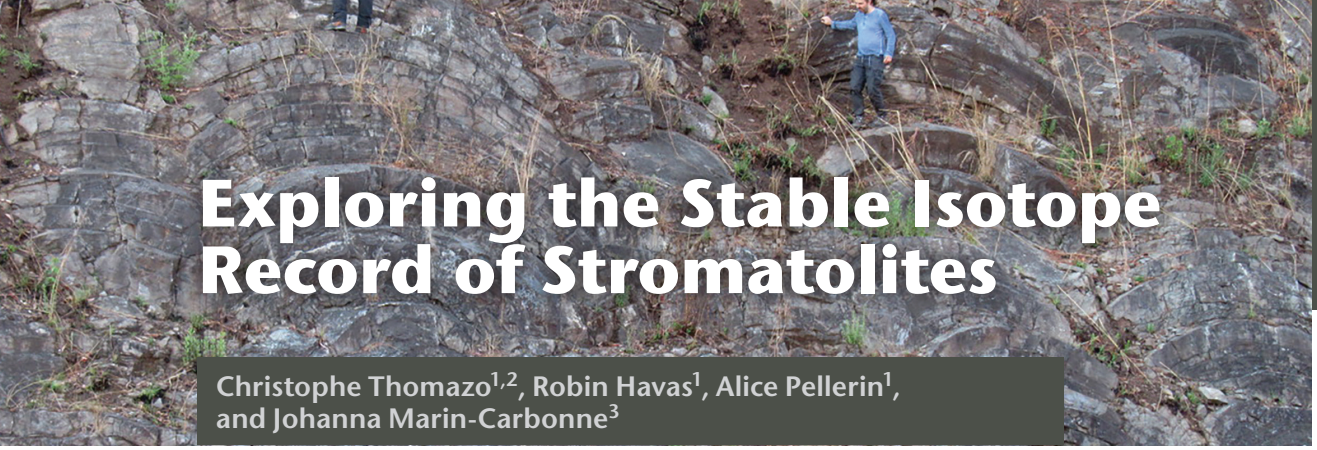

Exploring the Stable Isotope Record of Stromatolites

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Campbellrand-Malmani giant stromatolites at Sudwala Pass (South Africa).

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Stromatolites rank among the most productive ecosystems on Earth, with extremely high rates of element cycling, especially carbon, oxygen, nitrogen, iron, and sulfur. Their study provides critical insights into early microbial life evolution, environmental conditions, and associated biogeochemical cycling. Specifically, carbon, nitrogen, and sulfur isotopes can be used to assess metabolic activities of microbial communities, including those that may regulate the formation and preservation of modern and ancient stromatolites, such as photosynthesis, nitrogen fixation, and sulfate reduction. Moreover, while Precambrian stromatolites' isotopic signals can record microbial communities that both influence and adapt to changing major redox conditions such as the Great Oxidation Event, local and secondary processes can open a window onto microbial evolution such as the early evolution of bacterial sulfate reduction.

KEYWORDS: carbon isotopes; nitrogen isotopes; sulfur isotopes; early life; biogeochemistry; geobiology

INTRODUCTION

Stromatolites provide an unparalleled, continuous 3.5-billion-year archive of life's history. To decipher the biogeochemical signals within this record, geochemists often turn to well-preserved Archean deposits. Notable examples include the Strelley Pool (3.4 Ga) and Tumbiana formations (2.7 Ga) in Australia, the Cheshire Formation (2.73 Ga) in Zimbabwe, and the Campbellrand-Malmani platform (2.5 Ga) in South Africa. In this chapter, we focus on the latter three, which retain carbonate mineralogy, unlike the older Strelley Pool Formation, which has been extensively silicified. Interpreting these ancient traces of life profoundly relies on the study of modern microbialites. Understanding the development, preservation, and associated biosignatures of such modern analogue microbialites is crucial for elucidating the evolution of early microbial communities and past environmental conditions, and for providing a blueprint for planetary studies (see Perspective).

Carbon, nitrogen, and sulfur (C, N, and S) are elements central to life and their isotope geochemistry (see Toolkit) has been widely used to trace biological activity in deep time. This approach relies on comparing the rock record with modern analogues, linking measurable isotopic signals to primary biogeochemical processes such as photosynthesis, nitrogen fixation, and sulfate reduction (see Toolkit). Provided that the reactant is not completely converted to the product, these reactions generate distinct isotopic fractionations. These signatures become recorded in geologic materials, including organic matter, carbonates, and sulfides. However, transposing this isotopic framework to deep time rests on critical assumptions: (i) pathway continuity: the ancient metabolic pathways were similar to modern ones; (ii) efficient preservation: the primary isotopic signals survive with minimal alteration; and (iii) source inference: the isotopic composition of ancient substrate reservoirs can be estimated.

Systematically understanding how primary isotopic signals are recorded in modern stromatolite-forming environments is therefore essential. It provides the foundation for assessing their formation mechanisms and refining paleoenvironmental and paleoecological interpretations throughout the geological record. In this contribution, we detail the framework and concepts associated with the use of carbon, nitrogen, and sulfur isotopes within microbialites, taking examples from both modern settings and the Precambrian rock record. The aim of this review is to highlight the variety and complexity of messages that can be conveyed by microbialite isotopic records.

