, a prior methane-rich greenhouse would (over the aeon) have led to H loss to space and irreversibly oxidized the upper part of the planet (Catling *et al.*, 2001), easing the eventual conversion to an oxygen-rich environment. However, sustained H loss from the water inventory may be dangerous to the biological health of a planet: eventually the mid-ocean ridges would be exposed. This would end both hydrothermal regulation of ocean chemistry and hydrothermal hydration of the oceanic crust. In turn, arc volcanism over subduction zones would decline, and with it, erosional inputs to ocean chemistry. Perhaps it was fortunate for all life (not just animals), and for water-fluxed arc volcanism, that oxygenesis evolved.

When oxygenesis began, the initial new atmospheric oxygen inventory created by the first bloom must have been less than the mole equivalent of the prior inventory of atmospheric CO₂: the oxygen would have been released first into water, where much would have gone into reaction with reduced anoxic deeper water or reduced minerals, or taken up quickly by respiration, and only a remnant would have reached the air. In the biologically active photic zone, oxygen emission would have created an oxygenated water body (including sulphate) under a reducing sky.

The upward release of oxidation power by oxygenesis is necessarily balanced by the creation of reduction power in newly reduced organic carbon that eventually falls to the seabed. Oxygen production by cyanobacterial plankton, and sulphate-driven anaerobic oxidation, would be balanced by the large inventory of reduction power building up in anoxic deeper water. Biology is adept at creating niches into which contrasting

chemistry can be sequestered (as in the Black Sea, or the mud beneath any pond) and the relatively small inventory that is the atmosphere may have been relatively isolated from the crustal and upper mantle inventories. The overall impact of the onset of oxygenesis would therefore likely have been a sharp increase in respiration in the upper layers of the water, population blooms of archaea in the deeper photic zone (e.g. Karner *et al.*, 2001), and an increase in biological debris falling to the sea floor, with some emission of oxygen to air, balanced by sequestration of reduced carbon in the seabed sediment.

Early biogenic oxygen may therefore have been a transient gas in the photic zone, in close vertical proximity to anoxic habitats, not a major component of the air. Thus, after the evolution of oxygenesis, most of the oxygen may have existed in a specific depth range in the photic zone water, especially in coastal lagoons where it would be protected from mixing with upwelling anoxic bottom water. Biology would flourish at the interface between oxic and anoxic waters.

Free oxygen in the air itself (as opposed to seawater) would only occur when new volcanic emission of CO₂ permitted photosynthetic blooms from which O₂ release to air was markedly more rapid than the consequent competing sedimentation of organic matter and eventual emission and return to air of methanogenic methane. Recycling to the air of the reduction power from decaying organic matter created by the new biological productivity would be likely to maintain both deep-water and atmospheric anoxia.

A Yo-Yo Ocean?

Oxygenesis may also have been self-limiting through its greenhouse impact in a process that could lead to a 'Yo-Yo' oscillation. Global CO₂ drawdown and methane oxidation would reduce greenhouse warming. This may have been enough to initiate glaciation and hence, by converting ocean to ice, to inhibit the cyanobacterial blooms and slow further carbon capture by life, unless CH₄ release from organic matter in sediment restored warmth. Form I Rubisco thus carries with it some capacity for forcing glacial/interglacial cycles. The possible sequence is as follows:

1. Release of oxygen even in trace quantities would increase the oxidative power of the atmosphere. Increased sulphate would promote anaerobic methane oxidation. This would reduce atmospheric methane, cutting the greenhouse warming that sustained global temperatures.
2. In the colder temperatures, methane clathrates would form, reducing methane emission from sediment. Also, Rubisco's slightly increased specificity in cold conditions (Tcherkez *et al.*, 2006) would initially exacerbate the draw down of atmospheric CO₂.
3. Glaciation would result, eventually inhibiting further biological production by limiting cyanobacterial habitat under ice-covered oceans. Cold oxygenated water would sink to fumigate (borrowing a term from atmospheric chemistry)

and oxidize the surface of bottom muds. Deeper in the sediment pile, methane from organic matter in muds would be stabilized by clathrate traps in the sediment under large amounts of clastic glacial debris.

4. Eventually warmth would be restored when clathrates, destabilized by depressurization as sea-level fell, broke down and CH₄ emission overwhelmed oxygen in the air. The extended CH₄ lifetime would restore a methane greenhouse. This methane would come from the deep stores of reduced organic debris on the continental edge and proximal oceanic shelves that had been enriched in organic matter by the earlier productive microbial ecology.

