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Microbial lithification in marine stromatolites and hypersaline mats

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Lithification in microbial ecosystems occurs when precipitation of minerals outweighs dissolution. Although the formation of various minerals can result from microbial metabolism, carbonate precipitation is possibly the most important process that impacts global carbon cycling. Recent investigations have produced models for stromatolite formation in open marine environments and lithification in shallow hypersaline lakes, which could be highly relevant for interpreting the rock record and searching for extraterrestrial life. Two factors that are controlled by microbial processes and physicochemical characteristics determine precipitation: exopolymeric substances and the saturation index, the latter being determined by the pH, $\{Ca^{2+}\}$ and $\{CO_3^{2-}\}$. Here, we evaluate community metabolism in microbial mats and hypothesize why these organosedimentary biofilms sometimes lithify and sometimes do not.

Lithification in the history of the Earth

Approximately 3.5–3.8 billion years ago, at the onset of life on our planet, complex microbial communities formed. These microbial communities orchestrated the precipitation of calcium carbonate and the formation of stromatolites, recording a permanent imprint of their presence on the Earth [1,2]. Stromatolites are lithifying organosedimentary structures formed by trapping and binding of sediment and/or the net carbonate-precipitating activities of microorganisms, resulting in a layered structure. As the first photosynthetic communities, proliferating in the shallow zone of the oceans, they consumed the greenhouse gas CO_2, and produced free O_2 [3,4] and H_2 [5]. The evolutionary processes that drove the formation of these lithifying prokaryotic communities remain largely uncharacterized [6], although it has been speculated that microbial lithification resulted as a by-product of metabolism [7] or as a direct consequence of microbes harvesting energy from protons released during calcium carbonate ($CaCO_3$) precipitation [8]. Regardless of origin, microbial lithification represents what could be seen as a major evolutionary advance that enabled stromatolites to thrive for almost 85% of the Earth's history [1], having a crucial role in regulating sedimentation and global biogeochemical cycles.

After the decline of stromatolites in the late Proterozoic (ca. 543 million years before present), microbially induced and/or controlled precipitation continued throughout the geological record as an active and essential player in most aquatic ecosystems [9,10]. Although less abundant than in the Precambrian, microbial precipitation is observed in a multitude of semi-confined (physically or chemically) to confined macro- and micro-environments, from the deep sea to shallow platforms and terrestrial deposits. Microbial precipitation is proposed to have a role in the formation of sedimentary carbonate sedimentary particles [11,12], and terrestrial consortia of microorganisms through oxalate-carbonate transformation can sequester large amounts of CO_2 by precipitating $CaCO_3$ [13,14]. Lithifying microbial communities seem to be crucial to the settlement and edification of reefs through stabilization of sediments and in filling the porosity inside the reef body through precipitation. In Mesozoic coral and sponge reefs, for example, microbially induced precipitation supported construction of a rigid framestone by 'cementing' the macrofauna inside the reef [15,16]. Even in the absence of macroscopic metazoans, microbes continue to build reef-like structures, such as mud mounds in the deeper oceans, the ecological implications of which are still poorly understood [10,17].

Stromatolitic structures are among the earliest macroscopic evidence for life on Earth [10,18], which raises the question of how precipitation and dissolution of minerals is facilitated by microorganisms. Microbially mediated precipitation is not limited to carbonates but might also comprise other minerals, such as silicates and sulfates (e.g. gypsum, anhydrite) [9]. Here, we explore calcium carbonate precipitation and dissolution as a result of coupled microbial metabolism (altering, for example the carbonate alkalinity and pH) and geochemical reactions. We focus on sedimentary biofilms in modern marine and hypersaline environments to potentially aid interpretation of the rock record and search for extraterrestrial life.

Requirements for lithification

There are several models for lithification, ranging from entirely microbial [19–22] to purely chemically mediated mechanisms [23]. Cyanobacteria have been implicated in $CaCO_3$ precipitation (for example, see Ref. [13]) (Figure 1),