CARBON ISOTOPES: A THREE-WAY RELATIONSHIP BETWEEN DISSOLVED INORGANIC CARBON, ORGANIC CARBON, AND CARBONATES

Framework From Modern Examples

Carbon plays a central role in both physico-chemical and biological processes in the environment. Carbon, as a major component of microbialites, both as organic carbon and carbonate minerals, provides critical insights into isotope fractionations associated with autotrophic carbon assimilation pathways (FIG. 1A). The offset between the isotopic composition of organic carbon ($\delta^{13}C_{OC}$; primarily reflecting the primary producers' metabolic activity) and dissolved inorganic carbon ($\delta^{13}C_{DIC}$) reflects metabolic pathways and serves as a proxy for biogeochemical activity. For instance, microbialites dominated by green sulfur bacteria (GSB), which employ the reductive citric acid cycle for anoxygenic

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photosynthesis, typically exhibit less depleted $\delta^{13}C_{OC}$ values than those dominated by cyanobacteria and purple sulfur bacteria (PSB), which use the Calvin–Benson–Bassham (CBB) cycle, assuming both utilize the same DIC source (Havas et al. 2025). Conversely, when carbon is sourced from biogenic methane, methanotrophs produce highly depleted $\delta^{13}C_{OC}$ signatures (Thomazo et al. 2009, 2013; FIG. 1A).

In modern stromatolites and other microbialites, spatially resolved carbon isotope analyses can discriminate between heterotrophic organic carbon degradation and autotrophic fixation. However, microbialite architecture (e.g., microbial mats) can impose significant CO_2 diffusion limitations, reducing the extent of net fractionation (ε; see Toolkit), and leading to $\delta^{13}C_{OC}$ values that are higher than those of free-floating cells (Havas et al. 2023, 2025). This may complicate the use of microbialite $\delta^{13}C_{OC}$ for applications such as pCO_2 reconstructions.

Carbon assimilation pathways also influence carbonate biomineralization in microbialites, primarily through pH and alkalinity modulation. Whether mineralization is driven by autotrophy or heterotrophy will drive ^{13}C enrichments or depletions, respectively, in the resulting carbonates relative to isotopic equilibrium (FIG. 1A). While heterotrophy tends to lower the isotopic composition of carbonate ($\delta^{13}X_{carb}$), primary production elevates it (photosynthetic pooling effect; FIG. 1B; Havas et al. 2025). This effect is amplified in microbialites, which function as partially closed systems.

The preservation of metabolic isotopic biosignatures in microbialite carbonates is further modulated by environmental factors, particularly water-carbonate saturation states (Havas et al. 2025). Many modern microbialites form in semi-closed basins where high alkalinity buffers the $\delta^{13}X_{carb}$ value toward equilibrium, obscuring metabolic signals (FIG. 1B). Facies-dependent isotopic analyses of carbonates within and around microbialites may provide more specific biological or climatic insights (Ingalls et al. 2020).

Although isotopic analyses cannot capture the full microbial diversity of microbialites, they offer a time-integrated perspective on the biotic and abiotic processes shaping their formation. Specifically, microbialite $\delta^{13}C_{carb}$ values reflect the balance between microbialite net primary production (mNPP) and physicochemical precipitation (PCp; FIG. 1B). The microbialite $\delta^{13}X_{carb}$ value is primarily controlled by the isotopic composition of the precipitating DIC, making it a valuable archive of global or regional carbon cycling (Fogret et al. 2024). Yet, we show that it remains a powerful tool for deciphering the interplay of metabolic and environmental drivers in microbialite formation.

Extending Carbon Isotopes to Ancient Microbialite Records

In geological samples, including ancient stromatolites, the difference between the $\delta^{13}C_{carb}$ and $\delta^{13}C_{OC}$ isotope compositions (i.e., $\Delta^{13}C_{carb\text{-}oc} = \delta^{13}C_{carb} - \delta^{13}C_{OC}$; see Toolkit) depends on multiple factors including lithology, CO_2 abundance, alteration, and diagenesis (Hayes et al. 1989). FIGURE 2 compares paired $\delta^{13}C_{Carb}$–$\delta^{13}C_{OC}$ records from three Neoarchean stromatolites with the range observed in modern Lake Alchichica microbialites. These examples exhibit markedly different isotopic signals, illustrating the challenges in interpreting early rock records in terms of metabolic activity, environmental conditions, and preservation. Calcitic stromatolites from the Tumbiana Formation display $\delta^{13}C_{carb}$ values near 0‰, like in modern marine environments, and $\Delta^{13}C_{DIC\text{-}OC}$ consistent with biomass derived from both CO_2 and CH_4 (Thomazo et al. 2009; FIGS. 1A and 2). Tumbiana's extreme $\Delta^{13}C_{carb\text{-}oc}$ signal, up to 48‰, is often cited as evidence for methane cycling in early Earth ecosystems, consistent with the "Faint Young Sun paradox": the sun was dimmer in Earth's early history, yet estimates of warmer surface temperatures imply a stronger greenhouse effect (Sagan and Mullen 1972).

