BIOFILMS

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INTRODUCTION

Most microorganisms found in natural, clinical, and industrial environments prevail associated with surfaces rather than as free-living (planktonic) organisms (Costerton & others, 1995; Flemming & Wuertz, 2019). These communities can develop as biofilms in a diverse range of environments (e.g. living tissues, indwelling medical devices, water distribution systems, natural aquatic and sediment systems, rocks, surfaces of buildings, stromatolites, etc.). Biofilms are "aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPS) adhere to each other and/or to a surface" (Vert & others, 2012, p. 383). The aggregation of cells can result in highly structured microbial communities that allow for cell-to-cell contact. This proximity of the cells, the intra- and intercellular interactions within the microbial community, and the properties of the EPS matrix can confer distinct emergent properties upon the biofilm substantially different from planktonic communities (Flemming & others, 2016). Biofilms are characterized by their unique: 1) physicochemical and biological heterogeneity, which provides habitat diversity; 2) services provided by the EPS matrix, which provides architecture and stability to the biofilm and acts as a protective barrier; 3) physical and social interactions, which in conjunction determine the survival strategies for the community, such as quorum sensing, gene exchange, EPS production, or coordination of metabolic action; and 4) increased tolerance and/or resistance to survive environmental stress (Costerton, Stewart, & Greenberg, 1999; Flemming & others, 2016). The biological and physicochemical characteristics of biofilms (e.g., structure, EPS production, and cell biomass) are the result of the environment, the nutritional and physical conditions in which the biofilm develops (Nielsen, Jahn, & Palmgren, 1997).

The significance of biofilms in the geological record of life was recognized by Noffke (2010). Examples of the manifestation of biofilms in the geological record include microbially induced sedimentary structures (MISS) and stromatolites (Astafieva, 2013; Noffke, 2010). These structures suggest that biofilms have existed throughout the geological record of life (Costerton & Stoodley, 2003; Noffke, 2010). Considering that cells within a biofilm can exhibit different phenotypes and change their metabolic activities compared to their planktonic counterparts, it is possible that biofilms induce distinct characteristics (e.g., structures, textures, chemical signatures) in the consolidated rock record. Thus, a better understanding of the biofilm way of life can aid in reconstructing the evolution of prokaryotes throughout Earth history.

BIOFILM FORMATION AND DEVELOPMENT

Biofilm formation follows a number of progressive steps including initial microbial attachment to a surface, microcolony formation, development of a three-dimensional community structure, maturation, and detachment.

ATTACHMENT OF MICROORGANISMS

The first step in biofilm formation is microbial attachment, which includes planktonic cells being able to find, interact with, and adhere to a surface. Microbial attachment is influenced by several factors, including the type of substratum (e.g. an inert surface or living tissue), hydrodynamics

of the aqueous medium, physicochemical characteristics of the medium (e.g., pH, nutrient levels, temperature), and properties of the cell surface and cell motility (Bouwer & others, 2000; Donlan, 2002; Palmer, Flint, & Brooks, 2007). Attachment is more likely to occur on surfaces that are rough, hydrophobic, and coated by conditioning films (i.e., surfaces in nature and industry are often at least partially coated by compounds—including polymers—from the liquid medium) (Donlan, 2002; Palmer, Flint, & Brooks, 2007).

FORMATION OF MICROCOLONIES

With the initial attachment of cells, microbial association to the surface (substratum) begins and—given appropriate growth conditions—becomes suitable for microcolony formation. During this stage of biofilm development, microbial cells undergo growth, which is usually accompanied by the excretion of EPS, resulting in the formation of aggregates or microcolonies. EPS production aids in promoting the irreversible attachment of cells to a substratum (Flemming & Wingender, 2010). Microbial aggregation also occurs as a result of the interaction of already attached cells and the recruitment of planktonic cells from the surrounding medium (McLean & others, 1997). Initial EPS production can be a response to attachment and environmental conditions such as osmotic pressure, pH, temperature, starvation and likely other factors (Flemming & others, 2016).

FORMATION OF THREE-DIMENSIONAL STRUCTURE AND MATURATION

Given suitable growth conditions, microcolonies develop into an organized structure over time and differentiate into true biofilms. Mature biofilms are typically comprised of multilayered microcolonies encased in EPS and separated by interspersed water channels. The EPS matrix has an active role in microbial attachment to surfaces, acts as a glue that keeps cells together, and allows for the development of a three-dimensional structure (Flemming & Wingender, 2010).

DETACHMENT

As the biofilm matures, detachment or dispersal occurs, which is crucial to the biofilm life cycle. Detachment of microbial cells occurs due to multiple factors including the lack of nutrients, competition, hydrodynamic stresses, among others (Stewart, 1993). The release and dispersion of microbial cells can lead to the formation of new biofilms (Stewart, 1993). Detachment can occur as a rapid, extensive loss of parts of the biofilm known as sloughing, or as continuous loss of single cells (small fractions of the biofilm) known as erosion (Bryers, 1988; Stewart, 1993). Detachment can influence the competition in biofilms (Morgenroth & Wilderer, 2000) and the biofilm morphology (Picioreanu, van Loosdrecht, & Heijnen, 2001). For instance, erosion can result in smoother biofilms, whereas sloughing usually increases the morphological heterogeneity of the biofilm (Picioreanu, van Loosdrecht, & Heijnen, 2001).

THE BIOFILM MATRIX

The biofilm matrix is a conglomeration of different extracellular biopolymers in which the biofilm cells are embedded. The microbial extracellular material, known as extracellular polymeric substances or EPS, typically accounts for ~90% of the biofilm, and the rest corresponds to biomass as well as minor components such as particulates, gas bubbles, etc. (Flemming & Wingender, 2010). EPS are comprised mostly of water (up to ~97%) (Zhang, Bishop, & Kupferle, 1998) and are usually a mixture of polysaccharides, proteins, lipids, nucleic acids, and other organic compounds (Flemming & Wingender, 2010; More & others, 2014). The EPS composition within a biofilm can vary greatly; it can be strain-dependent but can also be affected by the nutritional and physical conditions in which the biofilm develops (Nielsen, Jahn, &

Palmgren, 1997). It has also been suggested that the presence of microenvironments within biofilms may lead to the production of various mixtures of polysaccharides by specific subpopulations (Sutherland, 2001).

The presence of EPS does not seem to be key for the initial attachment of microbial cells to surfaces (Gaylarde & Gaylarde, 2005). However, EPS production is essential for the development of the architecture of any biofilm (Flemming & Wingender, 2010; Sutherland, 2001). EPS production appears to begin after the initial attachment of the microbial cells and the formation of the first microcolonies; production of EPS is often associated with the so-called irreversible attachment of cells (Flemming & Wingender, 2010).

Although the production of EPS can also occur during planktonic growth (e.g., microbial aggregates) (More & others, 2014), EPS provide biofilms with many of their unique physical characteristics. The EPS matrix has different functions in biofilms, including: 1) adhesion, cohesion, and aggregation of microbial cells—the EPS immobilize cells and keep them close allowing for cell-cell communication; 2) architecture and stability of the biofilm—formation of the structural support of the biofilm is a continuous and dynamic process that results in the spatial organization of biofilms; 3) protective barrier for cells and retention of water to prevent desiccation, which increases the tolerance and/or resistance to antimicrobials and other stressors; 4) resource capture (nutrients, organic compounds and inorganic ions) by sorption; 5) enzyme retention, which provides digestive capabilities; 6) exchange of genetic information; 7) function as electron donor or acceptor; 8) export of cell components; 9) sink for excess energy; and 10) binding of enzymes (Flemming & Wingender, 2010; Flemming & others, 2016). For excellent reviews summarizing the possible services the EPS matrix can provide to biofilms, see Flemming and Wingender, 2010; More and others, 2014; and Sutherland, 2001.

CHARACTERISTICS OF BIOFILMS

HETEROGENEITY

Biofilms are comprised of dense clusters of microbial cells (microcolonies) held together by the EPS matrix with fluid channels formed within the biofilm through which nutrients circulate. This structural organization leads to the formation of numerous microenvironments within the biofilm with different microbial composition, activity, cell density, pH, EPS production, water content, presence of channels, and solute concentrations (Stewart & Franklin, 2008). As a result, biofilms are physically, chemically, and biologically heterogeneous.

Mature biofilms are characterized by the presence of concentration gradients of metabolic substrates and products, resulting in chemical heterogeneity within the biofilm matrix. Specific patterns of chemical heterogeneity can be observed within biofilms due to reaction-diffusion interactions for metabolic substrates, metabolic products, and metabolic intermediates (Stewart & Franklin, 2008) (Fig. 1). As biofilms grow, the microbial cell density often increases, leading to an increase in the demand of nutrients (metabolic substrate). In general, cells located closest to the substratum are more limited for nutrients, whereas cells closest to the surrounding environment (e.g., farthest from the substratum) have higher availability of nutrients (Stewart & Franklin, 2008). Opposite to nutrients, metabolic products are usually present at higher concentrations inside the biofilm with decreasing concentrations in the outer layers. Metabolic intermediates can be produced and consumed in the biofilms, leading to concentration profiles with maxima somewhere within the biofilm; for instance, in a multi-species biofilm, the waste product of one species can serve as substrate for another species (Fig. 1) (Stewart & Franklin, 2008).

Under well-mixed conditions, planktonic microorganisms show fairly uniform physiological activity, whereas the chemical

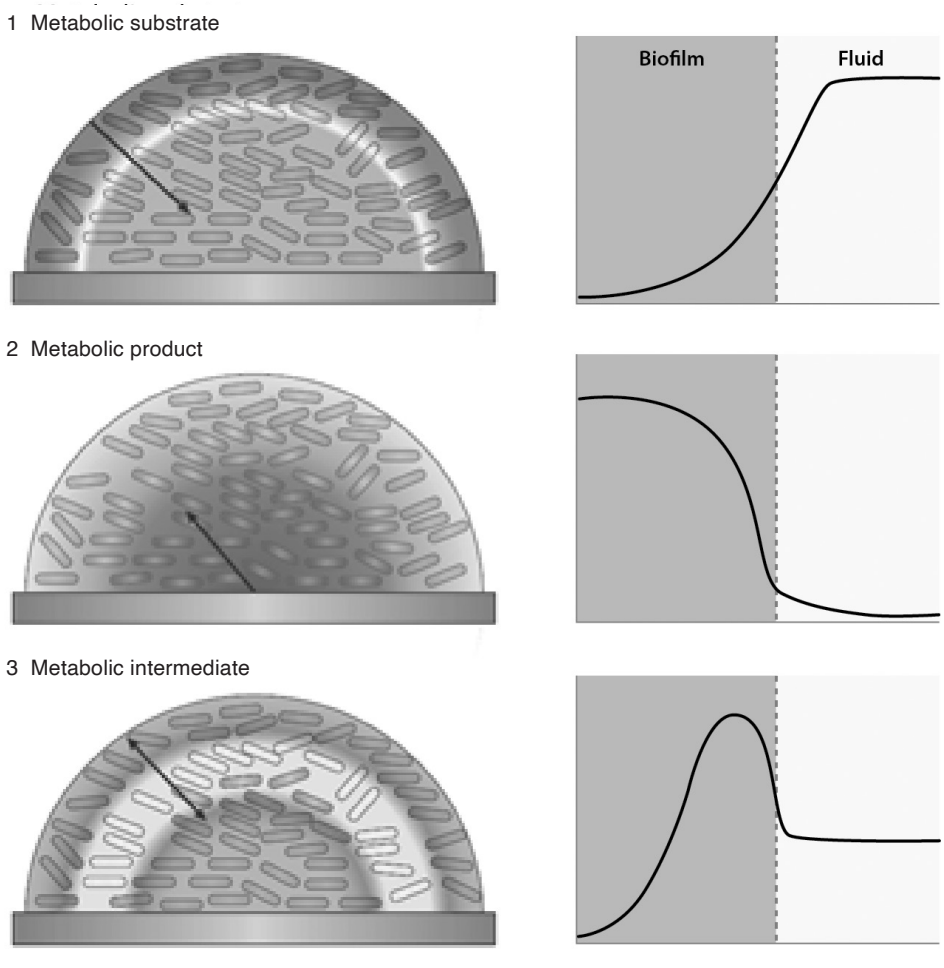

FIG. 1. Chemical heterogeneity in biofilms. Three qualitatively distinct patterns of chemical heterogeneity arise in biofilms owing to reaction-diffusion interactions for a metabolic substrate, *light gray* (*1*); a metabolic product, *medium gray* (*2*); and a metabolic intermediate, *darker gray* (*3*). *1,* The concentration of a substrate that is consumed inside the biofilm decreases with depth into the biofilm and distance away from the bulk fluid. *2,* Conversely, a metabolic product is more concentrated inside the biofilm. *3,* A metabolic intermediate that is both consumed and produced within the biofilm can exhibit concentration profiles that have local maxima (reprinted by permission from Springer Nature Customer Service Center, Nature Reviews Microbiology, Stewart & Franklin, 2008, fig. 2). Color version available in *Treatise Online* 147 (paleo.ku.edu/treatiseonline).