Perhaps after an initial outburst of productivity at 2.9 Ga that partially drew down a pre-existing inventory of, say, ~1000 ppm CO₂ and released matching oxygen, an oscillation occurred between methane return from sediments and temperature-restricted oxygenic photosynthesis. This view is consistent with the Yo-Yo record of mass independent fractionation of sulphur isotopes (Farquhar & Wing, 2003; Kasting & Ono, 2006; Knauth, 2006; Ohmoto *et al.*, 2006).

More generally, Lowe & Tice (2007) make cogent arguments that tectonic factors, through the formation of large blocks of continental crust, provided a fundamental control on the timing and directions of biological evolution throughout the Earth's history. They apply this hypothesis especially to the Archaean and the evolution of cyanobacteria. Indeed, tectonic events must have been crucial in shaping biological evolution, but we now make the opposite argument, that biological evolution has shaped the atmosphere, and thence the surface conditions (including erosion and subduction). The two factors – tectonics and biology – are inextricably interdependent, a Möbius surface.

Rubisco specificity, compensation controls in organisms, and atmospheric CO₂:O₂ mixing ratios

'It is clear that there will be a limiting minimum concentration of carbon dioxide, below which it is impossible for vegetation to live' (Macgregor, 1927). There is a limit of CO₂ partial pressure, set by disequilibrium thermodynamics, below which carbon capture fails to compete with respiration and growth stops. If CO₂ is too low, life cannot extract it from the air or from dissolved oceanic sources. If O₂ is too high the living organism self-oxidizes and instead of extracting carbon *from* the air, it gives up CO₂ *to* the air, a process which, if unduly prolonged, is fatal to the cell. Equally significantly, there is an environmental limit set by the greenhouse: on the modern Earth, with oxygen-rich air, if CO₂ fell below about 150 ppm there would likely be catastrophic glaciation.

The balance between CO₂ uptake and CO₂ emission by cells carrying out oxygenic photosynthesis is subtle (Tolbert *et al.*, 1995). CO₂ uptake is controlled by Form I Rubisco's specificity for carbon over oxygen (Gutteridge & Pierce, 2006; Tcherkez *et al.*, 2006). Organisms performing carbon capture and release

by Forms I and II Rubisco are subject to ‘compensation’ limits (Berry *et al.*, 1994; Tolbert, 1994; Tolbert *et al.*, 1995), which are the outward (organism-scale) expression of Rubisco’s molecular specificity. The CO₂ compensation point is the CO₂ concentration at which net CO₂ fixation is zero. Modern oxygenic organisms have very sophisticated carbon-capturing mechanisms (e.g. in the C₄ plants) and carbon sequestration systems (e.g. the carboxysomes in cyanobacteria), which enable them to exist in conditions of low ambient CO₂. The compensation limits are set by the import of CO₂ into the cell, by the transport of the carbon inside the cell to the enzyme (e.g. as HCO₃⁻ and then reconverted to CO₂) and ultimately by Rubisco’s specificity. More generally, the compensation controls define a limiting field for the permissible relative burdens of CO₂ and O₂ in the air if oxygenic photosynthesis is to occur.

Rubisco is exquisitely finely tuned to its task (Gutteridge & Pierce, 2006; Tcherkez *et al.*, 2006). Given the rates of replication and evolutionary change possible in bacteria, which have rapid generational turnover in comparison to geological timescales, this fine tuning of Rubisco may be very ancient. It is also probable that the carbon concentrating mechanisms involving forms of CA are ancient: indeed, the existence of ancient CA based on Fe²⁺ (Tripp *et al.*, 2004) suggests that CA may long pre-date oxygenesis, as Fe²⁺ would have been readily available in anoxic settings.

In general, discussion of CO₂:O₂ ratios in the geological literature focuses on controls by erosion and inorganic chemistry rather than biochemistry. On today’s planet, biology’s ability to sequester and partition reductant from oxidant always trumps erosional homogenization. Biology determines the playing field for chemistry, and the uniformitarian hypothesis is surely that this has been the case ever since a global-scale biosphere developed. However, if oxygenesis by cells using Form I Rubisco did indeed exist in the period between ~2.9 Ga and ~2.4 Ga ago, as the isotopic and geological facies evidence above suggests, then these oxygenic organisms, and the atmospheric greenhouse, would have been subject to, and interactive with, the specificity of Form I Rubisco and to the consequent compensation limits on organisms.