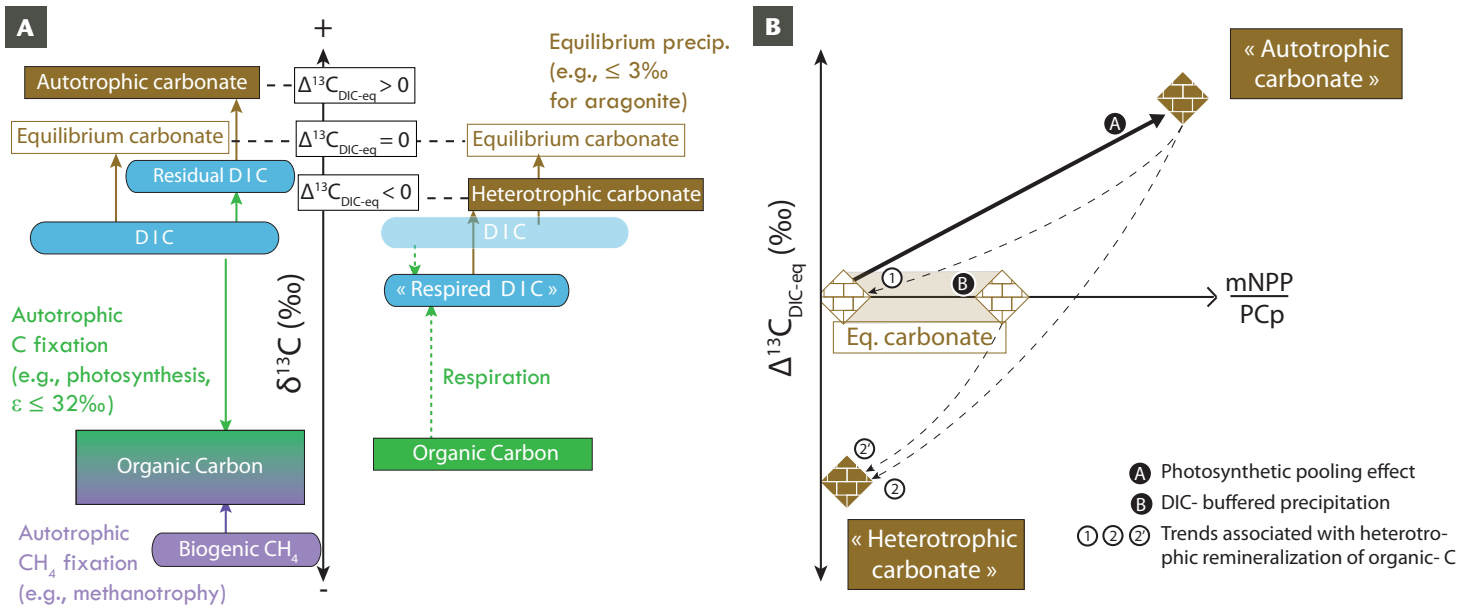

FIGURE 1 Schematic view of the isotope exchanges recorded by organic and inorganic carbon in microbialites. (**A**) Metabolic reactions consume or produce dissolved inorganic carbon (DIC), affecting its isotope composition and hence that of microbialite carbonates precipitating from it. The difference of $\delta^{13}C$ values between these carbonates (brown boxes) and those precipitating in equilibrium with a DIC pool unaffected by metabolic activities (white boxes) is represented with the $\Delta^{13}C_{DIC\text{-}eq}$ notation. Metabolic fractionations (ε) are expressed as: $\varepsilon = \delta^{13}C_{reactant} - \delta^{13}C_{product}$. Autotrophic fixation of biogenic CH_4 (which bears strongly negative isotopic signatures) can significantly decrease the microbialite $\delta^{13}C_{OC}$. (**B**) The $\Delta^{13}C_{DIC\text{-}eq}$ recorded by microbialite carbonates varies according to the ratio between microbialite net primary productivity (mNPP) and physicochemical precipitation (PCp, i.e., from non-biologically-cycled DIC), which is favored in highly super-saturated solutions.