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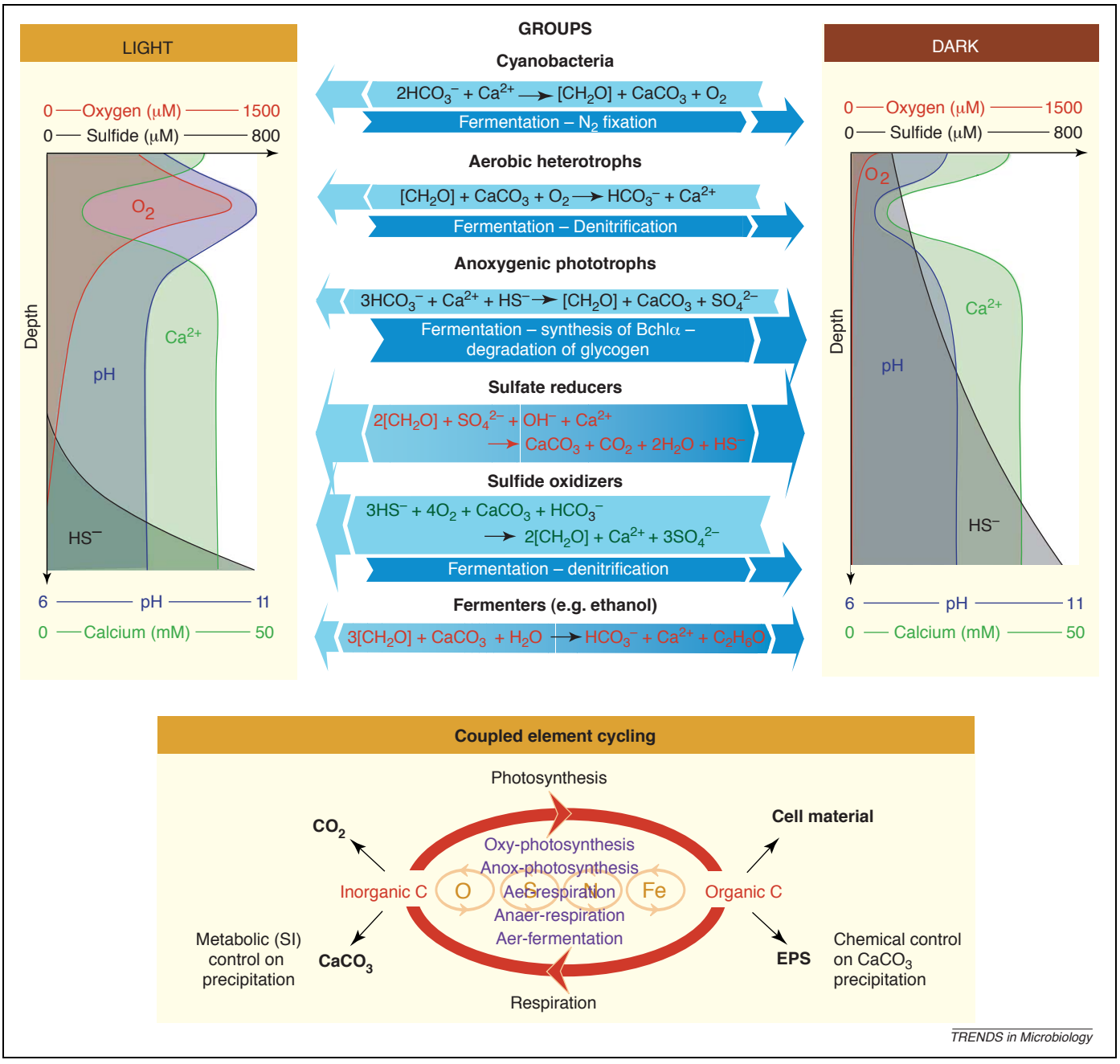

Figure 1. Metabolic pathways and geochemical gradients in a lithifying microbial mat. Six major groups of microbes composing the microbial mat community impact calcium carbonate precipitation and dissolution through metabolic activities (as outlined in the coupled microbial–geochemical equations presented in the main text). Combined metabolic activities determine the net precipitation potential and also the vertical geochemical gradients (left and right panels). Note the extreme diel fluctuations of oxygen, sulfide and pH caused by dependence of photosynthesis on light. Differences in day and night metabolism (temporal separation; redrawn from actual measurements) and a certain degree of vertical structuring of the various metabolic reactions (spatial separation) cause local differences in the saturation index (SI), ultimately regulating chemical precipitation. The calcium profile shows a minimum due to sequestration by the exopolymeric substances (EPS), which is most abundant in the layer of maximum photosynthesis. Carbon cycling (bottom part of figure) is coupled to element cycles of O, S and N, as these provide electron donors for C-fixation and electron acceptors for respiration, respectively. Preferential use of a certain cycle over another yields a greater precipitation potential (e.g. S > O) [21,27,28]. Similarly, excess carbon fixation enables, for example, EPS build-up, whereas excess anaerobic respiration favors precipitation through an increased SI [28].

as have aerobic heterotrophs [12] and sulfate-reducing bacteria (SRB) [24–27]. Although precipitation has been well-studied and dissolution of carbonates has been largely neglected, it should be noted that net precipitation results from the balance of these two processes [28]. Pinckney and Reid [20] proposed a balance between photosynthesis (P) and respiration (R): if P > R then precipitation would take place; if P < R, dissolution would follow.

Two crucial factors that support $CaCO_3$ precipitation emerge: (i) a geochemical facet, the saturation index (SI), and (ii) a biological–chemical facet, exopolymeric substances (EPS). The saturation index is defined as $SI = \log(IAP/K_{sp})$, where IAP denotes the ion activity product (i.e. $\{Ca^{2+}\} \times \{CO_3^{2-}\}$) and K_{sp}, the solubility product of the corresponding mineral ($10^{-6.19}$ and $10^{-6.37}$ for aragonite and calcite, respectively, at 25°C, 1 bar atmospheric pressure and 35 PSU salinity [29]). If $IAP > K_{SP}$, then the solution is supersaturated, and when $SI > 0.8$, then $CaCO_3$ precipitates [30]. The $[CO_3^{2-}]$ depends on the carbonate equilibrium ($H_2CO_3 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$; p$K_a$ of 5.9