If so, the compensation limits on atmospheric composition are worth investigating.

CO₂ and O₂ compensation

For a typical organism depending on oxygenic photosynthesis, the CO₂ compensation point CO₂ Γ (Berry *et al.*, 1994; Tolbert *et al.*, 1995) varies linearly with ambient O₂ (Fig. 5, plotted from data in Tolbert *et al.*, 1995). Below the CO₂ Γ line in Fig. 5 there is net CO₂ loss from photorespiration. Similarly, the O₂ Γ line is the line at which organismal or tissue-specific oxygen production rates balance O₂ consumption.

To locate the lines Tolbert *et al.* (1995) studied chloroplasts in tobacco seedlings in varying atmospheres (Fig. 5). The tobacco chloroplast is a valid, if distant, model for Form I Rubisco in

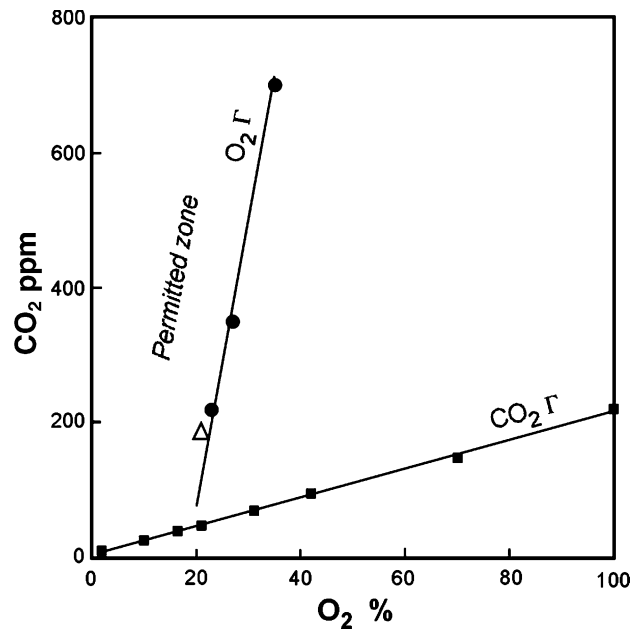


Fig. 5 Compensation lines for tobacco chloroplasts (plotted from data in Tolbert *et al.*, 1995, who worked at lab temperature and pressure).

CO₂ Γ is near-horizontal line, with CO₂ (ppm) = 2.13 O₂ % + 3.89

O₂ Γ is steep line with O₂ % = 0.0246CO₂ ppm + 17.94

For net growth, atmospheric CO₂ levels must be on or above the CO₂ Γ line, and O₂ on or below O₂ Γ. CO₂ Γ is in parts per million, while O₂ Γ is in percentage. ‘Permitted zone’ shows region in which Form I Rubisco organisms are capable of sustaining oxygenic photosynthesis. In practice, in water, photic zone CO₂ is quickly depleted. Resupply must come from air so, even with efficient carbonic anhydrase, there must be a gradient (i.e. the effective atmospheric CO₂ Γ line may be parallel to, but is above, that shown). Triangle shows approximate composition of air in last glacial maximum.

cyanobacteria (to which it is phylogenetically related). Tobacco seedlings capture the CO₂ from air, which convects and mixes rapidly. The seedlings are thus a ‘limiting case’ model for the CO₂ Γ in plants, as CO₂ may partly enter the seedling chloroplasts by direct diffusion rather than nascent stomata. In adult plants, the entry of the CO₂ may be more complex and difficult. Note that the trends measured by Tolbert *et al.* (1995) and shown as lines in Fig. 5 are only loosely illustrative, not in any way quantitative for Archaean conditions because the results, from plants, not cyanobacteria, are for 20 °C only and are temperature and pressure dependent.

Organisms use carbon concentrating mechanisms (CCM) based on CA to help gain access to CO₂, an ancient process (e.g. Smith *et al.*, 1999). Smith & Griffiths (2000) investigated the impact on compensation if plants were deprived of the help of CA. They grew bryophytes (moss-like plants) under mist on peat at 17 °C. Under these conditions, normal *Phaoceros laevis* (a hornwort) showed CO₂ compensation at about 22 ppm CO₂ at 1 atm pressure. Smith & Griffiths (2000) then inhibited CA by incubating the *Phaoceros laevis* in the membrane-permeable CA inhibitor ethoxylzolamide. For

these plants that lacked the assistance of their CCMs, the CO₂ compensation point was about 205 ppm.