gradients within biofilms are commonly accompanied by physiological heterogeneity (GU & others, 2013; JENSEN & others, 2017). Due to limitations in metabolic substrates and oxygen (or other electron acceptor) availability, there are usually regions of slow microbial growth and activity within a biofilm. Furthermore, as a response to microenvironments inside a biofilm, microorganisms can modify gene expression patterns and physiological activities, favor the growth of particular microbial species, and select for fitter strains that can adapt to and survive in particular conditions (STEWART & FRANKLIN, 2008).

As an illustration of the various biogeochemical gradients that can be found in a biofilm, consider a mixed-species microbial mat, which may be viewed as complex biofilms (STOLZ, 2000) growing in (and producing)

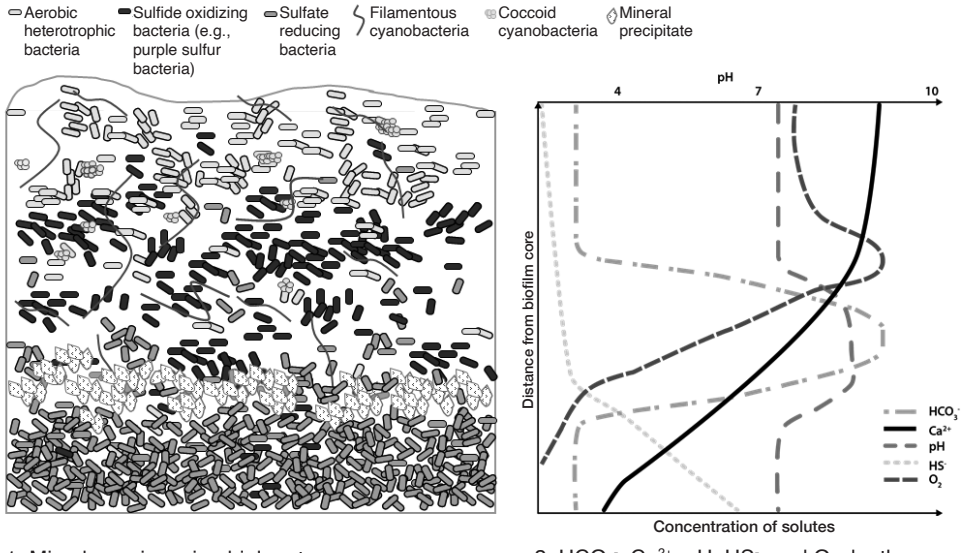

FIG. 2. Microbial diversity in biofilms. *1,* Conceptual representation of the microbial diversity observed in a mat similar to the one in the superficial layer of the stromatolite in the Cayo Coco Lagoonal Network described by Pace and others (2018); various groups of microorganisms are distributed within the mat and are located based on their physiological preferences, including photosynthetic microorganisms (filamentous and coccoid cyanobacteria), aerobic heterotrophic bacteria, and sulfate-reducing and sulfide-oxidizing bacteria. *2,* Sketch of chemical microenvironments developing within the mat indicated by the HCO_3^-, Ca^{2+}, pH, HS^-, and O_2 depth profiles. Mineral precipitation is observed in the oxygenic-anoxygenic photosynthetic interface as a result of a pH maximum induced by the microbial activity. (adapted from Pace & others, 2018, fig. 3 and fig. 8). Color version available in *Treatise Online* 147 (paleo.ku.edu/treatiseonline).

a lithifying stromatolite (Fig. 2) (PACE & others, 2018). Stromatolite growth can be the result of dynamic and successive cycles of sedimentation and microbial lithification in which the metabolism of microbial mats plays a key role (REID & others, 2000). Early studies reported the formation of chemical micro-gradients within microbial mats due to the metabolic activity of various microbial groups (VISSCHER & VAN GEMERDEN, 1993; STAL, GEMERDEN, & KRUMBEIN, 1985; JØRGENSEN, REVSBECH, & COHEN, 1983; JØRGENSEN & REVSBECH, 1983). PACE and others (2018) collected an actively growing microbial mat from a lithifying stromatolite in the hypersaline Cayo Coco Lagoonal Network (Fig. 2.1–2.2). Based on confocal laser scanning microscopy, microbial community analysis, dissolved oxygen (O_2), sulfide (H_2S/HS-/S^{2-}) concentration, and pH profiles, various chemical microenvironments were observed along a vertical profile in the stromatolite (Fig. 2.2). Microbial activity in the upper layers of the stromatolite is indicated by the O_2 and bicarbonate profiles (Fig. 2.2); and within the first few millimeters from the surface, O_2 concentration peak and bicarbonate concentrations are low due to oxygenic photosynthesis by, most likely, cyanobacteria. Below ~3 mm depth, bicarbonate concentrations increase and O_2 decreases rapidly due to reduced photosynthetic activity and increased net-aerobic respiration creating an oxic-anoxic interface at about 5 mm depth. Sulfide appears below the oxic-anoxic interface.

TOLERANCE AND RESISTANCE TO ENVIRONMENTAL STRESS

One of the unique properties of biofilm-grown cells is their enhanced tolerance

and/or resistance to antimicrobials (e.g. disinfectants, toxic compounds, antibiotics) and stresses compared to their planktonic counterparts. Flemming and others (2016) described biofilms as fortresses due to the ability of biofilm-grown cells to survive exposure to antimicrobials as well as desiccation. We refer here to resistance as the inherited ability of microorganisms to survive exposure to concentrations of antimicrobials that can be lethal (Sholar & Pratt, 2000) and that remains even when cells in the biofilm are dispersed. The term tolerance is described as the ability of the cells to survive transient exposure to compounds or stresses that could be lethal (Kester & Fortune, 2014), a phenomenon that is uniquely observed when cells grow as biofilms (Olsen, 2015).

Tolerance in biofilms is often attributed to the role of the EPS matrix acting as a protective barrier as well as to the development of regions with low metabolic activity created as a result of the intrinsically heterogeneous nature of biofilms. The EPS matrix acts as a protective barrier by: 1) quenching the activity of antimicrobials that diffuse through the biofilms via diffusion-reaction inhibition (Daddi Oubekka & others, 2012); this could involve the binding of the antimicrobials to components of the biofilm matrix or to microbial membranes (Chiang & others, 2013) as well as degradation of antimicrobials by enzymes contained in the EPS (Høiby & others, 2010), and 2) acting as a hydrogel that holds water protecting the organisms from desiccation (Flemming & Wingender, 2010). The intrinsic heterogeneity of the biofilms promotes the creation of zones of low metabolic activity and dormancy, which can decrease the susceptibility of the biofilm to harmful substances and increase the resistance of the biofilm to changing environmental conditions (Brown, Allison, & Gilbert, 1988; Stewart & Franklin, 2008). Cells in these zones of low metabolic activity and dormancy have reduced susceptibility to antimicrobials that depend on the microbial metabolism for their activities (Amato & others, 2014). Furthermore, biofilms can contain inactive microbial subpopulations (up to 1%) known as persisters that appear to exhibit unique phenotypic traits that make them more tolerant to antimicrobials (Wood, Knabel, & Kwan, 2013).

Microbial diversity within biofilms is a factor that can further increase the tolerance of biofilm-grown cells. Biofilms comprised of multiple species are affected by cross-species interactions, which can influence the development and structure of the microbial species within the biofilms and, in turn, provide an increased tolerance to stresses compared to their single-species biofilms (Lee & others, 2014; Moons, Michiels, & Aertsen, 2009).

DIVISION OF LABOR

Biofilm-grown cells can demonstrate division of labor (Armbruster & others, 2019; Dragoš & others, 2018; van Gestel, Vlamakis, & Kolter, 2015; Vlamakis & others, 2008), which refers to the specialization of subpopulations of cells to perform different tasks within a microbial community. Division of labor appears to be based on three conditions: 1) development of different microbial phenotypes (task allocation); 2) associated microorganisms having a cooperative interaction; and 3) all partners involved in the interactions gaining inclusive fitness benefits (West & Cooper, 2016).

An example of division of labor can be found in *Bacillus subtilis* biofilms, which have subpopulations that are genetically similar but are able to perform different specialized activities including motility, matrix production, and sporulation, which in conjunction are key for the successful development of the biofilm (Dragoš & others, 2018; van Gestel, Vlamakis, & Kolter, 2015; Vlamakis & others, 2008). In *B. subtilis* biofilms, flagellum-independent migration is achieved by two different cell types: surfactin-producing cells that aid lubricating the substratum and matrix-producing cells, which agglomerate as bundles (van

Gogh bundles) that are able to move away from the colony; these bundles can migrate greater distances compared to what would be possible without the division of labor (van Gestel, Vlamakis, & Kolter, 2015).

BIOFILMS AS COMPLEX MICROBIAL COMMUNITIES

Biofilms in the environment typically consist of complex microbial communities that host multiple species. Subaerial biofilms, biofilms that grow on solid mineral surfaces exposed to the atmosphere (e.g., rocks, surface of buildings, stromatolites), are perfect examples of complex communities with different cross-species interactions. A diverse community of microorganisms is usually present in subaerial biofilms, including algae, bacteria, fungi, protozoa, and even microscopic animals such as mites and insects (Gaylarde & Gaylarde, 2005; Gorbushina & Petersen, 2000). Interactions among different microbial species in mixed-biofilm communities seem to influence the development, structure, and functions of these communities (Moons, Michiels, & Aertsen, 2009). Cross-species interactions in mixed biofilms can range from synergistic (cooperative) to antagonistic (competitive) (Elias & Banin, 2012), and they can lead to a number of microbial adaptations by promoting horizontal gene transfer events, cell-cell communication (quorum-sensing abilities) (Davies & others, 1998; Parsek & Greenberg, 2005), and can induce protein secretion systems resulting in phenotypic changes that can affect the survival, dynamics, spatial distribution, and coexistence of the microbial communities (Elias & Banin, 2012).