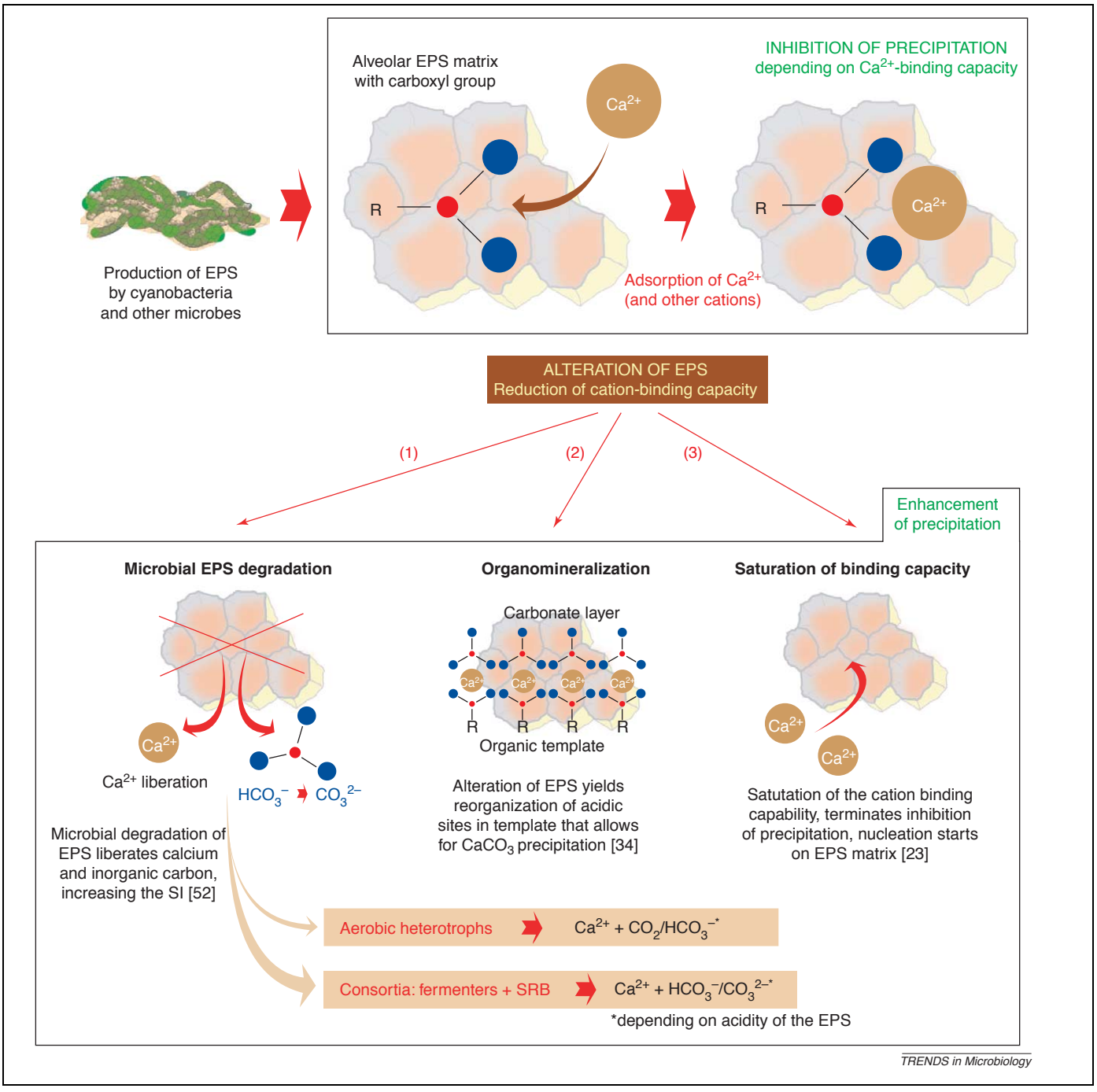

Figure 2. The role of exopolymeric substances in calcium carbonate precipitation. Initially, EPS produced by various microbes, predominantly cyanobacteria, binds cations, including Ca^{2+}, which inhibits precipitation. Following this, microbial and/or chemical alteration via the three different pathways enables $CaCO_3$ to precipitate.

and 8.9 at 25 °C, 1 bar pressure and 35 PSU salinity [29], respectively), and hence, the pH. Thus, when evaluating the role of microbial metabolism in $CaCO_3$ precipitation, production and/or consumption of inorganic carbon and the pH change of the various reactions needs to be considered [25,28] (Figure 1).

The EPS matrix represents an extension of the microbial cell [31] and a pliant matrix for structuring associations within microbial communities. EPS consists of a variety of molecules such as polysaccharides and amino acids. Although cyanobacteria are believed to produce the bulk of the production, other microorganisms excrete EPS with different composition and structure, the content of which might vary with different stressors and/or environmental conditions [31,32]. The specific EPS characteristics might regulate physiological processes and interactions within the microbial community. EPS functions as a chelator for cations and the template for crystal nucleation [31–34] (Figure 2). Some EPS macromolecules contain hydroxyl and/or carboxyl groups that strongly bind Ca^{2+} and other cations, such as Mg^{2+}, inhibiting $CaCO_3$ precipitation [33]. EPS is under constant modification through physicochemical (e.g. by UV radiation, pH, free radicals) and/or microbial degradation (e.g. through hydrolysis, decarboxylation).