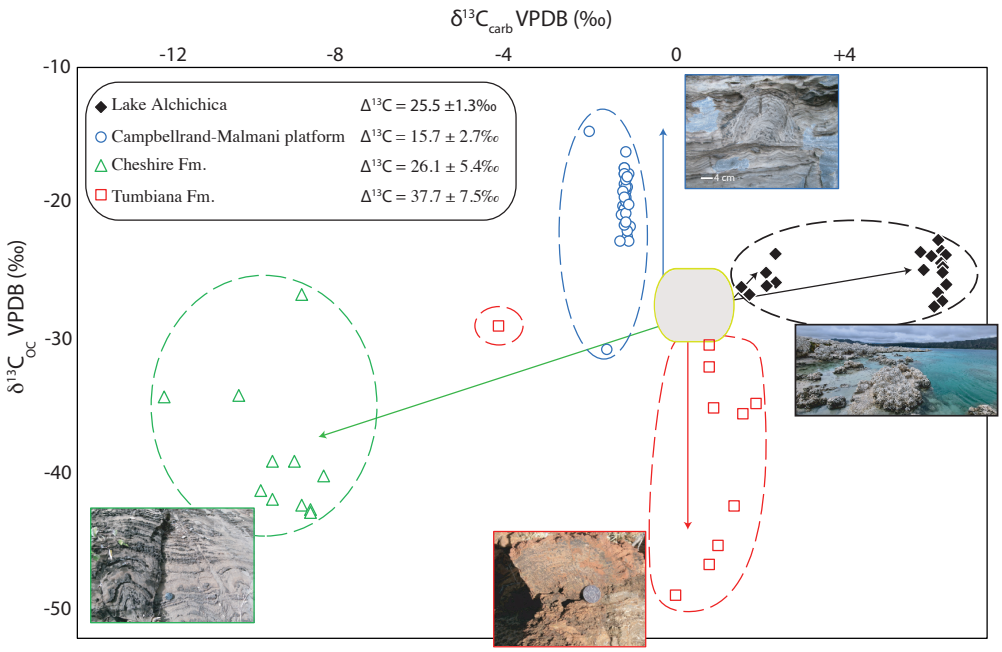

Figure 2 Paired $\delta^{13}C_{Carb}$–$\delta^{13}C_{OC}$ records of three Neoarchean stromatolites and modern microbialites from Lake Alchichica, Mexico, and marine sediments (gray shaded area; Krissansen-Totton et al. 2015). Mean and standard deviation of $\Delta^{13}C_{DIC\text{-}OC}$ (equal to $\delta^{13}C_{DIC} - \delta^{13}C_{OC}$) are reported; considering a temperature of 30 °C, HCO_3^- as the major DIC species, and a difference between Neoarchean carbonates and DIC of 1‰ and 3‰ for calcite and dolomite, respectively (see Havas et al. 2025 for calculation details). Deviations from typical marine records are underlined with arrows and reflect the complex interplay between carbon source, metabolic pathways of carbon fixation, and secondary processes that can shift both carbonate and organic matter isotopic signals. Black arrows reflect the effects of continentality and local sources generating heterogeneity in Alchichica's microbialites $\delta^{13}C_{Carb}$ (Havas et al. 2025).

Dolomitic stromatolites from the Cheshire Formation deviate from modern reference because both $\delta^{13}C_{OC}$ and $\delta^{13}X_{carb}$ are strongly ^{12}C-enriched. However, their $\Delta^{13}C_{DIC\text{-}OC}$ values fall within the range of modern microbialites using the CBB cycle. Given their contemporaneity with the Tumbiana Formation, their heavy dolomitization and association with organic-rich shales, the most plausible interpretation is that the $\delta^{13}C_{OC}$ reflects methane cycling, while the $\delta^{13}C_{carb}$ records secondary recrystallization from ^{12}C-enriched fluids (e.g., from organic matter oxidation such as "heterotrophic carbonate"; Fig. 1A; Thomazo et al. 2013). In this case, $\Delta^{13}C_{DIC\text{-}OC}$ is inconclusive.

Dolomitic stromatolites from the Campbellrand-Malmani carbonate platform exemplify further interpretive complexity due to high-temperature metamorphism (greenschist facies, ~395 ± 30 °C). At such temperatures, $\delta^{13}C_{OC}$ values can be significantly altered through graphitization and carbonate-graphite isotopic exchange, inducing fractionations of a few permil to 20‰ (Kitchen and Valley 1995). Nonetheless, part of this $\delta^{13}C_{OC}$ heterogeneity may also reflect the variability of fractionation factors associated with different autotrophic C fixation pathways (Eroglu et al. 2017).

Overall, the Tumbiana stromatolites provide evidence of a sustained methane biosphere, whereas the Cheshire and Campbellrand-Malmani stromatolites need careful assessment of secondary processes.

NITROGEN ISOTOPES: A REDOX PROXY

Nitrogen is an essential nutrient for life, and its isotope composition ($\delta^{15}N$) serves as a powerful tracer of redox conditions due to the diverse N-based metabolic pathways that operate under different redox states and its relative resistance to alteration by metamorphism (Pellerin et al. 2024; Stüeken et al. 2024; Fig. 3). The primary source of fixed N in aqueous environments is biological nitrogen fixation, which converts atmospheric N_2 ($\delta^{15}N \approx 0‰$) into bioavailable forms. Subsequent remineralization of organic matter to NH_4^+ (ammonification) occurs with minimal isotopic fractionation ($\delta^{15}N < \pm 2‰$). However, further transformations such as the oxidation of NH_4^+ to NO_2^- and NO_3^- (nitrification) and the reduction of NO_3^- to gaseous N_2O or N (via denitrification, dissimilatory nitrate reduction, or anammox) can induce substantial isotopic fractionation (up to 55‰). The expression of these fractionations depends on the dynamics and size of the N reservoirs. For instance, no fractionation occurs if the conversion is complete.

Recent $\delta^{15}N$ measurements in modern microbialites from Lake Alchichica, a redox-stratified, monomictic lake, reveal a systematic increase in $\delta^{15}N$ values from +1‰ to +12‰ across the redox gradient (Fig. 4). This trend likely reflects the influence of redox-dependent nitrogen cycling, with higher $\delta^{15}N$ values corresponding to microbial processes under increasingly anoxic conditions.

The low $\delta^{15}N$ values (near 0‰) recorded in both planktonic and microbialite organic matter of Lake Alchichica surface waters, point towards biological nitrogen fixation (diazotrophy). By contrast, deep microbialites, particularly those at or below the redoxcline, exhibit significantly higher $\delta^{15}N$ values (>10‰; Fig. 4). This enrichment arises from active nitrate cycling at the redoxcline (Pajares et al. 2017), where coupled nitrification-denitrification reactions generate ^{15}N-enriched ammonium in the anoxic bottom waters during stratification. Subsequent assimilation of this isotopically heavy ammonium into microbialite biomass explains the observed $\delta^{15}N$ depth gradient. The relatively low $\delta^{15}N$ values of the early stratification period (as recorded by deep plankton samples) suggest that anoxic microbialites from a permanently stratified water column could record even stronger ^{15}N enrichments. The systematic variability in microbialite $\delta^{15}N$ may thus serve as a potential proxy for (1) their formation depth (redox conditions) and (2) the stability of water-column stratification (Newell et al. 2017).