In contrast to plants taking up CO₂ from the well-mixed air, pelagic cyanobacteria must capture CO₂ from slowly mixing seawater (which is in eventual equilibrium with the upper surface of the water and hence the atmospheric CO₂ mixing ratio). In practice, access to CO₂ for marine organisms is difficult. The local CO₂ partial pressure in the water next to the cell may be far lower than in the ambient air above the sea surface. This means that while a cell may be operating in water with CO₂ on the compensation point, the air above the sea may have a much higher CO₂ mixing ratio.

A cell that is taking up CO₂ from surrounding water depletes its proximal environment below the ambient CO₂ concentration of the wider environment, and competes with neighbouring cells. For much of the daylight time, cells are in water that has been depleted of CO₂ by the uptake by surrounding life. This leads to diurnal cycles, with strong depletion of CO₂ in the photic zone of seawater during daylight uptake, and then restoration of CO₂ levels overnight. The limiting constraint will be the local availability of the CO₂ in the water around the cell. Thus in upper photic zone water, the *ambient* CO₂ (e.g. night-time CO₂ in equilibrium with air) needed to support cyanobacterial communities must be higher than the partial pressure experienced during daytime growth by cells, living among competing plankton and surviving by extracting CO₂ from depleted water. In contrast, chloroplasts in land plant cells, such as Tolbert *et al.*'s tobacco seedlings, are in direct contact with moving air, and do not experience such sharp daytime environmental cycles of CO₂ availability.

Collectively, compensation is set by the broad global community of photosynthesing organisms, which can only live long and prosper if dioxygen concentrations are below communally set O₂ Γ and also if CO₂ is above communally set CO₂ Γ . Although cyanobacteria have sophisticated CA-based carbon capturing mechanisms to introduce CO₂ to the Rubisco, the much faster mixing of CO₂ in air than in water suggests the compensation line for tobacco seedlings acquiring CO₂ from air can be regarded as an illustrative lower limiting case. Even if tobacco chloroplasts and cyanobacteria were biochemically identical, the effective CO₂ Γ line for cyanobacteria (i.e. the limiting line of growth for CO₂ dissolved in ambient water) would be at a significantly higher atmospheric CO₂ mixing ratio, given the diurnal mixing gradients between water and air.

The ~200 ppm CO₂ Γ for bryophytes when CA is inhibited (Smith & Griffiths, 2000) may be illustrative: this is the 'unassisted' mixing ratio that supports growth. Though eukaryotic hornworts are far from cyanobacteria, this may very roughly illustrate the minimum atmospheric level at which Rubisco can operate without help from carbon concentrating mechanisms.

For autotrophs dependent on Form I Rubisco, the specific CO₂ Γ and O₂ Γ lines set permissive habitat limits for dioxygen and CO₂ (Fig. 5). Within the permissive zone, evolution by natural selection will tend to maximize productivity and hence

take up carbon into organic matter, driving the CO₂ mixing ratio downwards towards the CO₂ Γ line. Thus the atmospheric CO₂ mixing ratio is likely to be close to the effective CO₂ Γ , providing the supply of other nutrients is adequate. There is striking disproportion: in the permissive zone (Fig. 5), O₂ is in per cent, but CO₂ is in parts per million, a difference of three orders of magnitude.

The Quaternary ice core record of minimum CO₂ is not far from the O₂ Γ /CO₂ Γ intersect (Fig. 5) for the seedlings studied (Tolbert *et al.*, 1995). It is interesting that on the modern Earth, 190–200 ppm is the CO₂ level typical of the air during major glaciations. Faced with a relatively low CO₂ glacial atmosphere, C4 plants, in particular, have evolved preconcentration mechanisms to increase the carbon yield of CO₂ brought to the Rubisco. This increased yield means that the isotopic selectivity is far less than in the rival C3 plants. This suggests that the modern inventory of atmospheric O₂, with a lifetime of millions of years, is tuned to the long-term average CO₂ of the past few million years (Quaternary/late Tertiary) (Tolbert *et al.*, 1995). In our dominantly glacial Earth (over the 10⁷ year timeframe), global productivity may be maximized close to the compensation barriers, with the air below 200 ppm CO₂, at 21% O₂.