Three different types of EPS alteration leading to $CaCO_3$ precipitation have been proposed (Figure 2): (i) microbially

mediated decomposition of EPS, liberating HCO_3^- and Ca^{2+}, both of which activities will increase the SI, producing 'hotspots' of precipitation (further discussed in subsequent sections); (ii) so-called organomineralization [34], during which the EPS matrix is altered, either through chemical or microbial activity. This reorganizes acid binding sites, creating a template, which enables $CaCO_3$ to precipitate; and (iii) precipitation regulated by the balance of the external cation concentration and binding capacity of EPS: when the available negatively charged groups are saturated with Ca^{2+}, precipitation can commence [23]. The mineralogy (calcite versus vaterite) and morphology (spherulites versus rhombohedra) of crystalline phase are controlled by the quality and quantity of EPS [14]. In practice, all three types of EPS alteration could result, at least in part, from microbial activity.

Microbial mats

Although discussions of the biogenicity of stromatolites continue [1], these layered sedimentary structures bear great resemblance to contemporary microbial mats [18]. As such, microbial mats have been investigated extensively as analogues for ancient stromatolites [35]. Microbial mats are vertically laminated, sedimentary biofilms found in lagoons, marine intertidal and subtidal zones, hypersaline ponds, hot springs and fresh water rivers and lakes [36]. The classic view of a mat is that each layer contains different microorganisms with distinct metabolic activities [37]. This view has been revised as, for example, active SRB have been found at the surface of mats [38,39] (Box 1).

Microbial mats are primarily composed of six functional groups of microbes [21,28,40] (Figure 1): (i) oxygenic phototrophs (cyanobacteria) are the primary producers, coupling light energy to CO_2 fixation, and sometimes fix N_2 [41]. Through EPS production and other mechanisms, filamentous cyanobacteria, and to a lesser extent coccoid forms, have an important role in trapping and binding of sediment; (ii) anoxygenic phototrophs (purple and green bacteria), use HS^- as electron donor for photosynthesis, and some fix N_2; (iii) aerobic heterotrophic bacteria gain energy from respiration of O_2 and organic carbon; (iv) fermenters, use organic carbon or sulfur compounds [42,43] as electron donor and acceptor; (v) anaerobic heterotrophs, predominantly SRB, respire organic carbon with SO_4^{2-} while producing HS^-; and (vi) sulfide oxidizing bacteria (SOB), many of which are chemolithoautotrophs that oxidize reduced sulfur compounds with O_2 or nitrate while fixing CO_2. This view of the mat composition might have to be revised as nucleic acid sequences will undoubtedly reveal a great diversity and complex community structure. Microbial mats are efficient in element cycling and, once developed, require little more than light to function. As such, they can be viewed as semi-closed systems [22], making it fairly easy to create mass balances and study element cycling. Compared with other benthic ecosystems, microbial mats harbor the highest metabolic rates (e.g. of photosynthesis, aerobic respiration and sulfate reduction) [44], which, per surface area, rival that of rain forests [45].

Cyanobacteria are the driving force of biogeochemical cycling in the mats: CO_2 and N_2 fixation by these organisms provide the crucial components for the system to function [22]. The high photosynthetic rates results in supersaturated [O_2] and high pH values during the late afternoon (Figure 1). Usually, chemolithotrophs (SOB) and anoxyphototrophs have a minor role in C fixation [40,45]. Aerobic heterotrophs respire during the daytime when O_2 is abundant, rapidly causing anoxia at the end of the light period. During daytime and nightime, fermenters and SRB also degrade organic carbon. These two groups work in concert (Figure 2), the former partially degrading complex organic molecules (including polysaccharides), benefiting the SRB, which typically rely on small organic molecules (e.g. short-chain fatty acids, alcohols). Respiration using O_2 and SO_4^{2-}, in most cases, consumes equal amounts of carbon in marine and hypersaline mats, whereas the role of fermentation, although acknowledged, is undetermined. Several studies [22,40,46] have reviewed the interactions among the functional groups. The combined community activity results in steep vertical geochemical gradients with extreme diel fluctuations (Figure 1).

To understand the role of microbial mats in precipitation and dissolution, it is important to determine both the abundance and metabolic activity of the key functional groups, and possibly even that of individual species within the functional groups. The metabolic activity of the community ultimately determines the quality and quantity of EPS, in addition to the pH, concentration of Ca^{2+} and CO_3^{2-}, and thus the SI of $CaCO_3$.

Lithifying versus non-lithifying mats

Microbial communities produce a range of carbonate precipitates with a composition and crystallography that is influenced by environmental conditions and species composition. In fresh water precipitates, particular cyanobacterial species are associated with specific crystal morphologies, often showing sheaths impregnated with calcite [47]. By contrast, few cyanobacterial casts are found in recent and fossil marine environments [1]. Marine microbial deposits exhibit a wide range of mineral compositions (e.g. low-to-high Mg calcite, aragonite) with many distinct crystal microstructures (amorphous, cryptocrystalline to microsparite, rods or needles), mesostructures (dense micritic, peloidal and agglutinated forms) and macrostructures (organized in laminated, clotted or 'structureless' macrostructures) [12,15,48]. Several microbial mat systems produce carbonate phases: travertine in hot springs in Yellowstone [49], dolomite in Lagoa Vermelha, Brazil [26], an unknown carbonate phase in Lake Chiprana, Spain [50], high Mg-calcite in Storr's Lake [51] and Salt Pan, Bahamas [52] and aragonite in modern marine stromatolite mats of Highborne Cay, Bahamas [53]. However, when exploring contemporary mats, most of which trap and bind sediments, it is clear that not all lithify and the fossilization potential varies vastly (Figure 3).