These principles extend to ancient stromatolites, where $\delta^{15}N$ trends have been used to reconstruct the evolution of the nitrogen cycle across major Earth redox transitions from an anoxic Archean world, through redox-stratified Proterozoic oceans, to modern oxygenated conditions (Stüeken et al. 2024). Notably, $\delta^{15}N$ signals from the Tumbiana, Cheshire, and Campbellrand-Malmani stromatolitic carbonates suggest distinct microbial metabolisms (Figs. 3 and 5),

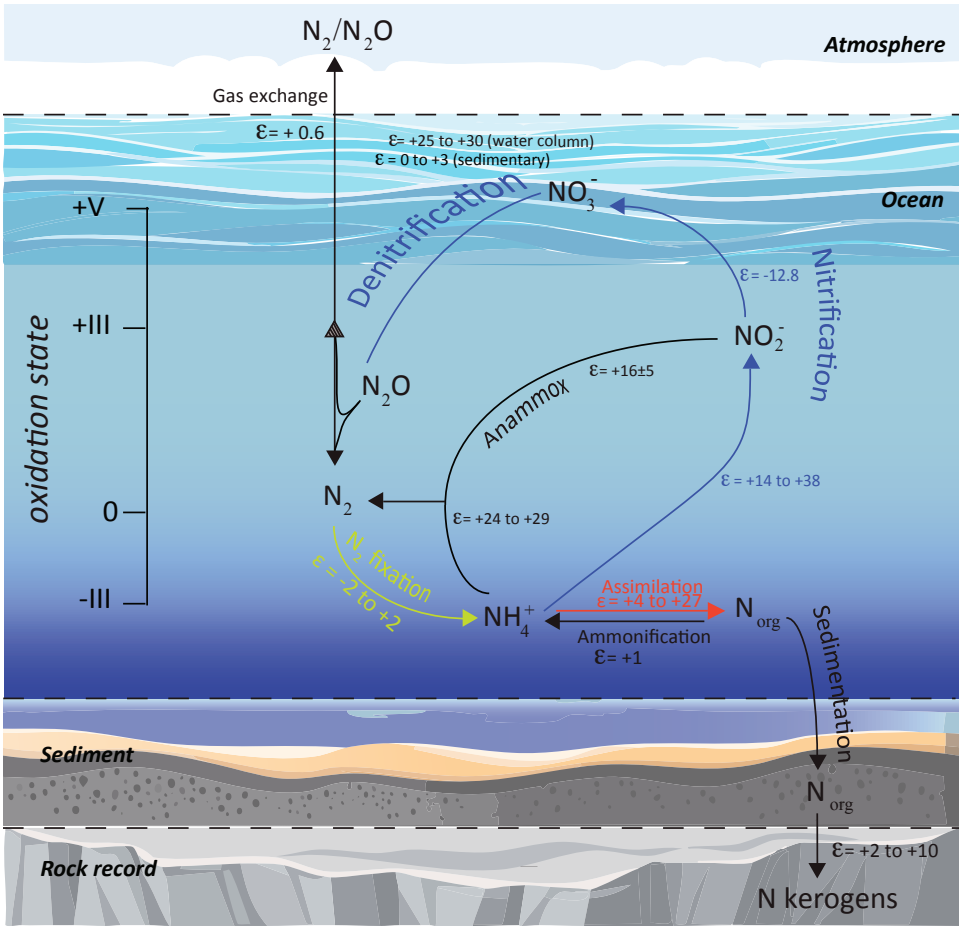

Figure 3 Simplified representation of key marine redox reactions in the biogeochemical nitrogen cycle. Isotopic fractionation (ε) is expressed as: $\varepsilon = \delta^{15}N_{reactant} - \delta^{15}N_{product}$. (For a comprehensive review of marine N cycling and associated isotopic fractionation, see Stüeken et al. 2024).

possibly linked to local redox heterogeneity or specific nitrogen-transforming pathways in late Archean microbial ecosystems.

The stromatolitic record of the Tumbiana Formation shows exceptionally high $\delta^{15}N$ values ranging from +15.1‰ to +35.4‰ (Fig. 5). This pronounced ^{15}N enrichment has been interpreted as evidence for ammonium oxidation (nitrification) coupled with denitrification, i.e., the microbial reduction of nitrite/nitrate to N_2 (Thomazo et al. 2011; Fig. 3). These data suggest the emergence of oxidative nitrogen cycling processes prior to the Great Oxidation Event, implying localized oxygen production sufficient to sustain nitrification. Supporting this interpretation, contemporaneous deep marine deposits from 2.7 Ga show similarly elevated $\delta^{15}N$ signatures (Pellerin et al. 2024). Together, these records indicate that: (i) oxygenic photosynthesis was likely well established by 2.7 Ga; (ii) resultant O_2 production enabled an intermediate nitrogen cycle where (iii) ammonium oxidation occurred in oxygenated surface waters, while (iv) nitrite/nitrate were subsequently reduced in adjacent anoxic zones. The Cheshire Formation stromatolites show an even higher average nitrogen isotope signal with $\delta^{15}N$ values ranging from +25.97‰ to 34.98‰ (Martin et al. 2025; Fig. 5). This extreme enrichment likely reflects a hydrothermal-driven nitrogen cycle where volcanic activity released deep-water ammonium that underwent progressive ^{15}N enrichment through partial assimilation in the water column. Upwelling transported this heavy ammonium to shallow stromatolite-forming environments, where ammonium oxidation in locally oxic conditions further amplified the isotopic signal. The hydrothermal system may have played a dual role by both supplying ^{15}N-enriched ammonium and delivering nutrients such as iron that stimulated primary productivity, potentially supporting oxygenic photosynthesis in shallow waters.