Cross-species interactions can influence the development and structure of microbial species within the biofilms, which can provide an increased resistance to stresses compared to their single-species biofilms (Lee & others, 2014; Moons, Michiels, & Aertsen, 2009). Lee and others (2014) tested the response of mixed-species biofilms, comprised of *Pseudomonas aeruginosa, Pseudomonas protegens,* and *Klebsella pneumoniae* to their exposure to two antimicrobials—sodium dodecyl sulfate and tobramycin. Compared to single-species biofilms, the mixed-species biofilm was more adept at maximizing and optimizing the use of nutrients to enhance their growth and persistence, which made it more resilient to these antimicrobials. Furthermore, the increased tolerance observed in the mixed-species biofilm was suggested to be a result of a cross protection effect provided by the resistant species to all other members of the microbial community, rather than selecting for the least sensitive species in the biofilm (Lee & others, 2014). The way microorganisms interact within biofilms can indeed influence the spatial organization of the biofilm (see Fig. 3). Liu and others (2016), for instance, described that 1) species exhibiting strong cooperation appear to develop intermixed distributions or layered structures without patchy patterning; 2) in the absence of nutrient or space limitation, species with weak interdependence tend to interspecifically segregate; 3) exploitation by one of the species can result in the formation of layered structures with patchy patterning; and 4) competition appears to lead to an overall decrease in biomass with patchy patterning or interspecific segregation (Fig. 3.1–3.4) (Liu & others, 2016).

Whereas biofilms in the environment can be dominated by a particular species, other secondary species are almost always present. Dominance by one species in a biofilm is determined by: 1) the particular location within the biofilm; 2) the environmental conditions; and 3) the specific stage in the development of the biofilm. In the example of the microbial mat studied from the lithifying stromatolite in the hypersaline Cayo Coco Lagoonal Network, dominance of a particular species varied according to the specific location within the biofilm. The green lamina of the stromatolite (top layer of the biofilm) was dominated by cyanobacteria, whereas deeper layers (mineralized lamina)

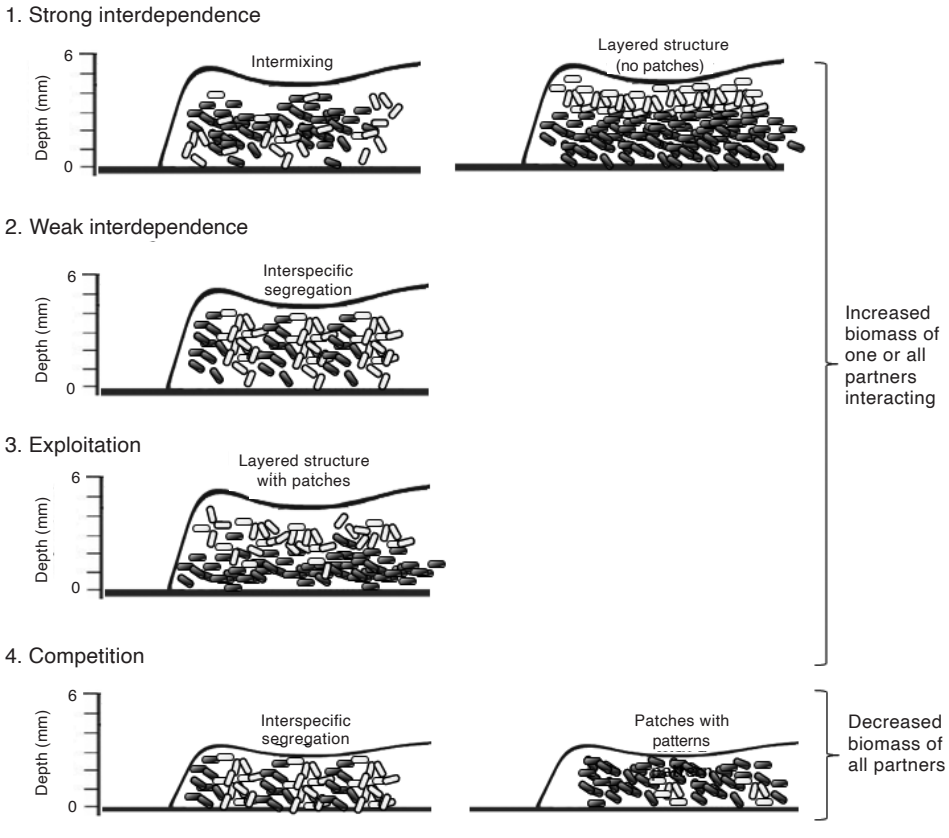

FIG. 3. Cross-species interactions influence the spatial organization of mixed-species biofilms. *1,* Strong interdependence (cooperation) leads to the formation of intermixing or layered structures. *2,* Weak interdependence results in interspecific segregation. *3,* Exploitation results in layered structures with patches. *4,* Competition can lead to species segregation and the formation of patches with patterns. Negative interactions (competition) can result in the overall decrease of biomass (new; based on information in Liu & others, 2016). Color version available in *Treatise Online* 147 (paleo.ku.edu/treatiseonline).

were dominated by purple sulfur (sulfide-oxidizing) bacteria (PACE & others, 2018) (see Fig. 2.1). The development of freshwater phototrophic biofilms can also be influenced by environmental conditions, such as the presence of light (ROESELERS, VAN LOOSDRECHT, & MUYZER, 2007). For instance, under high light conditions, initial colonizers can predominantly consist of green algae, whereas under low light intensities, heterotrophic bacteria tend to colonize. Moreover, over time, as the biofilm matures, filamentous cyanobacteria can become predominant in these phototrophic biofilms (ROESELERS, VAN LOOSDRECHT, & MUYZER, 2007).

BIOFILMS AND MINERAL PRECIPITATION

Microbially induced precipitation of minerals (biomineralization) is a relevant process in various biological, geological, medical, and engineered systems (PHILLIPS & others, 2013). Of importance for the study of the evolution of prokaryotes throughout Earth history, is the understanding of carbonate biomineralization. The formation of carbonate sediments in

different environments (e.g., marine reefs, fluviatile tufas, hot springs, travertines, etc.) seems to be influenced by microbial mineralization.

Various microbial metabolic processes, including photosynthesis, sulfate reduction, urea hydrolysis, ammonification, denitrification, and methane oxidation, affect the solution chemistry of the surrounding environment (e.g., increase carbonate alkalinity, pH values, or dissolved inorganic carbon), which in turn can induce carbonate or other mineral precipitation (Dupraz & others, 2009; Zhu & Dittrich, 2016).

Biomineralization is a common event in microbial mats or biofilms (Braissant & others, 2003; Handley & others, 2008; Shiraishi & others, 2008). Chemical heterogeneity in biofilms can lead to the formation of microenvironments that create gradients of alkalinity and/or supersaturation, which can facilitate mineral precipitation within the biofilm. Furthermore, the presence of EPS in biofilms can influence the biomineralization process by providing nucleation sites for mineral precipitation, regulating the patterns of mineralization and the types of minerals produced (Braissant & others, 2003; Decho, 2010). Certain functional groups in the EPS can inhibit carbonate precipitation: negatively charged groups can bind with mineral ions such as Ca^{2+} and Mg^{2+}, thus, a high binding capacity of the EPS can potentially inhibit carbonate precipitation (Flemming, 1995). Release of cations from the EPS can occur due to EPS degradation or after release from the binding sites through an external trigger (e.g., change in ionic strength, salinity), which can lead to carbonate and other mineral precipitation (Decho, 2010; Dupraz & others, 2009).

As an illustration of the various metabolic processes that can promote mineral precipitation, consider again the example of the stromatolite in the Cayo Coco Lagoonal Network described by Pace and others (2018) (see Fig. 2). As mentioned earlier, stromatolites result from successive cycles of microbial mineralization triggered by the metabolism of biofilm forming microbiota. Pace and others (2018) suggested that mat formation starts with the development of biofilms comprised of coccoid and filamentous cyanobacteria-fixing CO_2, leading to the formation of biomass and the production of O_2 through oxygenic photosynthesis. Oxygenic photosynthesis also consumes CO_2 and increases the pH, which can result in the precipitation of (calcium) carbonates. In the top layer of the microbial mat, aerobic heterotrophs consume O_2; in the anoxic depths, sulfate-reducing bacteria produce HS^- from sulfate. Sulfate reduction can increase carbonate alkalinity (in the form of bicarbonate, HCO_3^-). In an intermediate zone, both sulfide and O_2 are present. Purple sulfur (sulfide-oxidizing) bacteria are involved in recycling the sulfide back to sulfate, and other microbes are involved in this process as well. The microbial activity of cyanobacteria, sulfate-reducing and sulfide-oxidizing bacteria creates a daytime pH maximum, which promotes the precipitation of magnesium calcite from dissolved ions in the lagoon. Mineral precipitates are mostly located at the oxygenic-anoxygenic photosynthetic interface. Figure 2.2 shows the different chemical profiles in the microbial that can be created due to the different microbial activities. The repetition of these series of physicochemical and biological steps along with the upward growth of the biofilm led to the formation of stromatolites in the studied lagoon (Pace & others, 2018).

Pace and colleagues suggested a role of the EPS in the different mineralization steps, hypothesizing that cyanobacterial EPS acts as a binding agent for calcium, thus inhibiting carbonate precipitation in the green lamina of the stromatolite (upper layer of the mat). EPS in the oxic-anoxic zone appears to have a decreased cation-binding capacity, which would make Ca^{2+} more available for carbonate precipitation or indicate that the EPS in these layers is saturated with multivalent cations.

SUMMARY

Most microorganisms persist associated with surfaces in the natural environment, most likely in the form of biofilms. Biofilms are complex microbial communities attached to surfaces and embedded in a matrix of extracellular polymeric substances (EPS). The presence of EPS provides architecture, stability, and protection to the microbial communities within the biofilm. Furthermore, these microbial communities typically contain multiple species that interact with each other and with the environment.

Due to the spatial arrangement of the microbial communities, biofilms develop microenvironments, which result in highly physically, chemically, and biologically heterogeneous arrangements. Biofilm-grown cells can exhibit different phenotypes and change their metabolic activities compared to their planktonic counterparts.

Considering that biofilm-grown cells exhibit characteristics distinct from their corresponding planktonic communities, it is possible that biofilms produce specific marks in the consolidated rock record. Thus, a better understanding of the biofilm way of life can aid in the reconstruction of the evolution of prokaryotes throughout Earth history.

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MICROBIAL MATS

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INTRODUCTION

Microbial mats are laminated organosedimentary structures that develop as benthic biofilms in a wide range of aquatic environments (Fig. 4). They are robust, complex ecosystems, primarily comprised of bacteria and archaea, semi-isolated from their immediate environment by a self-constructed matrix of extracellular polymer substances (EPS) (Nicholson, Stolz, & Pierson, 1987). Understanding present-day microbial mat development and preservation is valuable for interpreting signs of life through geologic time. Microbial mats stabilize sediments, precipitate minerals, and typically include remnants of organisms, making them important to the paleontology field. Several processes, such as binding and trapping and *in situ* precipitation of minerals, can lead to complete lithification of microbial mats, forming microbialites,

Microbial mat populations exhibit large metabolic diversities, driven by the availability of various electron donors and acceptors and mediated by environmental controls. Their diverse biochemical pathways convey an extraordinary capacity to fill every available niche. Because the microbial rates utilizing metabolites are higher than the limited diffusion rate afforded by the polymer matrix, elements are recycled rapidly (Des Marais, 2003), and nutrients are contained within the mat.