These regularly laminated, often dome-shaped structures are quintessential examples of lithified mats because they are easily recognized in the fossil record.

Box 1. Sulfur cycling and regulation of carbonate precipitation.

Sulfate-reduction activities and carbonate precipitation in a Highborne Cay stromatolite (Figure I).

Sulfate reduction

Sulfur metabolism impacts precipitation and dissolution in different ways. The equations shown here are examples of sulfate reduction, using a variety of low-molecular weight organic carbon compounds (Eqns 1, 2 and 3) and hydrogen (Eqn 4). SRB can be divided based on their capability to fully (completely) or partially (incompletely) oxidize organic carbon. Note the difference on calcium carbonate precipitation of the different reactions. For example, complete sulfate reduction (Eqn 1) produces three times as much $CaCO_3$ per lactate then incomplete sulfate reduction (Eqn 2) does, and ethanol oxidation (Eqn 3) precipitates less than H_2 oxidation (Eqn 4) does.

Lactate – complete

$$2C_3H_5O_3^- + 3SO_4^{2-} + 3Ca^{2+} \rightarrow 3HS^- + 3H_2O + 3CaCO_3 + 3CO_2 + H^+ \quad \text{(Eqn 1)}$$

Lactate – incomplete

$$2C_3H_5O_3^- + SO_4^{2-} + Ca^{2+} \rightarrow HS^- + H_2O + 2C_2H_3O_2^- + CaCO_3 + CO_2 + H^+ \quad \text{(Eqn 2)}$$

Ethanol

$$2C_2H_6O + 3SO_4^{2-} + OH^- + 2Ca^{2+} \rightarrow 3HS^- + 5H_2O + 2CaCO_3 + 2CO_2 \quad \text{(Eqn 3)}$$

H_2 – autotrophic

$$6H_2 + SO_4^{2-} + 2HCO_3^- + Ca^{2+} \rightarrow HS^- + [CH_2O] + 5H_2O + CaCO_3 \quad \text{(Eqn 4)}$$

Sulfide oxidation

Once produced during sulfate reduction, sulfide is reoxidized via several pathways (Eqns 5–8). Autotrophic SOB group according to high or low carbon-fixation yield (i.e. efficiency) [28] (Eqns 5 and 6). When oxygen is low, sulfide is high (e.g. at the end of the day; see Figure 1 in main text), incomplete oxidation follows, yielding intermediately oxidized S-species [28,39,40] that might diffuse away. This reaction precipitates carbonate. When oxygen is high (middle–late day), the intermediate S compounds are fully oxidized, dissolving carbonate. This two-step oxidation enables spatial and temporal separation of metabolic processes, which is characteristic for microbial metabolism associated with the C-S cycles (not for the C-O cycles, which is limited to daytime; see Figure 1 in main text).

High yield

$$3HS^- + 4O_2 + CaCO_3 + HCO_3^- \rightarrow 2[CH_2O] + Ca^{2+} + 3SO_4^{2-} \quad \text{(Eqn 5)}$$

Low yield

$$5HS^- + 8O_2 + 3CaCO_3 \rightarrow 2[CH_2O] + 3Ca^{2+} + 5SO_4^{2-} + HCO_3^- \quad \text{(Eqn 6)}$$

Step 1 – low O_2: polythionate production (no CO_2 fixation)

$$8HS^- + 6.5O_2 + 6HCO_3^- + 6Ca^{2+} \rightarrow S_8O_6^{2-} + 6CaCO_3 + 7H_2O \quad \text{(Eqn 7)}$$

Step 2 – high O_2: polythionate consumption (no CO_2 fixation)

$$S_8O_6^{2-} + 9.5O_2 + 14CaCO_3 + 7H_2O \rightarrow 8SO_4^{2-} + 14HCO_3^- + 14Ca^{2+} \quad \text{(Eqn 8)}$$

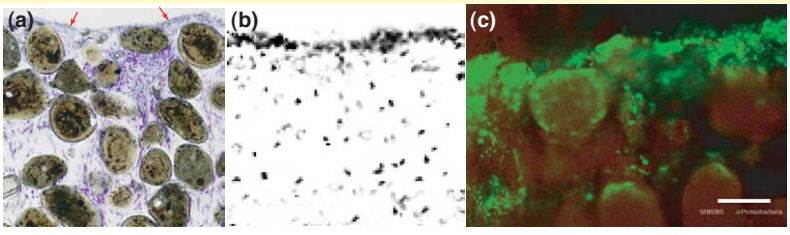

Figure I. (a) Low-magnification photomicrograph of a surface mat thin-section. Red arrows indicate the continuous thin micritic crust, precipitated on top of trapped and bound ooids. This thin surface crust is characteristic for stromatolite lamination. **(b)** Sulfate-reduction activity (dark pixels), mapped using ^{35}S-Ag-foil [27]. Density and size of pixels indicate location and relative activity (darker = higher). **(c)** Fluorescence *in situ* hybridization (FISH) image using probe to target the δ-proteobacteria with confocal scanning microscopy. The maximum fluorescence near the surface corresponds with the location of the micritic (a) and the maximum sulfate reduction rate (b).