Finally, the Campbellrand-Malmani stromatolites, studied as potential archives of oxygenic photosynthesis during the Great Oxidation Event, present an intriguing paradox. While Neoarchean shallow platforms were presumably favorable niches for early aerobic ecosystems, these carbonates exhibit $\delta^{15}N$ values consistently near 0‰ (Fig. 5), contrasting with the positive $\delta^{15}N$ signatures (up to +10‰) in contemporaneous basinal shales and the oxidative nitrogen cycling deduced from the 2.7 Ga $\delta^{15}N$ records. This stark environmental dichotomy may reveal fundamentally different nitrogen cycling regimes between open-water systems, as recorded by shales and microbialite microenvironments. The 0‰ values could be explained by several non-exclusive hypotheses: (i) cryptic nitrate cycling (nitrate that is fully consumed within microbial mats) leaving only a N_2 diazotrophic isotopic signal (Fig. 5); (ii) variable ammonium assimilation rates in the microbial mats or in their vicinity;

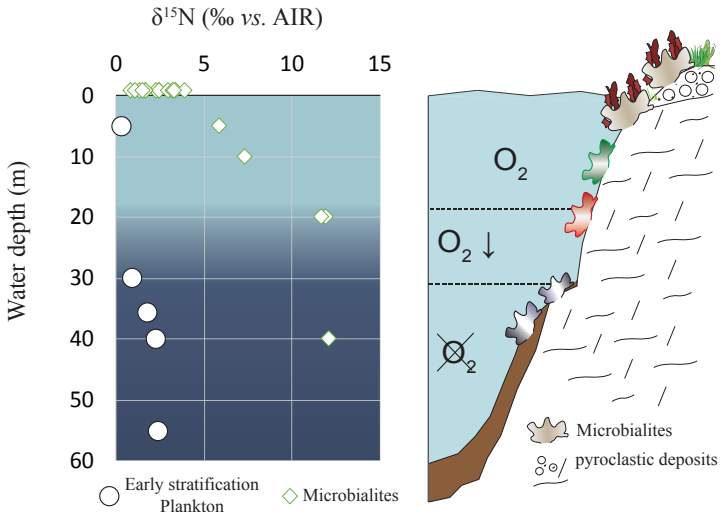

Figure 4 Bulk nitrogen isotope compositions ($\delta^{15}N$) of plankton and microbialites from the modern and redox-stratified Lake Alchichica (monomictic), sampled during the early stratification period of the lake (May). Microbialite variations in colors with water depth correspond to change in the nature of surface layer biofilms (Havas et al. 2025). Both show increasing $\delta^{15}N$ with depth and reducing conditions, reaching +12‰ in the microbialites growing at or below the oxycline.

or (iii) differential preservation of nitrogen isotopes in the highly dolomitized stromatolites of the Campbellrand-Malmani platform. We notice that microbialites forming in the fully oxic surface waters of Lake Alchichica similarly record low $\delta^{15}N$ (<4‰; FIG. 4). These findings suggest that Campbellrand-Malmani stromatolites may predominantly capture localized, short-timescale microbial processes rather than global-scale oxygenation trends suggested by the contemporaneous open-water record.

SULFUR ISOTOPES: A WINDOW TO MICROBIAL MAT ACTIVITY

Fool's Gold Within Microbialites!

The sulfur cycle is tightly coupled to oxygen cycling in microbialites and plays a pivotal role in their formation, primarily driven by the metabolic activities of sulfur-oxidizing and sulfate-reducing bacteria (SRB). These microorganisms influence the carbonate saturation state by changing alkalinity, pH, and ion availability. Despite a generally redox-layered structure, in situ probing has revealed widespread occurrence and activity of SRB throughout microbial mats (Visscher et al. 2000). These bacteria use sulfate as an electron acceptor to oxidize organic matter, generating bicarbonate and hydrogen sulfide and locally decreasing the oxidation state. Microbially derived H_2S can further react with Fe^{2+} to form iron sulfides such as pyrite (FeS_2). These reduced minerals can form in microbialites even under fully oxic water columns because of the anoxic micro-niches that exist within microbialites. The formation of pyrite is mainly controlled by the availability of iron, which explains why some microbialites are devoid of pyrites.

Two types of pyrite morphologies can be found in microbial mats: (i) framboidal pyrites, which correspond to spherical aggregates of microcrystalline pyrites, and (ii) micropyrites, which are often associated with organic matter (Marin-Carbonne et al. 2022).

Resolving Microbialite Sulfur Cycling Through Isotopic Analysis

The sulfur isotope composition ($\delta^{34}S$) recorded in pyrites primarily depends on the fractionation produced by SRB, which can be up to −72‰ between the sulfate and sulfide, while sulfur-oxidizing bacteria (SOB) tend to induce smaller fractionations (FIG. 6). Microbial sulfur disproportionation (splitting sulfur into sulfide and sulfate) has also been suggested in microbialites but cannot be firmly resolved based on currently available $\delta^{34}S$ data. The extent of SRB fractionation is primarily governed by the cell-specific sulfate reduction rate (csSRR), sulfate concentration, electron donor availability, and microbial phylogeny (Sim et al. 2023). Insights into environmental and ecological factors can thus be gained by analyzing isotopic differences ($\Delta^{34}S$) between sulfate and sulfide (FIG. 6; Toolkit).

Because bacterial sulfate reduction is a spatially discrete and locally influenced process, bulk $\delta^{34}S$ measurements tend to average isotopic signals and mask fine-scale metabolic heterogeneity. Recent advances in in situ isotopic mapping using SIMS and NanoSIMS (nanoscale secondary ion mass spectrometry) have enabled high-resolution investigation of sulfur metabolism within modern microbial mats (e.g., Fike et al. 2009) and lithifying microbialites (Marin-Carbonne et al. 2022). These techniques reveal $\delta^{34}S$ variations at the sample scale (i.e., a whole microbialite) and/or the mineral scale (i.e., inside the pyrite grains), highlighting the role of microbial microniches in controlling parameters like oxidant and organic C supply, Fe availability, porewater connectivity, metabolic diversity, or S re-oxidative cycling. Accordingly, sample ranges of $\delta^{34}S$ composition reflect local sulfide accumulation, while $\delta^{34}S$ variations at the framboidal pyrite scale suggest possible reoxidation (Pasquier et al. 2025).