Because of the difference in size of the O₂ and CO₂ reservoirs, the CO₂ will respond quickly to any environmental change, on a timescale of a few centuries, but the dioxygen burden of the air will shift proportionately more slowly, over millions of years. This gives long-term stability to the system even though brief 'Yo-Yos' occur: over recent planetary history, glacial climate dominates. In engineering terms this is a 'ramp' type of control system. The huge O₂ reservoir in the modern air imposes long-term stability on CO₂. Short-term injections of CO₂, for example from volcanoes, make very little impact on oxygen over a geological timescale, though they may cause major transient (say 10–100 ka) greenhouse warming. The system is inherently stable. For example, doubling CO₂ from volcanoes would induce rapid photosynthetic release of dioxygen, bringing down the CO₂ again but barely shifting the oxygen reservoir. The system will regress again to the CO₂ Γ line (Fig. 5).

Compensation, Rubisco specificity and the greenhouse in the Late Archaean

The CO₂ Γ line suggests that in conditions with low ambient O₂, cyanobacteria could operate with local (i.e. vicinity of cell) CO₂ mixing ratios as low as a few tens of ppm or less. If Tolbert *et al.* (1995)'s work on modern tobacco seedlings can be taken as analogous to the limiting case for cyanobacteria, for growth at low O₂ concentrations, the CO₂ mixing ratio in the proximal water must be above the limit of 9 ppm (Tolbert *et al.*, 1995). Lower O₂ mixing ratios would permit even lower proximal CO₂ mixing ratios. This is an extreme lower limit, likely only to apply in local CO₂-depleted water to plankton

cells assisted by carbon concentrating mechanisms. The slowness of mixing in water would sustain transient differences (of the order of hundreds of ppm) between ambient CO₂ in the atmosphere and in the photic zone of the water. Even so, if life is capable of bringing CO₂ mixing ratios down as low as say 100 ppm, the modern greenhouse would fail: catastrophic glaciation would occur. For low CO₂ air, the climate has to be kept warm by another greenhouse gas, such as methane (though N₂O is also a candidate, being a strong greenhouse gas).

In the Archaean, under anoxic air, with abundant other nutrients, CO₂ uptake to compensation limits could have fostered so-called 'oxygen oases' (e.g. Knauth, 2006) in restricted settings in water bodies. In lagoons of active stromatolite growth, conditions may have been locally aerobic in the photic zone of the water, with O₂ release in the water creating oxic conditions even though the air above remained anoxic (the partial inverse of the modern Black Sea). It is possible to imagine an anoxic global atmosphere with a very low free O₂ mixing ratio, but with higher O₂ in photic zone waters and – necessarily to give the greenhouse warming to support liquid water – a high methane mixing ratio in the atmosphere above the water surface. In a world with oxygen oases in photic zone waters but anoxic subphotic sea, upwelling nutrients would create cyanobacterial blooms.

These compensation constraints suggest a possible Late Archaean microbial ecology of coastal and planktonic oxygenic cyanobacteria, and oxygen-tolerant anoxygenic photosynthesizers, above deeper anaerobic anoxygenic photosynthesizers (e.g. green sulphur bacteria). Archaea were probably widespread, with archaeal consortia carrying out anaerobic methane oxidation underneath the photic zone (compare Karner *et al.*, 2001). Productive cyanobacteria would sustain an ecology that sedimented large quantities of organic matter to the seabed, supporting abundant methanogens on the anoxic deep sea floor and consequent methane emission. The oxygen and sulphate in turn would provide substrate for methanotrophs and anaerobic methane oxidizing consortia, which would take up excess oxidant. Some methane would reach the air, some would be stored in clathrates or gas in sediment, eventually to be destroyed in subduction. Much of the methane would probably be taken up by anaerobic methane oxidation, using sulphate that in turn depended on the oxygenesis. Methane would be released from organic-rich sediments and also from deep-water sediment via overturn of anoxic deeper waters. In such a setting, free oxygen produced as waste from photosynthesis would compete with methane as a trace component of the air. In photic-zone waters, oxygen might build towards significant levels late in the afternoon, providing substrate for nocturnal respiration.