However, it should be noted that not all lithifying mats form stromatolites, only those that form layer upon layer do so. Modern stromatolites exist in freshwater systems [47], hypersaline and open marine environments in the Bahamas [51,53] and Shark Bay, Australia [54]. Based on microscopic observation, models of Shark Bay stromatolite formation were proposed [55] and, in separate studies, some microbial activities were reported [54]. Recently, the open marine stromatolites on Highborne Cay (Bahamas) were targeted in a geomicrobial investigation. Three crucial stages in the formation of these subtidal stromatolites were found, each of which having a characteristic community composition of the surface mats [21,27,53,56]: (i) a filamentous cyanobacterial (*Schizothrix* sp.) dominated community, which binds and traps ooids. This stage is distinguished by relatively low biomass, low photosynthetic rates and few heterotrophic organisms compared with the other two stages; (ii) a more developed community, where aerobic and anaerobic microbes are abundant and highly active. This community produces copious amounts of EPS and precipitates a thin crust of microcrystalline $CaCO_3$ (micrite) (Box 1); (iii) a highly developed community, which includes coccoid endolithic cyanobacteria (*Solentia* sp.) [53]; this community forms a thicker lithified layer through boring of the $CaCO_3$ grains and welding these together. The lamination, which can be preserved in the rock record, is the result of cycling of these three microbial community types.

Another lithifying microbial mat system that was investigated using a combination of geological and microbial techniques is the hypersaline pond system of Salt Pan in Eleuthera, Bahamas [52]. In this shallow (<60 cm) pond, with approximately constant salinity (averaging 90 ppt), a gradient from lithifying mats, starting several

Figure 3. Model of controls on lithification. Four microbial mat types: the bottom two lithify, the top two do not lithify. Mats on the left harbor lower biomass and trap and bind sediments, the ones on the right have higher biomass systems that trap and bind relatively little sediment, and produce more exopolymeric substances (EPS; brown). The degree of environmental controls (blue arrow to the right of mat boxes) decreases from bottom to top, resulting in net precipitation in the bottom two mats, and little or no precipitation in the top two mats.

meters from the shoreline, to gelatinous soft mats towards the middle is present. The shallow water column contains cyanobacterial pigments, such as phycoerythrin and scytonemin, which greatly quench the light. As a result, the microbial activities (photosynthesis, aerobic respiration, sulfate reduction) are higher and geochemical gradients are steeper in the shallower lithifying mats. Furthermore, EPS destruction by UV radiation is greater in the shallow mats, removing the inhibition of precipitation by this extra-cellular matrix (Figure 2; Scenario 1). The combination of these processes results in precipitation, the sequence of which is shown in Figure 4. By contrast, precipitation in hypersaline Lake Chiprana is believed to result from P>R owing to a daily export of 50% of the photosynthate from the mat, with no particular role for EPS in this system [50]; however, further investigations are required.

Production and consumption of EPS

EPS prevents desiccation of the mat, retains essential nutrients, protects against UV radiation, and provides water channels for transport of metabolites and signaling

compounds [31,32,57]. UV radiation might cause browning (Maillard) reactions, and other types of weathering mechanisms (e.g. dehydration, high pH) exist [35,37]. EPS production in a stromatolite mat accounted only for a small fraction (ca. 8%) of $^{14}HCO_3^-$ uptake during the light, and a rapid turnover followed during the dark [58]. This suggests that the net EPS production was low and that a dynamic balance between production and consumption exists. Following hydrolysis, the EPS components were readily consumed by the mat community, particularly anaerobes. Surprisingly, the stimulation of anaerobic heterotrophic activity in mats was greater than that of aerobic heterotrophs when *Schizothrix* EPS, xanthan, or sugar and amino acid monomers and polymers that comprise EPS were supplied [27,43,58]. The combined action of fermentative organisms and SRB (Figure 2 and Box 1) could be responsible for this high consumption rate. Oxygen levels are subject to rapid and extensive fluctuations when the light regime changes (daytime-nighttime, cloud cover) and O_2-consuming cell clusters in the EPS can produce anoxic microenvironments, therefore, the anaerobic pathway could be important in microbial EPS degradation.

In addition to liberating Ca^{2+} and HCO_3^- during microbial alteration, EPS itself can influence chemical gradients that affect the mineral phase. The EPS matrix can reduce the mobility of hydrated Mg^{2+} (8 Å diameter) relative to hydrated Ca^{2+} (6 Å diameter). The delay of Mg diffusion would lead to an initial Ca-enrichment, decreasing the Mg:Ca ratio of mineral products forming inside the EPS [13]. As a result, changes in the amount or type of EPS could influence the rate of precipitation or types of crystals formed, as was observed in a magnesium increase in sequential lithified layers of mats in Lagoa Vermelha, Brazil (C. Vasconcelos and P.T. Visscher, unpublished results).