The difference between microbial mats and biofilms is somewhat indistinct. Although some biofilms are referred to as microbial mats, they do not fit the definition of mats as described here. Microbial mats, while often considered a type of biofilm, are generally thicker, more developed and complex; they host a more diverse microbial community yet exhibit a relatively high degree of structure and permanence, whereas the simplest biofilm could consist of a single bacterial species colonizing any surface, even temporarily (Decho & Gutierrez, 2017; Flemming & Wingender, 2010). The focus of this review is on photosynthetic microbial mats, their composition, diversity, geochemistry, preservation, and significance for paleontological studies.

MICROBIAL MAT DISTRIBUTION

Photosynthetic microbial mats develop in a wide variety of environmental conditions and settings. Modern mat environments include hypersaline lagoons, hot springs, alkaline lakes, open marine, shallow intertidal sediments, salt marshes, and mine tailings (Seckbach & Oren 2010) (Fig. 5). Coherent microbial biofilms also exist in aphotic coastal marine environments (Gallardo, 1977; Schulz & others, 1999), permanently dark environments such as caves (Summers Engel & others, 2004), and deep-sea methane seeps (Paul & others, 2017) and vents (Jørgensen & Boetius, 2007; Crépeau & others, 2011).

A wide range of physicochemical conditions support development of microbial mats. Prior to the evolution of multicellular life, microbial mats developed in the photic zone of shallow aquatic environments (Stal, van Gemerden, & Krumbein, 1985; Dupraz, Reid, & Visscher, 2011; Knoll, 2016), such as hot springs, streams, rivers, and lakes and in the marine environment (Walter, 1976; Grotzinger & Knoll, 1999; Nisbet & Fowler, 1999;

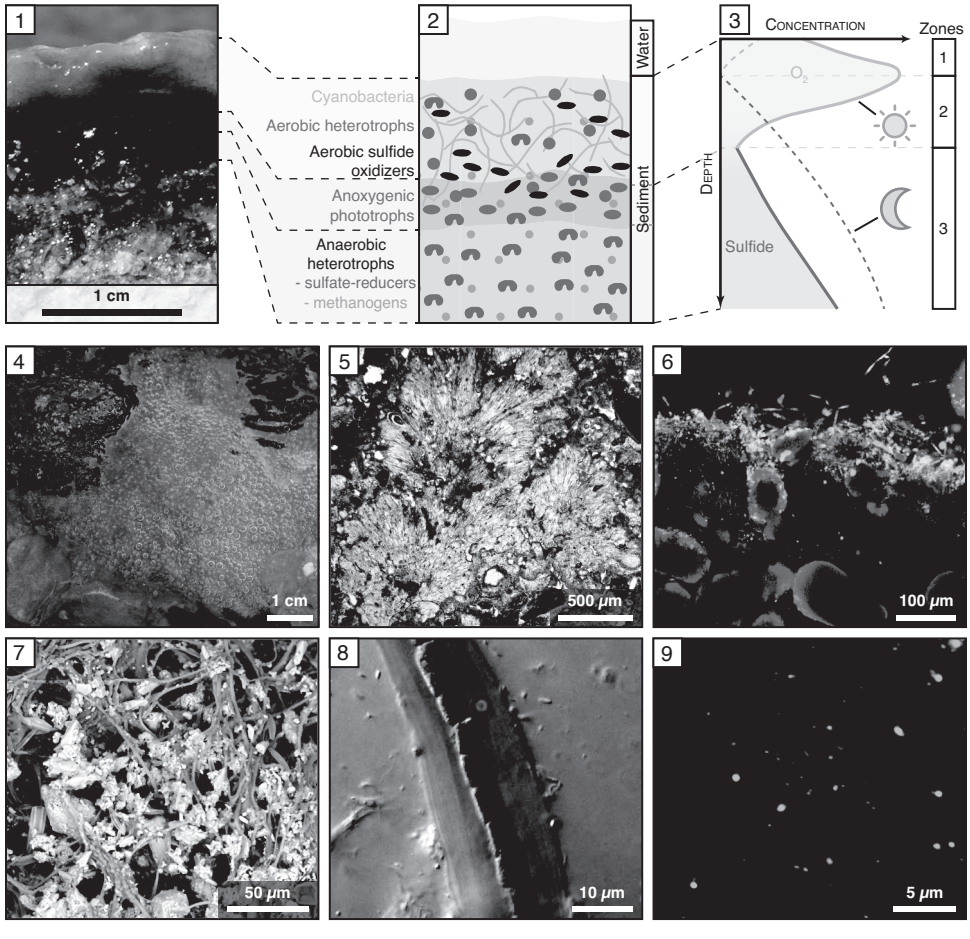

Fig. 4. Typical photosynthetic microbial mat characteristics. *1–3,* Properties of marine photosynthetic mats shown in cross section. *1,* Hand sample of a typical mat, showing exopolymeric substances near the surface. Top surface layer is dominated by cyanobacteria, the red (in color version) layer underneath by anoxygenic purple sulfur bacteria, and the deeper dark-colored sediment at the bottom is the anoxic part of the mat. *2,* Schematic representation showing the depth distribution of the major guilds of mat microbes. These are the key groups involved in element cycling. Note the presence of both sulfate-reducing bacteria and methanogenic bacteria in the oxic surface layer of the mat. *3,* Typical daytime (*solid lines*) and nighttime (*dashed lines*) depth profiles of oxygen (*green in color version*) and sulfide (*purple* in color version). Zones indicated on the right. Zone 1: Permanently oxic during a light-dark cycle; Zone 2: Oxic during the day and anoxic (and sulfidic) during the night; Zone 3: Continuously anoxic. The bottom and top of Zone 2 are defined by the oxic-anoxic (O_2/HS^-) interface during the day and night, respectively (new). *4–9,* Various imaging techniques used to study microbial mats and microbialites. *4,* Top view of a submerged lithifying microbial mat, showing oxygen bubbles at the surface; Great Salt Lake, Utah, USA. *5,* Polarized light petrographic thin section of a fossil microbialite (~14,500–12,500 cal ^{14}C BP) showing bundles of filamentous cyanobacteria (*Gloetrichia*/*Rivularia*-like nitrogen fixing cyanobacteria); pluvial Lake Bonneville; Stansbury Terrace, Oquirrh Mountains, Utah, USA. *6,* Confocal scanning laser microscopic image of a surface mat from a modern marine stromatolite (Highborne Cay, Bahamas) showing autofluorescent cyanobacteria (red; note the endoliths in the lower left of the image), sulfate-reducing bacteria, SRB385 probe (green), $CaCO_3$ ooids (blue) (image courtesy of Alan W. Decho, University of South Carolina, USA, see color image in *Treatise Online* 163. *7,* Scanning electron microscope image of filamentous cyanobacteria precipitating carbonate minerals; Green Lakes, NY, USA. *8,* Filamentous cyanobacterium *Oscillatoria* sp., surrounded by sheath of exopolymer (parallel to the filament) using Nomarski light microscopy; Cabo Rojo, Puerto Rico. *9,* Fluorescence microscopic image of viruses (*small dots*) and coccoid cyanobacteria (*larger dots*), *Synechococcus* spp. from a lithifying microbial mat; Green Lake, New York, USA. All images new. Color images available in *Treatise Online* 163 (paleo.ku.edu/treatiseonline).

Tice & Lowe, 2004; Dupraz & others, 2009; Djokic & others, 2017). The first microbial mats in the early Archean were most likely built by anoxygenic phototrophs (Guitiérrez-Preciado & others, 2018; Visscher & others, 2020). Oxygenic photosynthesis that evolved later, likely in microbial mats, resulted in the Great Oxidation Event (GOE) and allowed the development of multicellular life. Ironically, although the GOE created an opportunity for microbial life to develop and diversify beyond benthic biofilms (Knoll, 2016), it ended hundreds of million years of mats' dominance on Earth. Newly evolved multicellular life forms using aerobic respiration outcompeted mats for resources, such as space and nutrients. This and the burrowing activity and predation of animals (Walter & Heys 1985; Knoll 2016; Bosak, Knoll, & Petroff, 2013) forced mats to extreme environments (Seckbach & Oren, 2010; Dupraz, Reid, & Visscher, 2011). The physicochemical and geochemical conditions that define environments where mats develop today include:

1) *salinity*—0 to >300 ppt (Des Marais, 1995; Roche & others, 2019; Visscher & others, 2010; Preisner, Fichot, & Norman, 2016; Perillo & others, 2019);

2) *temperature*—from below zero (de los Ríos & others, 2015; Peeters & others, 2012; Sumner & others, 2015) to ~72–73°C, possibly short term up to 75°C (Meeks & Castenholz, 1971; Van der Meer & others, 2005; Beam & others, 2016; Bennett, Murugapiran, & Hamilton, 2020);

3) *specific chemical composition of the water and sediments*—iron, arsenic, zinc, copper, mercury, tungsten, sulfide (Pierson, Parenteau, & Griffin, 1999; Härtig & Planer-Friederich, 2012; Méndez-García & others, 2014; Visscher & van Gemerden, 1993; Beam & others, 2016; Fernández & others, 2016; Visscher & others 2020);

4) *hydrodynamics*—quiescent pools to rapidly flowing rivers (Cuadrado, Perillo, & Vitale, 2014; Roche & others, 2019;

5) pH—pH <1 to >9 (Visscher & others, 2010; Härtig & Planer-Friederich, 2012; Méndez-García & others, 2014; Bernstein & others, 2013; Prieto-Barajas, Valencia-Cantero, & Santoyo, 2017;

6) *desiccation* (Potts, 1999; Noffke, 2008; Dupraz, Reid, & Visscher, 2011; Perillo & others, 2019);

7) *light regime*—intensity of photosynthetically active radiation, <1 to >2000 $\mu E.m^{-2}.s^{-1}$ (Jørgensen, Cohen, & Des Marais, 1987; Visscher & others, 1998; Fernández & others, 2016);

8) *UV radiation*—<1 to 64 $W.m^{-2}$ (Farias & others 2017; Visscher & others, 2020);

9) *nutrient availability*—eutrophic to oligotrophic (Paerl, Joye, & Fitzpatrick, 1993; Pinckney, Paerl & Fitzpatrick, 1995; Rejmankova & Komárková, 2000; Smith & others, 2010; Varin & others, 2011; Kohler & others, 2016).

In summary, contemporary microbial mats have adapted to exist in many diverse environments where they often cope with multiple extremes such as alkaline and hypersaline conditions, elevated heavy metals concentrations and low pH, high UV radiation, arsenic and sulfide concentrations, and permanent anoxia. They have been successful in adapting to environments inhospitable to most multicellular life in many different geographic settings, thriving in early Earth environments and changing over time for billions of years.