Aerobic water may also have existed in the photic zone a few metres below the surface in the open ocean, maintained by picoplanktonic cyanobacteria at tightly controlled preferred photosynthetic depths. Such an ecology could perhaps support an atmosphere that contained a burden of a few hundred (say 200) ppm of CO₂. Though modern cyanobacterial clades

may not date back as far as the Precambrian, it is possible that the original evolution of carboxysomes in cyanobacteria may have occurred in cyanobacterial plankton during an Archaean CO₂ crisis caused by oxygenic cyanobacterial blooms. Cells with carboxysomes, aided by CA (So *et al.*, 2004), would have great advantage in the later CO₂-depleted part of the day and would have outcompeted neighbouring cells for the remaining CO₂.

If so, life would have been capable of drawing down CO₂ to low levels close to CO₂ Γ. Even today, in the light of a more powerful sun, this means glaciation. Given the evidence in the Archaean record for clastic sediment deposited by liquid water, methane must have been the crucial atmospheric greenhouse gas, perhaps in similar mixing ratios to CO₂. Zahnle *et al.* (2006) showed that transition from an oxygen-poor to an oxygen-rich state took place most easily when methane levels collapsed. This may have taken place multiply, in repeated Snowball Earth events (Claire *et al.*, 2006).

In the absence of oxygen and furthermore in a dry atmosphere without modern plant transpiration, the methane lifetime would have been very much longer than today (Kharecha *et al.*, 2005). Thus a large methane burden in the air could have been supported by emissions that were not substantially greater than today, provided the methane emissions were in balance with the net oxygen emissions (i.e. oxygenesis minus respiration and inorganic uptake of O₂).

The main challenge to the stability of such an ecology would be the over-exuberant emission of oxygen, to a level that threatened the methane greenhouse and induced glaciation. This, however, would normally be self-correcting to maintain anoxia, both because it would be matched by increased respiration and O₂ uptake, and also because any over-supply that produced free atmospheric O₂ would challenge the greenhouse inventory of CO₂ and CH₄, inducing glaciation (see earlier discussion). To recapitulate, glaciation would suppress oxygenesis in shallow waters while methane emission from large sediment piles would occur. As ice accumulated, sea-level drop would destabilize clathrates. Thus the glaciation would be limited by CH₄. Only catastrophic failure of this correction mechanism could tip the system to a long-term runaway glaciation or Snowball Earth of the sort envisaged by Hoffman *et al.* (1998).

Oxygenic photosynthesis thus can occur either:

1. Under an anoxic atmosphere, with oxygen levels so low that a long methane lifetime and hence a high methane burden is possible, or
2. Under oxic air, in which a high CO₂ burden (necessarily much further up the cells' CO₂ Γ line), sustains the greenhouse, with only trace methane.

Form I Rubisco's relationship with the greenhouse thus permits two stable states of the air (Nisbet, 2002) in which oxygenic photosynthesis occurs. One state is anoxic with long-lived methane as the greenhouse gas, low CO₂ and very low O₂. The methane emission flux is not necessarily high as the lifetime is long. The other state is further up the CO₂ Γ line. This necessarily means

that the air is oxic, shortening the lifetime of methane. In this state, a higher CO₂ burden must provide the main greenhouse warming and methane can only be a trace component.

Either stable state can sustain liquid water on the ocean surface. Intermediate states are not stable in the long term as the coexistence of high oxygen and high methane mixing ratios is improbable. However, methane could have a multidecade atmospheric lifetime provided the troposphere had low OH formation (OH is the main methane sink), and emissions of catalytic Cl and Br from the ocean surface were low. It is possible to imagine a very dry planet, in the light of a faint young Sun, with very low atmospheric OH. In this 'Cold Dune' model, a high oxygen burden in the air might coexist with high methane, sustained by emission from previously formed organic-rich sediment accumulations on the continental shelf sediment. Such conditions could occur in strong glacial planet that was cool and very dry.

Goldblatt *et al.* (2006) showed that bistability is also imposed on the atmosphere by the constraints of ozone chemistry. The bistability arises because ultraviolet shielding of the troposphere by ozone occurs effectively when the oxygen burden is above 10⁻⁵ times present atmospheric level. Once this happens, there is a nonlinear increase in oxygen lifetime and the air is driven to a high oxygen state. A change as small as a 3% increase in organic carbon burial could trigger the change to a high oxygen state.

The three sets of constraints: – (i) Rubisco compensation and its greenhouse implications; (ii) the nonlinear increase in oxygen lifetime when ultraviolet shielding by ozone occurs; and (iii) the reduction of the methane lifetime as oxygen increases – all work together to permit only very discreet stability states for the air: one state with low oxygen, modest CO₂ and high CH₄, and the other state with high oxygen and significant CO₂ but only trace CH₄.