Microbial metabolism and saturation index

Simple reduction–oxidation reactions form the basis of microbial metabolism. These metabolic reactions often involve C and either O, S or N (Figure 1). Daytime and nighttime metabolism of the six key functional groups is typically different, especially when there is a dependency on O_2 (i.e. light). Chemical alterations of the (micro)-environment that result from different metabolic reactions might change the alkalinity and thus facilitate carbonate precipitation or dissolution [28]. Especially in microbial mats where the metabolic activities are extremely high, it can be anticipated that the SI changes rapidly, despite the buffering capacity of the carbonate system. Cyanobacterial photosynthesis, for example, fixes CO_2 ($CO_2 + H_2O \rightarrow CH_2O + O_2$). High rates of photosynthesis deplete CO_2, which necessitates a reestablishment of the carbonate equilibrium ($HCO_3^- \rightarrow CO_2 + OH^-$), and the alkalinity increase that results enables $CaCO_3$ precipitation through removal of the H^+ that is produced in the latter reaction ($Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$ and $OH^- + H^+ \rightarrow H_2O$). The combined microbial–chemical reaction is: $2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CH_2O + O_2$. Details of combined microbial–chemical reactions and their impact on SI, and thus $CaCO_3$ precipitation and dissolution, are published elsewhere [21,28] and overall reactions are provided in Figure 1. It should be noted that in these reactions, organic carbon is assumed to be CH_2O (a simplified denotation for photosynthate), and different outcomes are expected with different organic compounds. For example, the decomposition of carboxylic acids (e.g. acetate,

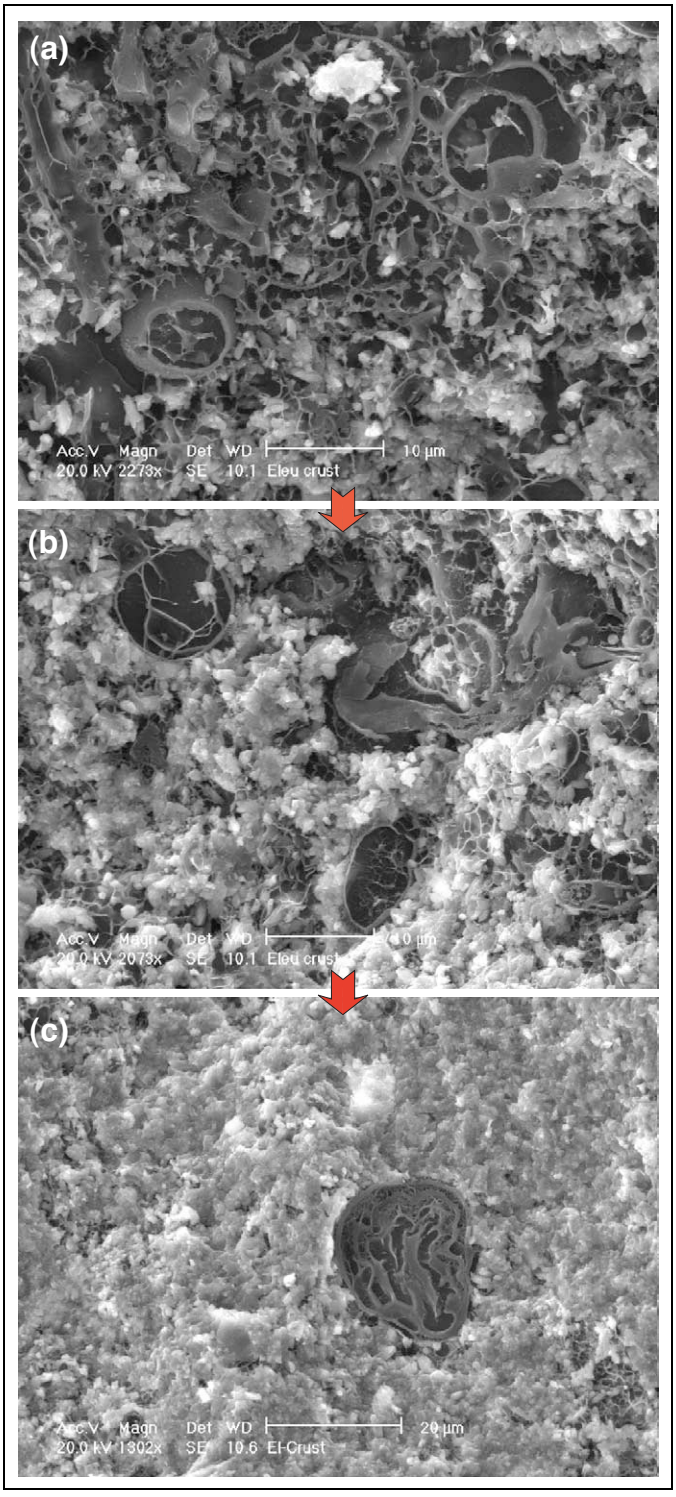

Figure 4. Photomicrographs showing the sequence of precipitation. The alveolar exopolymeric substances (EPS) structure is progressively replaced by high-Mg calcite. **(a)** Coccoid cyanobacteria embedded in EPS, in which precipitation is in the early stage. **(b)** EPS is gradually replaced by micrite. **(c)** Cocci (*Gloeocapsa* sp.) are completely surrounded by microcrystalline high-Mg calcite when the replacement of the EPS is complete. (Photomicrographs taken using low-temperature scanning electron microscopy using cryofixation.)

butyrate and lactate), producing CO_2 potentially results in an additional increase in carbonate alkalinity through CO_2 degassing (open/semi-closed system [14,29]). This could be the reason that heterotrophic aerobes have been shown to precipitate $CaCO_3$ [12,59]. Figure 1 outlines the overall (combined microbial–chemical) reaction for the six functional groups considered here.