Similar to pelagic sediments, petrographic characterization of pyrite in parallel with micro-scale isotopic analyses is essential for interpreting the primary and secondary processes that influence their isotopic signatures. For example, micropyrites can record near-equilibrium fractionation of SRB under low cell-specific sulfate reduction rates (under a wide range of ambient sulfate

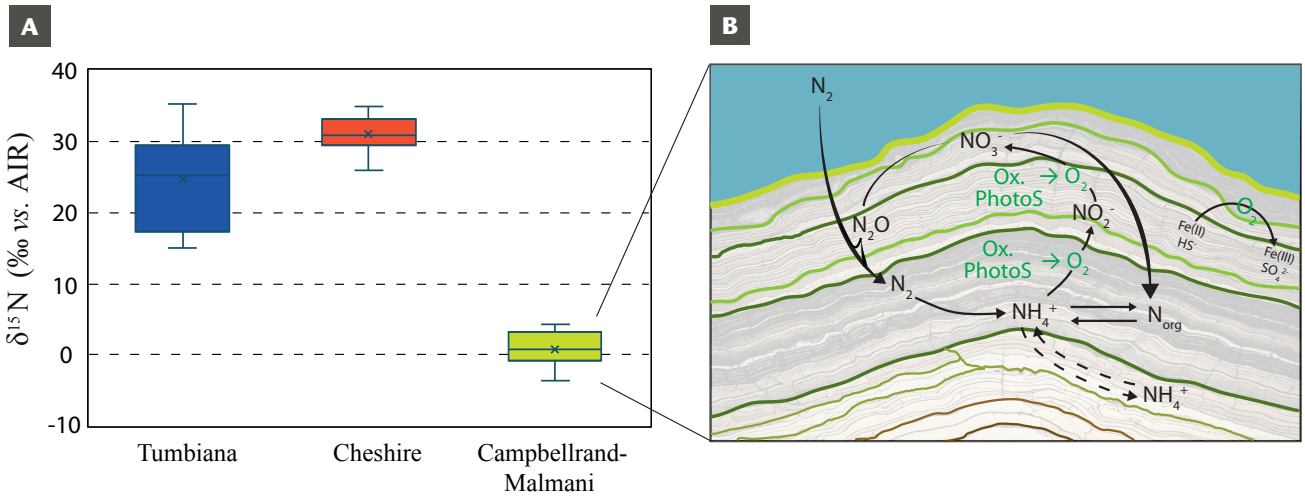

FIGURE 5 Boxplots of $\delta^{15}N$ values from three Neoarchean stromatolite formations and interpretative view of nitrogen cycling in the Campbellrand-Malmani stromatolites (**A**) Boxes show interquartile ranges, whiskers span 5th–95th percentiles (Thomazo et al. 2011; Martin et al. 2025). Differences in the three locations reflect distinct nitrogen-cycling regimes in Neoarchean microbial ecosystems. (**B**) Atmospheric N_2 is first fixed by photosynthetic organisms and converted to NH_4^+. Oxygen production in the mat oxidizes NH_4^+ to NO_3^-, which may be denitrified back to N_2. The isotopic fractionations associated with these reactions are suppressed due to a full consumption of NO_3^- (and residual NH_4^+) within the mat, causing the mat to record only the N source: atmospheric N_2 (0‰). Dashed arrows show that the $\delta^{15}N$ variability observed in Campbellrand-Malmani stromatolites could result from differential assimilation rates of NH_4^+ in distinct microniches of the mats. Oxygen cycling may also be cryptic if O_2 is fully consumed by the nitrification reactions and oxidation of other reduced species in the mat (e.g., Fe(II), HS^-).