THE ROLE OF SUBSTRATE IN MAT DEVELOPMENT

The development of microbial mats is influenced by physical and chemical properties of the substrate, which has major implications for their preservation (Roche & others, 2019). Some physical properties that affect mat development are grain size and sediment stability. For example, filamentous cyanobacteria form mats faster on the fine fraction (<125 μm) than on larger particle sizes (Rozenstein & others, 2014). The absence of microbialites developing on mud, silt, or sand in streambeds of rivers is due to a low lithification potential of microbial mats in these conditions (Roche & others, 2019). Harder and more extensive substrates

result in more stable microbialites (Moore & Burne, 1994). Increasing roughness of the substrate enhances the growth rate of microbial mats and microbialites (Tuchman & Stevenson, 1980). Turbulence and flow velocity over the microbialite surface affect the colonization of grazers, such as ciliates (Primc-Habdija, Habdija, & Plenkovic-Moraj, 2001). In addition, the chemical composition of the substrate may affect the colonization by microbial communities, the ability to mineralize, and preservation potential (Gradzinski, 2010; Chafetz, Rush, & Utech, 1991; Parsiegla & Katz, 2000). For example, microbial deposits grow faster on a limestone substrate than on elemental copper, due to the toxicity of the metal (Parsiegla & Katz, 2000). However, the effects of physical (roughness) and chemical properties of the substrate on mat adherence are often difficult to separate (Diaz & others, 2007).

BIOGEOCHEMISTRY

Chemical and physical environmental conditions determine the initial composition of the microbial community (Baas Becking, 1934). The metabolisms of this pioneer microbial community in turn change the microenvironmental conditions. This creates a feedback that likely affects the community metabolism and/or composition, possibly leading to a predictable oscillation (Dupraz & others, 2009). In a photosynthetic microbial mat lacking major disturbances, this results in a community adapted to extreme diel fluctuations (between daylight and nighttime conditions) of oxygen, sulfide, and pH (see Fig. 4.3).

THE MICROBIAL MAT COMMUNITY

Within microbial mats, biogeochemical processes can be described by a small number of functional groups or guilds, including oxygenic and anoxygenic phototrophs, aerobic chemoorganoheterotrophs, chemolithoautotrophic sulfur oxidizers, fermenters, and anaerobic chemoorganoheterotrophs (notably sulfate reducers and methanogens; see Fig. 4) (van Gemerden 1993; Ward & others, 1998; Decker & others 2005; Taffs & others, 2009; Pace & others, 2018). Their actual diversity is extremely high, exceeding 4,000 operational taxonomic units (OTUs) (Ley & others, 2006; Baumgartner, Dupraz, & others, 2009; Baumgartner, Spear, & others, 2009; Armitage & others, 2012; Bolhuis, Cretoiu, & Stal, 2014; Wong, Ahmed-Cox, & Burns, 2016). Interactions between mat-inhabiting organisms have been described in detail in several laboratory studies (e.g., De Wit & van Gemerden, 1987; Visscher & others, 1992; Caumette, 1993; Van den Ende, Laverman, & van Gemerden, 1996; Massé, Pringault, & de Wit, 2002; Müller & Overmann, 2011), and the effect of their combined metabolic activities on geochemistry of the mat has been measured *in situ* (e.g., Revsbech & others, 1983; Visscher, Taylor, & Kiene, 1995; Visscher & others, 1998, 2003, 2010, 2020; Ferris & others, 1997; Jonkers & others, 2003; Wieland & others, 2005, Pace & others, 2018; Maegaard, Nielsen, & Revsbech, 2017). Field measurements using microelectrodes demonstrate the dynamic nature of photosynthetic mats (Jørgensen, Cohen, & Revsbech, 1986; De Wit & others, 1989; Kühl, Lassen, & Jørgensen 1994; Visscher & van Gemerden, 1993), and oxygen and sulfide concentrations and depth distribution fluctuate vastly over a diel cycle (see Fig. 4.3).

EFFECT OF LIGHT REGIME AND PHOTOTROPHY

Layering within the microbial mat is a consequence of the changes in available light with depth (Jørgensen, Cohen, & Des Marais, 1987). Light of longer wavelengths penetrates deeper into the sediment, with near infrared penetrating the deepest, typically >1 cm (Kühl & Fenchel, 2000). This differential irradiance penetration accommodates a discrete vertical distribution of oxygenic and anoxygenic photosynthetic

organisms with light harvesting pigments that absorb different parts of the light spectrum. For example, cyanobacteria near the surface are oxygenic phototrophs with chlorophylls, phycobilins, carotenoids, xanthophyll pigments absorbing light between ~500 nm and 700 nm. Some cyanobacteria in microbial mats contain chlorophyll *d* and *f*, absorbing light in the far-red spectrum and are able to survive in environments with little photosynthetically active radiation (Ohkubo & Miyashita, 2017). Green and purple (non)sulfur bacteria are anoxygenic phototrophs constrained to deeper layers and use bacteriochlorophyll pigments (green bacteria: ~680–820 nm; purple bacteria: 820–150 nm) and carotenoids (absorbing around 500–600 nm) (van Gemerden & Mas, 1995). Some anoxygenic phototrophs contain bacteriochlorophyll *b*, which can absorb infrared light of 1050 nm. The extracellular polymer substances (EPS) themselves typically do not absorb visible wavelengths and even promote the downward (i.e., forward) scattering of light into a mat, which is the so-called biofilm gel effect (Decho & others, 2003).

Oxygenic photosynthesis occurs in the upper layers and is accompanied by a strong peak of oxygen production during daylight (Fig. 4.1–4.3). It uses two photosystems and electrons from water. In contrast, anoxygenic photosynthesis generally occurs deeper in the mat. It depends on one photosystem and utilizes other electron donors, typically sulfide and other reduced sulfur compounds, although H_2, Fe(II), NO_2^- or As(III) may also be used to supply electrons for photosynthesis (Hamilton, 2019). Because water is not the electron donor, oxygen is not produced.

Oxygenic and anoxygenic phototrophs, as well as chemolithoautotrophs (e.g., sulfide oxidizing bacteria), are the primary producers of the microbial mat ecosystem, fixing CO_2 carbon and producing biomass. In most contemporary mats, benthic cyanobacteria are the dominant phototrophs. Cyanobacteria in microbial mats can photosynthesize under extremely low light intensities, as low as 1 $\mu E.m^{-2}.s^{-1}$ (Jørgensen, Cohen, & Des Marais, 1987). Consequently, the peak of photosynthetic activity during the early afternoon is not at the surface, but just beneath it, typically at 1–2 mm depth (van Gemerden, 1993; Dupraz, Reid, & Visscher, 2011). Microbial filaments, typically cyanobacterial, weave together to form a coherent organic sediment, for which the designation mat is given. Biomass produced during autotrophy fuels a community of heterotrophic organisms that recycle the organic carbon (cellular material, EPS, and exudates of photorespiration like glycolate; Fründ & Cohen, 1992; Bateson & Ward, 1988) to yield metabolic energy and, to a lesser extent, provide building blocks for biomass. In marine and hypersaline microbial mats, the two major pathways of organic carbon oxidation are aerobic respiration and sulfate reduction (O_2 and SO_4^{2-} respectively, as electron acceptors; Fründ & Cohen, 1992; Visscher & others, 1998; Pace & others, 2018; Wong & others, 2018). Sulfate reduction (discussed in detail later) yields hydrogen sulfide as a metabolic product, which is used by anoxyphototrophs and colorless sulfide-oxidizing bacteria. Depth profiles of oxygen and sulfide concentrations fluctuate over a diel cycle (Fig. 4) (De Wit & van Gemerden, 1987; Dupraz & Visscher, 2005) as oxygen is only produced during daylight, and sulfide production continues in the dark (provided there is sufficient electron donor, e.g., organic carbon or H_2). The deeper mat layers are permanently anoxic, an intermediate depth zone is oxic during the day and anoxic during the night, and a thin surface layer is mostly oxic due to O_2 diffusion from the overlying water or atmosphere and, in daylight, from production by oxygenic photosynthetic organisms below (De Wit & others, 1989; Visscher, Beukema, & van Gemerden, 1991, Visscher & others, 1998). The vertical compartmentalization of the different types of phototrophy results in different availabilities of

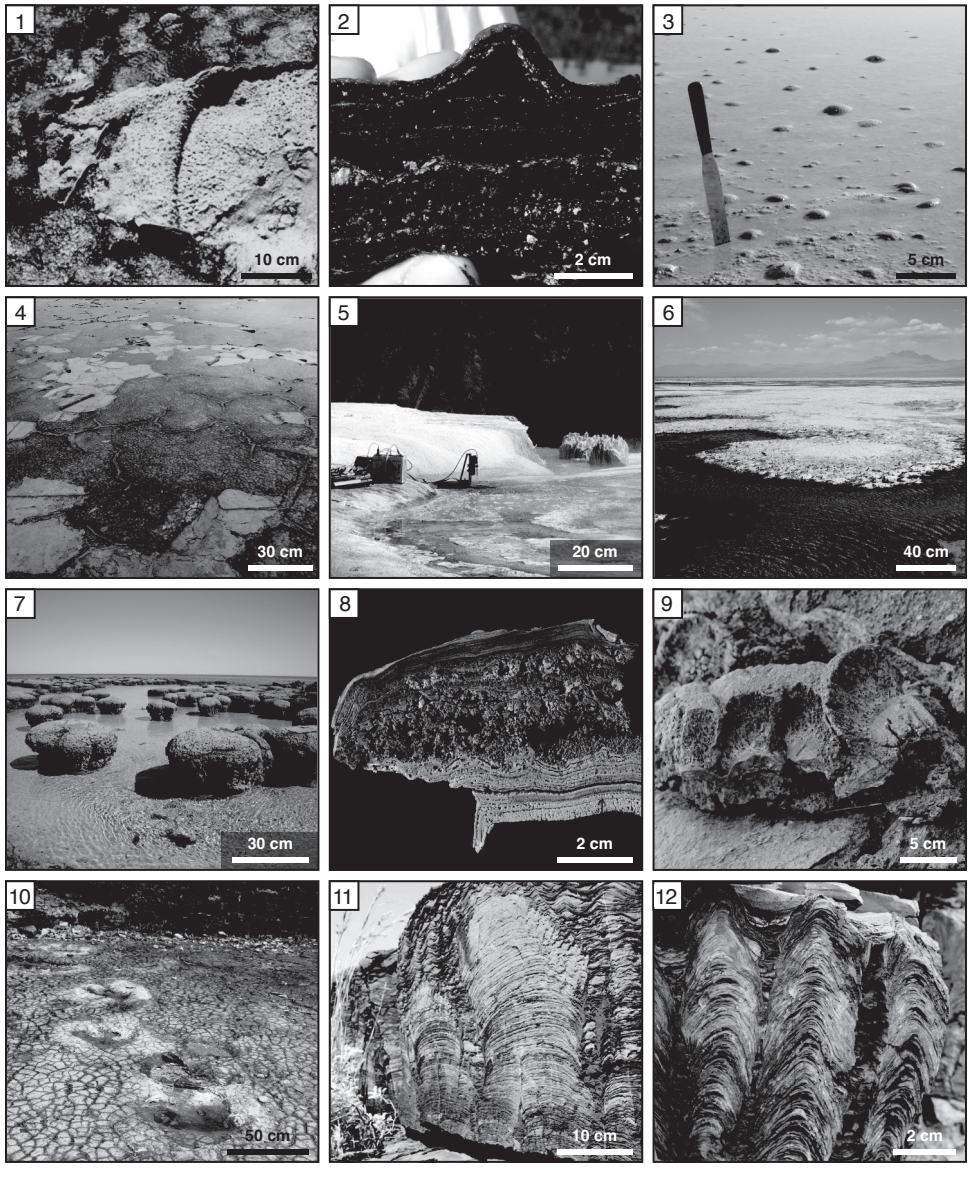

FIG. 5. Examples of contemporary (living) microbial mats (*1–6*), surface mats associated with microbialite structures (*7–8*), and recent and fossil microbialites of various ages (*9–12*) in diverse habitats. *1,* Top view of a temperate intertidal microbial mat showing the green cyanobacterial (*Microcoleus* sp.) surface with trapped sand and purple sulfur bacteria (*Thiocapsa* sp.) underneath; Orkney Islands, Scotland, UK. *2,* Cross section of a hypersaline, gelatinous mat with trapping and binding and *in situ* precipitation of carbonate minerals; Big Pond, Eleuthera, Bahamas. *3,* Microbial mat surface showing gas bubbles, in this particular case of trapped methane. The mat is lifting, showing the filamentous nature of the mats. Trapped sediment and extracellular polymer substances provide a gas-tight seal. This mat undergoes frequent salinity fluctuations from 35 ppt to 350 ppt; Cabo Rojo, Puerto Rico. *4,* Hypersaline mat that experiences wetting-drying (desiccation) cycles. During extensive dry periods, polygonal cracks form that often fill with exopolymeric substances. Recolonization and regrowth following wetting takes place within hours to days; James Pond, Eleuthera, Bahamas. *5,* Mats associated with a hydrothermal spring (72°C, the maximum temperature supporting photosynthesis) near a travertine platform; Mammoth Hot Spring, Montana, USA; image taken during microelectrode measurements. *6,* Permanently anoxic high-altitude microbial mats,