Compensation in snowball events and after

In any sustained global 'snowball' or near-snowball glaciation, catastrophic failure of the biosphere would sharply reduce organic productivity. If snowball models are correct, photosynthetic productivity must be very low for millions of years during glaciation until volcanic CO₂ accumulates to ~12% of the air (Caldeira & Kasting, 1992). Then the snowball breaks down when volcanic CO₂ builds up to the point that greenhouse warming eventually ends the glaciation.

After snowball collapse, with very high atmospheric CO₂, acidophile cyanobacteria would bloom in the newly warmed degassing oceans, releasing free oxygen. The water would be anoxic, acid, warm, and very rich in substrate nutrients, with a large inventory accumulated from submarine hydrothermal activity over the millions of years of the snowball. Nutrients such as dissolved iron would be particularly easily available. The huge nutrient inventory would be capable of sustaining an immense bloom of oxygenic photosynthesizers at the oxic/

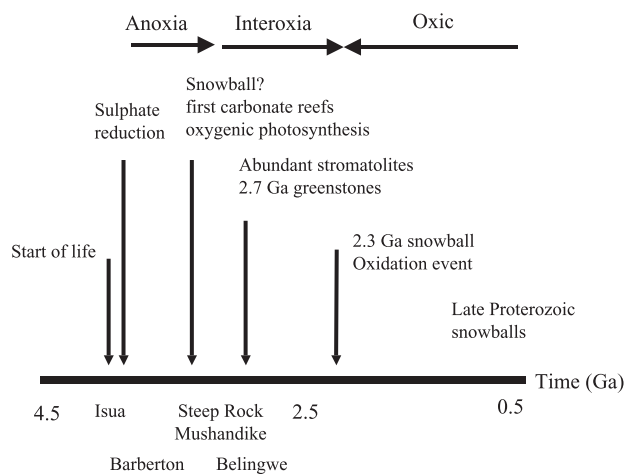


Fig. 6 Geological record of oxidation state.

Anoxia – Probably low O₂ (except see Rosing & Frei, 2004) before 2.9 Ga.

Interoxia – Between 2.9 Ga and 2.3 Ga. After the onset of oxygenic photosynthesis, but with anoxic methane-rich atmosphere, though note strong dissenting 'oxic' view (Ohmoto, 1997).

Oxic – Oxygen-rich air after 2.3 Ga.

anoxic interface that would rapidly extract CO₂ from the ocean/atmosphere system and precipitate carbon in reduced organic matter, emitting waste oxygen.

Conversion of a significant part of the snowball world's atmospheric inventory of (for example) 10–12% CO₂ to dioxygen would suddenly drive the O₂:CO₂ ratio far up the CO₂ Γ line, until oxygenesis was checked: either by using up a key prerequisite (e.g. available N, P or Fe in photic zone water); or by consuming the CO₂ inventory down to the CO₂ Γ at the mole equivalent O₂ level attained by the oxygenetic bloom; or by reaching the O₂ Γ line. This intersection with the compensation limiting line would necessarily be at a point sharply higher in oxygen burden than before the snowball. Being further up the CO₂ Γ line, the new state would have much stronger greenhouse warming from CO₂. The result could be a stable oxygen-rich system, with greenhouse warming supported by CO₂ alone (say >250 ppm), only weakly aided by transient methane (say 1–2 ppm) from seabed sources and microbial communities in terrestrial swamps.

A permanent rise in atmospheric oxygen (Fig. 6) may thus be consequential on the increase in the atmospheric inventory of CO₂ moles during the glaciation or snowball, when compensation constraints failed as oxygenic photosynthetic life retreated to a few liquid oases and CO₂ was able to increase unchecked. This may have first taken place in the ~2.4–2.3 Ga Huronian glaciation, though far less than today. Global glaciations in the late Proterozoic may then have allowed the final step up to an atmosphere with high O₂, when the CO₂ build-up in the snowballs permitted postsnowball compensation to be attained with high O₂ and high CO₂. The high CO₂, aided only by trace methane, would be able to induce a strong

H₂O-vapour greenhouse warming and the stable attainment of a warm liquid-ocean Earth, with the air close to the limiting O₂ Γ barrier. It may be that it was at this stage that in most organisms CA switched from Fe²⁺ to Zn²⁺ (see Tripp *et al.*, 2004).