Role of the sulfur cycle

Alteration of the SI in mats is clearly a combined community effort, however, evidence is compelling that alkalinity produced by SRB has a prominent role [24,25,27,54]. The crust in both Highborne Cay stromatolites and hypersaline Salt Pan mats coincides with the location of maximum sulfate reduction [27,52] and SRB abundance (Box 1). The use of different types of metabolic reactants (e.g. ethanol versus lactate) and products (e.g. HCO_3^- during complete versus acetate during incomplete oxidation), have different effects on the SI (Box 1). For example, complete oxidation by SRB potentially precipitates three times as much $CaCO_3$ as incomplete oxidation. Similarly, when considering CO_2 fixation in autotrophic SOB, the dissolution potential is reduced: CO_2 fixation supports precipitation, and the net effect is 0.5 mol (not 1 mol) $CaCO_3$ dissolved per HS^- oxidized. Furthermore, when the HS^- is only partially oxidized – for example, when $[O_2]$ is low – the incomplete S oxidation that results precipitates, not dissolves $CaCO_3$. When $[O_2]$ is high, oxidation of intermediate S compounds results in dissolution [28] (Box 1).

The above exemplifies that the impact of metabolic reactions is complicated, but also demonstrates that decoupling of the different metabolic processes in time and space controlled by physical (e.g. light, pH) and chemical (e.g. presence or absence of O_2) factors establishes the ideal conditions for net precipitation in a narrow horizon [21]. For example, SRB are active throughout the diel cycle, and although the maximum rates sulfate reduction are observed during daytime [38], dissolution by aerobic heterotrophs is absent during the night, creating the ideal scenario for net precipitation during that period.

Lithifying versus non-lithifying – a conceptual model

We have outlined how microbial mat communities can lithify but we have not addressed extensively why some mats do and others do not. Separation of metabolic processes in space and time is likely to contribute to net precipitation in modern marine stromatolites, where biomass is relatively low compared with (more) organic mats through 'dilution' by trapped and bound sediment (Figure 3). Additional extrinsic (e.g. local environmental conditions, including hydrodynamics, sediment transport, nutrients and light) and intrinsic (e.g. microbial growth rates, community succession and quorum sensing) factors cause the communities to cycle [56], which continually impacts SI and EPS properties and turnover within the stromatolite mats. The thin crusts of the hypersaline mats are formed through physicochemical and microbial controls on EPS: the depth of the overlying water column and, therefore, the amount of light and UV radiation, determines whether these high-biomass mats are crusty or soft ('bathymetric control'). Similarly, the high biomass hypersaline mats of Guerrero Negro, Mexico [60] are found at greater and constant water depth, which limits the environmental fluctuations, and thus the controls (e.g. light, UV radiation and ionic composition) on EPS and/or SI. By contrast, many modern intertidal mats trap and bind and lose sediments to tidal currents and winter storms, and precipitation is hampered by low biomass. These mats typically redevelop annually and accrete through trapping and binding of sediment, not through precipitation. A delicate balance of physicochemical processes and microbial activities is crucial in lithification. Further understanding of the intrinsic and extrinsic factors that drive precipitation and dissolution might provide insights into environmental conditions on Earth in deep time and aid in interpretation of the rock record on extraterrestrial planets [1,56]. The recent discoveries of ingredients for life on Mars (water, methane, sulfate minerals, and so on), possibly associated with shallow hypersaline pools [61], exemplifies the importance of unraveling microbe–mineral interactions.

The future of understanding the past

During the past decade, novel aspects of microbial carbonate precipitation have been uncovered. Pure culture studies and *in situ* measurements at increasingly smaller temporal and spatial scales, for the first time document differences between lithifying and non-lithifying microbial mats. An emerging pattern is that microbial processes through to metabolic processes impact mineral products. Changes in community composition and metabolism have been documented at the functional group level or in pure cultures at the species level. This leaves the need for a greater level of detail in field studies, in addition to a better linking of laboratory studies to the field. For example, we need additional information of the community composition at the species level, at short time intervals at small spatial scales. Molecular and other techniques face challenges from high mineral content and EPS matrices, although detailed 16S rRNA and gene sequence and *in situ* hybridization studies are well underway. The emerging field of glycobiology will undoubtedly aid the understanding of EPS. On a local scale, this EPS could have a crucial role in the regulation of microbial physiology through quorum sensing. As a matter of fact, assuming there is some link between modern and ancient stromatolites, the biofilms of these mats could hold important information about the long term properties of a highly successful ecosystem. Finally, group- or species-specific metabolism might be linked to particular mineral characteristics, which would provide a powerful geomicrobiological and paleoecological tool for understanding the past.

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