(*continued on facing page*)

FIG. 6. Global distribution of microbial mats and microbialites. Both living and fossil photosynthetic microbial mats and microbialites are present in terrestrial and marine settings across the planet within the shallow aquatic environments (including hot springs, lakes, rivers, the intertidal and subtidal zone of coastal marine environments). This is not meant to be a complete survey but merely to show the extensive geographic dispersal pattern of mats and microbialites.

electron donor, primarily low-molecular weight carbon and H_2, and electron acceptors. This generates characteristic steep geochemical gradients of O_2, sulfide, and pH that fluctuate depending on the available light (Fig. 4).

ELEMENT CYCLING

Microbial mats are among the most productive ecosystems on Earth, with extremely high rates of element cycling (JØRGENSEN, 2001), especially the major elements—carbon (C), oxygen (O), and sulfur (S) (CANFIELD & DES MARAIS, 1993) and, depending on the environment, nitrogen (N), arsenic (As), iron (Fe), and hydrogen (H) (EMERSON & REVSBECH, 1994; JOYE & PAERL, 1994; VISSCHER & others, 1998, 2020; DESNUES & others, 2007). The carbon cycle in microbial mats is coupled to other element cycles, such as O, S, N, Fe, and As (Fig. 7.1–7.3). Reduction of CO_2 is coupled to phototrophic oxidation of H_2O, HS^-, NO_2^-, Fe^{2+}, or As^{3+}. In turn, oxidation of organic carbon, including methane, is coupled to reduction of O_2, SO_4^{2-}, NO_3^- (producing mainly N_2), Fe^{3+} or As^{5+}. In most modern marine mats, sulfate is abundant relative to other electron acceptors, and respiratory processes rely mainly on O_2 and SO_4^{2-} as electron acceptors (Fig. 7.1) (VAN GEMERDEN, 1993). In non-marine environments, other available electron acceptors may be used preferentially. Iron cycling (Fig. 7.3) is abundant in some hot-spring mats (PIERSON, PARENTEAU, & GRIFFIN, 1999)

(*continued from facing page*) comprising purple-sulfur bacteria (anoxygenic phototroph *Ectothiorhodospira* sp.), cycling sulfur and arsenic; La Brava, Atacama, Chile. *7,* Intertidal living stromatolites; Hamelin Pool, Shark Bay, Australia. *8,* Cross section of a living hypersaline microbialite; Great Salt Lake, Utah, USA. The surface fabric is laminated (stromatolitic) with a clotted (thrombolitic) fabric underneath. *9,* Fossil microbial crust capping Cambrian rocks, (30,000–28,000 cal ^{14}C BP); Buffalo Terrace, Lake Bonneville, Utah, USA. The fabric of the inner part is laminated while the outer part depicts a clotted mesofabric. The microbialite displays a cauliflower-like macrofabric. *10,* Fossilized microbial mat showing mud cracks and dinosaur tracks, Late Jurassic; Loulle section, France. *11,* Outcrop showing fossil stromatolites (2.72 Ga), Meentheena Member, Tumbiana Formation, Australia; *12,* Fossil egg carton-like stromatolites; Meentheena Member, Tumbiana Formation, Australia. All images new, contributed by authors. Images *11* and *12,* courtesy of Dr. David Flannery, Queensland Institute of Technology, Australia. Color images available in *Treatise Online* 163 (paleo.ku.edu/treatiseonline).

and seeps (Emerson & Revsbech, 1994). Denitrification contributes to respiration in some mne and hypersaline mats (Joye & Paerl, 1994; Bouton & others, 2020), and arsenic cycling (Fig. 7.3) occurs in mats surrounding alkaline lakes (Hoeft & others, 2010; Visscher & others, 2020).

The distribution of aerobic and anaerobic chemoorganoheterotrophs depends on availability of reactants for their redox reactions. Both types of heterotrophs prefer to be in close proximity to cyanobacteria, which are typically the main carbon-fixing organisms in the mat. There they compete for organic carbon and hydrogen electron donors. Fermentation and other partial degradation pathways of complex carbon molecules support methanogens, acetogens, and sulfate reducers (Megonigal, Hines, & Visscher, 2003). A common misconception is that methanogens and sulfate reducers are confined to deeper anoxic layers. In fact, they frequently display maximum metabolic rates in the oxic zone of the mat (Canfield & Des Marais, 1991; Fründ & Cohen, 1992; Visscher, Prins, & van Gemerden, 1992; Hoehler, Bebout, & Des Marais, 2001; Buckley, Baumgartner, & Visscher, 2008). These anaerobes require physiological adaptations or formation of consortia to survive in the presence of oxygen. For example, sulfate-reducing bacteria (SRB) form microcolonies (Postgate, 1979; Petrisor & others, 2014), the center of which could be anoxic; or they form consortia with sulfide-oxidizers that remove the toxic oxygen by reaction with sulfide (Decho, Norman, & Visscher, 2010). Alternatively, some SRB can actually use oxygen or nitrate (Dilling & Cypionka, 1990; Sigalevich & others 2000; Baumgartner & others, 2006) or disproportionate intermediate sulfur compounds independent of O_2-sensitive enzymes (Bak & Pfennig, 1987). SRB can also survive by forming spores (Cypionka, Widdel, & Pfennig, 1985). Sulfate reducers and methanogens are at an energetic disadvantage compared to aerobes but can compete where organic carbon is in abundance (near cyanobacteria) and have an advantage at night when anoxic conditions prevail.

Chemolithoautotrophy is another metabolic strategy important to element cycling, notably through organic carbon production, and is common in microbial mats. It is supported by high concentrations of reduced sulfur compounds (thiosulfate, polysulfides, polythionates, zero-valent sulfur, etc.; Steudel & others, 1990; Visscher & van Gemerden, 1993; Findlay, 2016) and to some extent by H_2, NH_4^+, and Fe^{2+} (Visscher & Stolz, 2005; Fan, Bolhuis, & Stal, 2015; Chan & others, 2016). Sulfide oxidizing bacteria perform chemolithotrophic sulfide oxidation at the interface of O_2 and S^{2-}, but they can also use a range of intermediate sulfur compounds, including organosulfur compounds with oxygen and alternatively nitrate as electron acceptor (Kelly, 1982; Visscher & van Gemerden, 1993; Van den Ende & van Gemerden, 1993). Some purple sulfur bacteria can grow as chemolithotrophic sulfur oxidizers (De Wit & van Gemerden, 1987), thereby enhancing their competitive position in the mat ecosystem and even outcompete colorless sulfur bacteria (Visscher & others, 1992). H_2 is sometimes produced to release excess reducing equivalents (Burrow, Chaplen, & Ely, 2011), especially under anoxic conditions. Most of this is rapidly scavenged by sulfate reducers (Nielsen, Revsbech, & Kühl, 2015), but methanogens and acetogens can also use H_2 (Megonigal, Hines, & Visscher, 2003). Both hydrogen- and sulfur-oxidizing chemolithotrophs are generally autotrophs and, thus, can contribute to the primary production in the mats.

Other sulfur compounds have a role in microbial mats and are important in global sulfur cycling and climate regulation. Dimethylsulfoniopropionate (DMSP) is a non-competitive solute that also acts among others as an osmolyte and cryoprotectant. DMSP is present in some cyanobacteria and is the precursor to dimethyl sulfide (DMS)

in microbial mats (Fig. 7.1) (Visscher & van Gemerden, 1991; Vogt & others, 1998). DMS is further consumed by aerobic heterotrophs (methylotrophs), sulfate reducers, colorless sulfur bacteria, and methanogens (Visscher & Kiene, 1994; Visscher, Taylor, & Kiene, 1995; De Zwart & Kuenen, 1997). DMS and several thiols can be used as electron donors for photosynthesis in purple sulfur bacteria (Visscher & van Gemerden, 1991; Visscher & Taylor, 1993). DMS photooxidation yields dimethylsulfoxide, which is a common electron acceptor in marine mats (Taylor & Kiene, 1989). Importantly, the emission of volatile methylated sulfur compounds to the atmosphere has a significant role in climate regulation (Lovelock, Maggs, & Rasmussen, 1972; Kiene & others, 1996) by producing a cloud of condensation nuclei. Through the production and consumption of methylated sulfur compounds, methane, and carbon dioxide, microbial mats may therefore have played an important role in climate regulation through geologic time.

The nitrogen cycle (Fig. 7.2) provides metabolic energy but also has a critical role in biomass production. Nitrogen is typically a growth-limiting nutrient in the marine environment, and by fixing atmospheric N_2, various microorganisms in the mat enhance nitrogen supply (Steppe & others 1996; Paerl, Fitzpatrick, & Bebout, 1996; Steppe & Paerl, 2002; Olson, Litaker, & Paerl, 1999). The breakage of the triple bond of N_2 is energetically costly, and thus the capacity for nitrogen fixation is most prevalent in oxygenic and anoxygenic phototrophs. However, heterotrophs, including some clostridia, hyphomicrobia, azotobacters, sulfate reducers, and methanogens are also capable of nitrogen fixation in microbial mats (Fan, Bolhuis, & Stal, 2015; Finke & others, 2019). As biomass is degraded, ammonia can be released and either assimilated or used as an electron donor in energy metabolism. Chemolithotrophic oxidation of ammonium takes place during a two-step nitrification process, which has been found in coastal microbial mats (Fan, Bolhuis, & Stal, 2015; Bonin & Michotey, 2006). Under anoxic conditions, ammonium can be oxidized to N_2 with NO_2^- (anammox) (Jaeschke & others, 2009) or with sulfate (sulfammox) (Rios-Del Toro & others, 2018). However, the sulfammox reaction may be carried out by a consortium of different microbes (Bi & others, 2020). Nitrate can be assimilated into cell material or used as an electron acceptor for respiration with organic carbon and reduced sulfur. Based on ^{15}N-labeled isotope experiments, anammox is thought to be insignificant in intertidal mats (Bonin & Michotey, 2006), but may be prevalent in hot spring mats (Jaeschke & others, 2009). Nitrogen-containing compounds glycine betaine and ectoine are among common osmolytes in hypersaline mats (Oren, 1990; Stal & Reed, 1987; Welsh & others, 1996; Karsten, 1996; Wong & others, 2018). Ectoine transformations yield various amino acids, and ultimately ammonium as a degradation product (Reshetnikov & others, 2020). Glycine betaine catabolism produces trimethylamine (Oren 1990), a non-competitive compound that supports methanogenesis in mats (King, 1988; Orphan & others, 2008).