Organisms whose Rubisco has high CO₂ specificity only have this property at low temperatures: at higher temperatures the specificity drops somewhat (Tcherkez *et al.*, 2006), allowing for faster growth. Thus in warm high CO₂ times, community optimization by selective competition would, within the global population of organisms, broadly tend to favour those with low CO₂ specificity and high productivity. Consequently, isotopic fractionation into organic matter would be reduced in warmer periods, but total production and burial of organic carbon would increase. In the balancing carbonate reservoir, $\delta^{13}\text{C}_{\text{carb}}$ may thus be buffered by the specificity response, changing little despite the fluctuation in productivity.

Rubisco, the greenhouse, and the air

In conclusion, Rubisco and greenhouse controls on the global environment may jointly permit the Earth system to have two stable states – ‘anoxia’ low on the CO₂ Γ line with very little O₂ except in oases of oxygenated water, low CO₂ and with significant methane; and a second ‘oxia’ state close to the upper bound of the CO₂ Γ line where it intersects the O₂ Γ line, with high O₂, no atmospheric methane, and several hundred ppm CO₂. This argument for bistability is quite separate from the premise of Goldblatt *et al.* (2006), though the two hypotheses are fully compatible.

The geological record of CO₂ is usually studied through analysis of the dynamics of inorganic geochemistry. The rules of chemistry are certain, and the constraints of chemical equilibria are valid. The role of the biological control is different. Biology, by its exploitation of kinetics and the subtleties of thermodynamic disequilibrium, sets the wider framework – Is the atmosphere oxic or anoxic? Is the planet icy or warm? Is the ocean liquid? The geochemical engine, powered by the chemistry of the oceanic reagents, runs to firm equilibrium rules, but is subject to the disequilibrium gears and the steering direction chosen by the solar-powered biological driver of the planetary vehicle. The kinetic choices of that driver are difficult to predict.

Rubisco is the chief architect of the atmosphere. It designs the house of life, with a lower anoxic basement in the mud and an upper oxic habitation, and determines the surface temperature by its management of the carbon greenhouse gases. Once that basic design is built and the temperature is controlled, the laws of inorganic chemistry operate within the framework set by biology. The role of Rubisco’s partner enzyme, Nitrogenase, which by its control of N₂ sets atmospheric pressure, and is involved in the budget of N₂O, the third managing greenhouse gas, is closely linked to photosynthesis (Joshi & Tabita, 1996). Its role will be discussed by us elsewhere.

To summarize: anoxygenic photosynthesis is very old, dating back to the early Archaean. From it, the evidence discussed here implies that specifically oxygenic (Form I Rubisco) photosynthesis evolved ~2.9 Ga ago. The high productivity and draw-down of CO₂ permitted the development of marine carbonate reefs. We argue that since then, the balance between the carboxylase and oxygenase roles has meant that Form I Rubisco’s specificity (its supposed ‘inefficiency’) has controlled atmospheric CO₂ and the greenhouse, sustaining the disproportion between dioxygen and CO₂ in the air. In the emergence from the snowball catastrophes, after build-up of a very CO₂ rich atmosphere, that control stabilized the present aerobic surface biosphere and made possible the evolution of metazoa.

Analytical methods

$\delta^{13}\text{C}_{\text{red}}$ was analysed on a VG/Fisons/Micromass ‘Isochrom-EA’ system, consisting of an elemental analyser (EA1500 Series 2) on line to an Optima mass spectrometer operating in He continuous flow mode (Grassineau, 2006). Precision better than $\pm 0.1\%$ was obtained on hand-picked samples of 0.07 mg for pure carbon, to 30 mg for whole rock with 0.1 wt% C. Standards include NBS 21 and IAEA-CO9. Blank contamination from tin capsules has been measured at <34 ppm C in the RHUL laboratory. Pure carbonates (0.5 mg) were measured for $\delta^{13}\text{C}_{\text{carb}}$ using an Isocarb automated carousel connected to a PRISM mass spectrometer. Impure carbonates were analysed using a modified Micromass Multiflow connected to an Isoprime mass spectrometer. Internal precision is better than $\pm 0.07\%$ for $\delta^{13}\text{C}_{\text{carb}}$ for both systems. Standards are NBS 19 limestone and a laboratory calcite.

New C isotope results included in the histograms will be tabulated elsewhere (Grassineau *et al.*). Other data are from our prior work (Abell *et al.*, 1985a,b; Grassineau *et al.*, 2001, 2002, 2006) and McClory (1988).

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