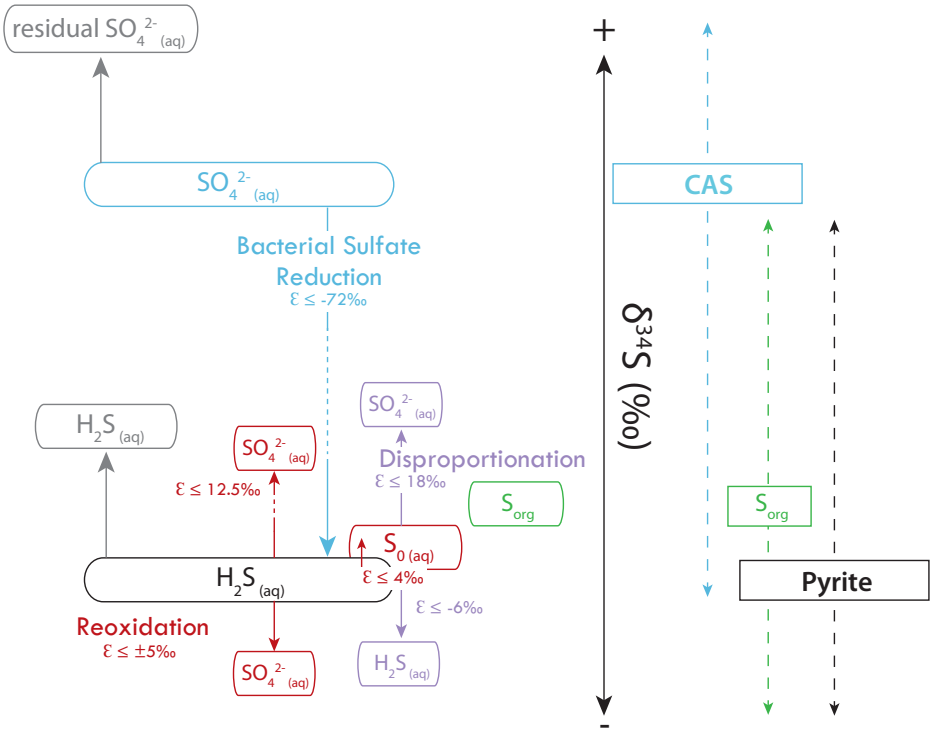

Figure 6 Schematic representation of the main reactions and associated isotopic signatures in the sulfur biogeochemical cycle of microbialites. Dissolved compounds and their corresponding mineral phases are represented on the left and right side of the vertical isotopic axis, respectively. Note that metabolic fractionations (ε) are expressed here as: $\varepsilon = \delta^{34}S_{product} - \delta^{34}S_{reactant}$. Values are provided to give a broad overview of ε fractionations' magnitude. Microbial sulfide (re)oxidation mostly remains between ±5‰ but can reach ≈ +12.5‰ (Pellerin et al. 2019). These fractionations, together with potential mixing between "primary" (blue) and "secondary" (red and purple) sulfates can lead to a huge range of $\delta^{34}S_{CAS}$. The grey arrows and boxes represent the effect of a Rayleigh distillation on a pool of residual "secondary" sulfate affected by SRB in a closed-system (e.g., in microbialites microniches, via porosity closure); sulfides produced from such distillation would follow the same increasing trend. Hence, $\delta^{34}S_{pyrite}$ can vary widely up to the composition of the initial sulfate source. Organic sulfur isotope compositions can show a wide range of variations but will approach that of sulfides (Fakhraee et al. 2025).

concentrations from 2 to 62 mM), whereas framboidal pyrites may record more significant ^{34}S enrichment due to porewater sulfate depletion and/or S oxidation (Fig. 6; Marin-Carbonne et al. 2022). Similarly, spatially resolved S isotope measurements have uncovered complex microbial interactions, including tight coupling between sulfate reducers, sulfide oxidizers, and phototrophic communities in the fossil record back to the Precambrian (Gomes et al. 2018).

Sulfur Isotope Composition in Archean Stromatolites: Insights Into Past Microbial Activity

The $\delta^{34}S$ signal preserved in Archean stromatolites provides critical insights into ancient microbial metabolism and the evolving redox conditions at Earth's surface prior to the Great Oxidation Event (see also Bruggmann et al. 2026 this issue). Stromatolites, as the oldest undisputed evidence of life, record the isotopic fingerprints of early sulfur-cycling microorganisms, particularly SRB. For example, micropyrites from the Tumbiana Formation analyzed using SIMS preserve strongly negative $\delta^{34}S$ values (down to −53‰), which are interpreted to reflect near-equilibrium sulfate reduction fractionation (Marin-Carbonne et al. 2018). However, disseminated pyrite in the stromatolite matrix, as well as the bulk sulfur isotope composition, carry near-zero $\delta^{34}S$ values (Marin-Carbonne et al. 2018; Thomazo et al. 2009), typical of the Archean siliciclastic record. The Tumbiana stromatolites are the only known Neoarchean stromatolites to record typical SRB isotopic signatures. The Cheshire stromatolites investigated at bulk level show a small range of variations centered around zero (between −2.1‰ and 1‰; Thomazo et al. 2013); nonetheless, negative $\delta^{34}S$, down to −15.2‰, occurred in the underlying Manjeri Formation black shales. To date, no SRB signatures have been detected in the stromatolites of the Campbellrand-Malmani platform. Although SRB are widely associated with modern microbialites, isotopic evidence of their metabolism remains rare in the geological record. This scarcity may result from micropyrite being frequently overlooked, the extensive alteration of pyrite's sulfur isotope composition by secondary fluid circulation, or the absence of conditions favorable for both pyrite formation and preservation.

Sulfate trapped in the carbonate lattice, named carbonate-associated sulfate (CAS), could be an interesting proxy for reconstructing ancient sulfate concentration. However, previous CAS isotope measurements from Archean microbial carbonates hint at complex interpretations due to the very low S concentration of such samples. Besides, local microbial S cycling and reoxidation (Fig. 6) make microbialites questionable archives to reconstruct sulfate reservoirs at the basin scale. Consequently, more studies on modern microbialite environments are essential.

CONCLUSIONS

Combined $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ records allow identification of the main metabolic groups participating in stromatolite formation: photosynthesis, methanogenesis/methanotrophy, sulfate reduction, and nitrogen fixation across 3.5 billion years of Earth's history. A sustained Neoarchean methane-based biosphere, for example, can be inferred from extreme $\Delta^{13}C_{Carb\text{-}OC}$ values. Nitrogen isotopes provide a valuable record of stepwise ocean oxygenation before the Great Oxidation Event, while sulfur isotopes in stromatolites preserve some of the oldest evidence of BSR on Earth. Modern analogues are essential for interpreting these isotopic signals and for understanding the spatial organization of metabolisms within the mat. Finally, diagenetic processes such as dolomitization, metamorphism, and fluid circulation can reset primary signals, requiring careful microscale assessment before drawing palaeobiological conclusions.

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