Concentrations of arsenic in microbial mats have been associated with biogenic metals: zinc (Zn), manganese (Mn), and copper (Cu) following early diagenesis (Sforna & others, 2017), indicating a biological role for this metalloid. In fact, arsenic provides a biogeochemical alternative to sulfur and iron (Fig. 7.3). Arsenite (As(III)) can be used as an electron donor for photosynthesis by some purple and green (non)sulfur bacteria (Kulp & others, 2008; Summers Engel, Johnson, & Porter, 2013; Hoeft McCann & others, 2016) and anaerobic denitrifying microbes (Oremland & Stolz, 2003). Arsenate (As(V)) is a product of As(III) oxidation, and can be used as an electron acceptor for the oxidation of organic carbon, H_2 or H_2S (Oremland & others, 2000; Hoeft & others, 2004). The

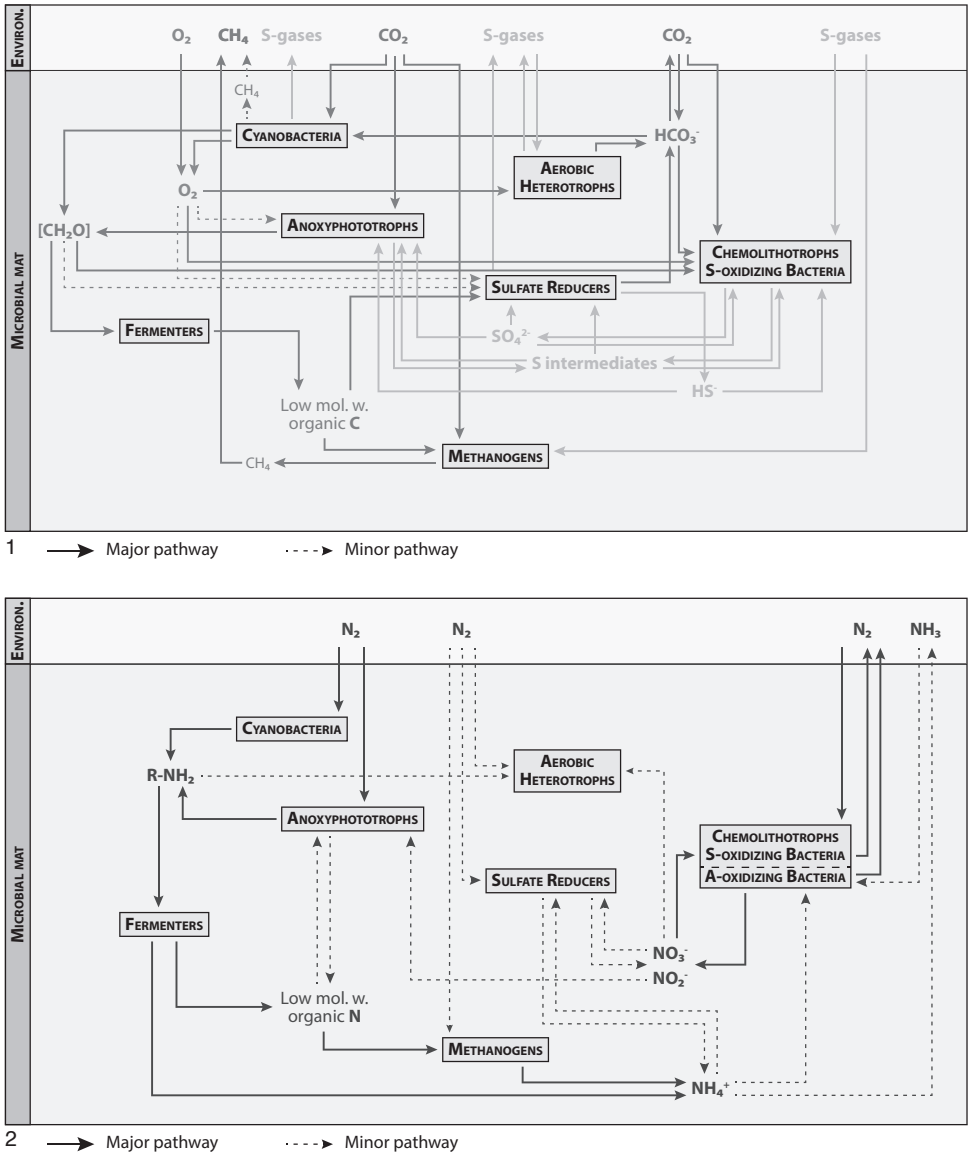

FIG. 7. Major element cycles in microbial mats showing intimate interactions between key guilds of microbes. *Solid lines* depict major biogeochemical transformations, *dashed lines* are minor pathways and *dot/dashed lines* are detoxification mechanisms (*3*). *1,* Cycles of oxygen and sulfur, the main elements supporting carbon cycling in modern photosynthetic microbial mats. *2,* Nitrogen cycling. *3,* Iron and arsenic cycling (on facing page).

presence of both As(III) and As(V) oxidation states in microbialites suggests that complete arsenic cycling could be involved in the lithification of these structures in Laguna Diamante, Argentina (SANCHO-TOMÁS & others, 2020). Arsenotrophic photosynthesis and arsenate-supported respiration may have existed before the GOE, supported by the association of arsenic with organic carbon globules in the 2.72-billion-year-old domal stromatolite laminae in the Tumbiana Formation in Australia (SFORNA & others, 2014).

At Laguna La Brava Atacama, Chile, mats proliferate under permanent anoxic condi-

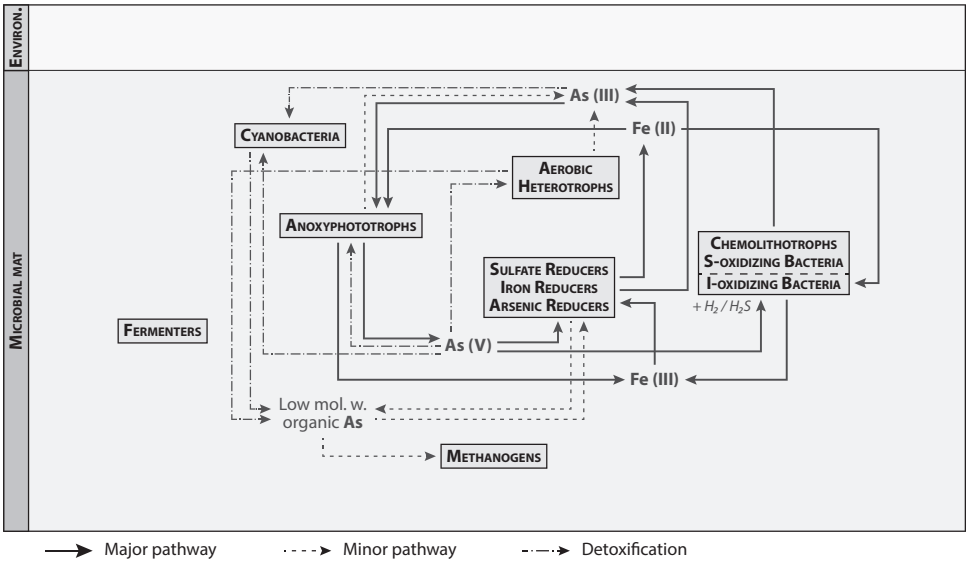

Fig. 7. (*continued*).

tions, using arsenic and sulfur cycling instead of oxygen (Visscher & others, 2020). Arsenic and sulfur cycles may have co-evolved. Several strains of phototrophs (e.g., *Ectothiorhodospira* sp.; Hoeft McCann & others, 2016; Visscher & others, 2020) that can use arsenite also use reduced sulfur compounds as electron donors. Similarly, known arsenate-respiring bacteria also use sulfate as an electron acceptor.

MICROBIAL DIVERSITY

Microbial mats are considered as functionally (van Gemerden, 1993) or biogeochemically simple ecosystems (Des Marais, 1995; Prieto-Barajas, Valencia-Cantero, & Santoyo, 2017), yet are very complex in their diversity and function at the genomic level (Kunin & others, 2008; Varin & others, 2011; Bonilla-Rosso & others, 2012; Khodadad & Foster, 2012; Bolhuis, Cretoiu & Stal, 2014; Ruvindy & others, 2015; White III & others, 2016; Babilonia & others, 2018; Wong & others, 2018, 2020). Over past decades, microbial diversity based on 16S rDNA sequencing has been studied extensively in a variety of microbial mats (Ley & others, 2006; Baumgartner, Dupraz & others, 2009; Bolhuis & Stal, 2011; Armitage & others, 2012; Farias & others, 2014, 2017; Cardoso & others, 2017; Louyakis & others, 2017; Greco & others, 2020) and estimates of operational taxonomic units (OTUs) typically present confirm that these ecosystems are in fact quite complex. In addition to diversity studies at the taxonomic level, several metagenomes of mats have also been assembled, providing a wealth of functional information. Previous metagenome-based studies may have targeted only specific ecophysiological responses (Varin & others, 2011, Lopéz-Lopéz & others, 2013; Mendes-Monteiro & others, 2019; Aubé & others, 2020) or had limited sequencing depth (Bolhuis, Cretoiu & Stal, 2014; Wong, Ahmed-Cox, & Burns, 2016). Furthermore, these studies generally lacked a clear biogeochemical context (Breitbart & others, 2009; Mobberley & others, 2015; Paul & others, 2017).

With advances in taxnomics, studies linking microbial diversity to mat function are beginning to develop. The spatial and/or temporal resolution of microbial diversity (i.e., species composition) in relation to selective geochemical data has been investigated at a few specific sites (Ley & others,

2006; Baumgartner, Dupraz & others, 2009, Baumgartner, Spear, & others, 2009; Visscher & others, 2010; Farias & others, 2014, 2017; Louyakis & others, 2017; Fernández & others, 2016), yet only a few metagenome studies with a similar focus exist (Breitbart & others 2009; Mobberley, Khodadad, & Foster, 2013; Wong & others, 2018; Paul & others, 2017). Metatranscriptomes of mats are even more sparse and suffer from the same shortcomings as those for metagenomes (Liu & others, 2011; Mobberley & others, 2015; Louyakis & others, 2017; Hörnlein & others, 2018; Campbell & others, 2020). However, studies at the metagenomic/metatranscriptomic level have the potential to delineate key microbial interactions and the role of gene transfer as well as identify novel organisms and pathways in microbial mat systems.

One intriguing area opened up through omic studies in microbial mats is that of understanding so-called microbial dark matter in these systems. Microbial dark matter is defined as the many unculturable community members in an ecosystem, and represents a huge untapped resource of biological information (Rinke & others, 2013). In the case of microbial mats, understanding the ecological roles of this dark matter is crucial for a complete understanding of the functioning of these systems through space and time. The only in-depth study on microbial dark matter in mats was undertaken in Shark Bay in Australia (Wong & others, 2020), with 115 potentially novel metagenome-assembled genomes affiliated with dark matter described. Detailed analyses of metabolic pathways allowed putative inferences about the roles of these microbes in recycling organic carbon and the potential for H_2, ribose and CO/CO_2 to become major energy currencies. Understanding these metabolic pathways may even provide key insights into the origin of eukaryotes (Wong & others, 2020). Indeed, a novel group of archaea—the Asgard archaea—represents the closest lineage of the archaeal ancestor of eukaryotes (Spang & others, 2015). The highest abundances of Asgard archaea found so far anywhere in the world are in microbial mats (Wong & others, 2018; Wong & others, 2020).

Viruses are another crucial component of microbial mats. Although modern sequencing approaches have helped our understanding of viral diversity and metabolisms in modern microbial mats and microbialites, their role through geologic time remains elusive. The virome of modern microbialites was first described by comparing viral communities in marine (Highborne Cay, Bahamas) and freshwater (Pozas Azules II and Rio Mesquites, Mexico) microbialites (Desnues & others, 2008). This study suggested some viruses appeared to be endemic to a given microbialite system, indicating biogeographical variability from one microbialite to another (Desnues & others, 2008). In a study on Pavillion Lake (British Columbia, Canada) microbialites (White III & others, 2016), most of the viruses within the microbialites were unclassified, consistent with earlier observations (Desnues & others, 2008). Interestingly, although the water column of Pavillion Lake had a great abundance of viruses, predominantly comprised of T4-like cyanophage (e.g., *Myoviridae*) and large algal viruses (e.g., *Phycodnaviridae*), few viral sequences were found within the microbialites. Instead, large numbers of antiviral phage genes were present (e.g., CRISPR-cas and phage shock proteins), suggesting that microbialites inhibit viral infection from the water column and surrounding sediments (White III & others, 2016). Similarly, a study of viruses in Shark Bay (Australia) stromatolites (White III & others, 2018), revealed few viral sequences and abundant antiviral genes (BREX, CRISPR-cas, DISARM).

The precise role of viruses in microbial mats is not known, although it is thought they may modulate microbial diversity or affect ecosystem function through the recycling of essential nutrients. Most recently, some have proposed that viruses may be key players in regulating the transition from

soft microbial mat to hard stromatolite (White III, Visscher, & Burns, 2021), including through mechanisms affecting carbonate precipitation and possibly also mineral composition (Perri & others, 2017; Slowakiewicz & others, 2021). Similarly, viruses may have influenced, transformed, and manipulated ancient microbial communities. Understanding the role of viruses in microbialites is needed to fully understand key biological processes and pathways relevant to early life on Earth, as well as to better interpret mineral biosignatures.

THE EXOPOLYMERIC MATRIX

All mats, including photosynthetic ones, typically form a slimy biofilm matrix. Large amounts of extracellular polymeric substances (EPS) excreted by cyanobacteria (Klock & others, 2007; Rossi & De Philippis, 2015) and other mat inhabitants (Braissant & others, 2007) make the microbial mat gelatinous and sticky. These EPS consist of polysaccharides, proteins, with small amounts of extracellular DNA, exoenzymes, trace metals and minerals. EPS facilitate adhesion to surfaces such as sediments (Grant & Gust, 1987; Yallop & others, 1994), protect the cells against desiccation and UV radiation, sequester nutrients such as essential trace metals, localize and facilitate cell-to-cell communication (quorum sensing) (Decho, 2015), and support gliding motility within the mat for phototaxis in cyanobacteria, and chemotaxis in sulfide-oxidizing bacteria. The EPS matrix also protects the organisms within by reducing predation and trapping viruses (Decho & Gutierrez, 2017; White III, Visscher, & Burns, 2021). The chemical composition of EPS varies with environmental conditions, microbial community, and biofilm maturity. Some EPS components such as uronic acids provide binding sites for calcium and other ions, potentially enhancing carbonate mineral precipitation (Dupraz & others, 2004; Decho, Visscher, & Reid, 2005; Braissant & others, 2007; Gallagher & others, 2011). The presence of authigenic minerals within the EPS matrix indicates its pivotal role in carbonate precipitation.

LITHIFICATION

Some microbial mats trap and bind sediments and skeletal grains and precipitate minerals directly within the polymer matrix (Dupraz & Visscher, 2005) or intracellularly (Couradeau & others, 2012). Over time, these minerals cement together, resulting in lithification, and enhanced potential for preservation of the mat morphology in the fossil record (Dupraz & others, 2009; Riding, 2011a; Finke & others, 2019). The oldest lithified examples of mats are stromatolites (Walter, Buick, & Dunlop, 1980), some arguably ~3.7 billion years old (Fig. **5**) (Nutman & others, 2016; Allwood & others, 2018; Shapiro & Wilmeth, 2020, see p. 56–60).

Another type, microbially-induced sedimentary structures (MISS), develop as a response of microbial mats to sediment dynamics (Noffke & Awramik, 2013; see p. 71–90). Carbonate precipitation and silicification of MISS can result in long term preservation of these sedimentary structures (Noffke, 2008; Fischer & Fralick, 2020), some dating back to 3.48 (Noffke & others, 2013) and 3.47 billion years (Hickman-Lewis & others, 2018), respectively.

CARBONATE SYSTEMS

Lithification of microbial mats can lead to formation of biogenic carbonate rocks, also known as microbialites (see *Precipitated Microbiolites*, p. 55–70). Of these, stromatolites have layers that are often compared with the lamination of microbial mats. This is deceiving; with only a few exceptions (Marin-Carbonne & others, 2018), a single layer in a stromatolite is more likely produced by an entire mat community, rather than by a single mat layer (Visscher & others, 1998; Reid & others, 2000). The precipitation of minerals within certain mats contributes to eventual mat lithification as does trapping and binding of sediment in the sticky extracellular polymer substances. Therefore, it is

generally accepted that microbial mats are the progenitors of microbialites.

Within mats, the precipitation of carbonate minerals is mediated by 1) the interaction of the EPS with calcium and magnesium ions, and 2) by the effect of combined microbial metabolisms on the carbonate saturation index. First, freshly produced EPS inhibits carbonate precipitation by binding cations such as calcium and magnesium. The maximum calcium binding capacity can reach 280–380 mg/g EPS (Braissant & others, 2007, 2009; Pace & others, 2018), which is about the amount present in six liters of water. The magnesium binding capacity of EPS is typically much lower (~5 times in Pace & others 2018), possibly because magnesium has a much larger hydration shell than calcium (Freytet & Verrecchia, 1998). EPS concentration decreases with depth in the mat (Braissant & others, 2009; Pace & others, 2018; Sforna & others, 2017) as it is consumed by aerobic and anaerobic heterotrophs (Braissant & others, 2009; Visscher & others, 1998; Decho, Visscher, & Reid, 2005). Anaerobic degradation of EPS, facilitated by fermenters and sulfate reducers, is about four to six times slower than aerobic consumption. Maximum potential consumption rates of EPS do not seem to change with depth in the upper four cm of the mat (Braissant & others, 2009).

Microbial degradation of EPS releases Ca^{2+} ions and, in case of microbial decomposition, locally produces dissolved inorganic carbon. This increases the saturation index of $CaCO_3$, thus favoring carbonate precipitation. Cryo SEM images of mat materials clearly show mineral precipitation associated with the organic matrix of the EPS, which acts as nucleation site for initial mineral growth (Dupraz & others, 2004; Dupraz & Visscher, 2005). In addition, intracellular carbonate precipitation found in several strains of cyanobacteria can add to the carbonate production in microbial mats (Benzerara & others, 2014).

SILICATE SYSTEMS

Similar to carbonate precipitation, silicate mineralization can also be facilitated by microbial mats (Ericksson & others, 2010; Zeyen & others, 2015; Pace & others, 2016). An increase of the pH through photosynthesis facilitates nucleation of a poorly crystallized Mg-Si phase in the EPS of Great Salt Lake (USA) mats. Silica formation within a mat involves the formation and dissolution of carbonate mineral phases. Below the photic zone in these mats, aragonite precipitates first during anaerobic degradation of EPS (Pace & others, 2016). Subsequently, this aragonite dissolves in acidic pockets deeper in the mat, where partially degraded EPS binds calcium (Ca) and magnesium (Mg), ultimately forming dolomite. Some of the liberated Ca and Mg may also form a complex silicon (Si). This sequential formation of aragonite followed by precipitation of a Mg-Si phase has been observed in Lake Clifton (Western Australia) thrombolites (Burne & others, 2014) and in Mexican lakes (Zeyen & others, 2015). Amorphous Ca-Mg-Si-Al nanoparticles, presumably permineralized viruses, formed in the surface of sabkha mats (Perri & others, 2017). These amorphous phases were the precursor of the Mg-rich clay palygorskite.

Micrometer-sized calcite, pyrite nanocrystals and framboids also precipitated in the sabkha mat. These mineral precipitation processes were interpreted as induced organomineralization, and may be a common feature in silica precipitation. However, this active organomineralization process differs from the silicification of microbial mats associated with some silicon-rich hot springs, which can produce stromatolite-like features (Schultze-Lam & others, 1995; Jones, Renaut, & Rosen, 1997; Berelson & others, 2011). In these systems, the EPS of the microbial sheath material may act as passive nucleation site, but microbial metabolisms do not have a role in mineralization (Konhauser & others, 2004; Yee &

others, 2013). Regardless of the mechanism, silicification of microbial mats was important in the preservation of early life (Awramik & Barghoorn, 1977; Walter & Awramik, 1979; Lowe, 1980; Manning-Berg & others, 2019), some as old as 3.5–3.3 billion years (Hickman-Lewis, Westall, & Cavalazzi, 2020).

Microbial activities can enhance mineral precipitation by increasing the alkalinity in the microbial mat on a microscale (Dupraz & Visscher, 2005; Petrisor & others, 2014). Some metabolic reactions, such as oxygenic and anoxygenic photosynthesis, certain types of sulfate and methanogenesis increase alkalinity (Visscher & others, 1998, 2020; Visscher & Stolz, 2005; Dupraz & Visscher, 2005; Gallagher & others, 2012; Gallagher, Dupraz, & Visscher, 2014) (Fig. 5). Other metabolic reactions decrease the alkalinity, e.g., aerobic respiration, fermentation, denitrification, and other types of sulfate reduction (Visscher & Stolz, 2005; Gallagher & others, 2012; Gallagher, Dupraz, & Visscher, 2014). In the case of sulfate reduction, the nature of the electron donor can drastically alter alkalinity—the use of hydrogen and formate increases alkalinity and mineral saturation more than other donors (Gallagher & others, 2012; Gallagher, Dupraz, & Visscher, 2014). So, if the community produces excess hydrogen (Skyring, Lynch, & Smith, 1988), the sulfate-reducing bacteria are more likely to increase the local mineral saturation leading to precipitation (Gallagher & others, 2012; Gallagher, Dupraz, & Visscher, 2014). It should, however, be noted that the metabolic reaction rates of all members of the microbial mat community determine the overall change in alkalinity and the dynamic balance between mineral precipitation and dissolution (Visscher & Stolz, 2005; Pace & others, 2018). In biofilm surface mats of modern stromatolites in the Bahamas, microcolonies—likely clusters of sulfate reducers (Petrisor & others, 2014)—can have a pronounced impact on the local alkalinity and could produce microcrystalline carbonates (Visscher, Reid, & Bebout, 2000; Reid & others 2000). Significant changes in microbial community metabolisms over a light-dark cycle need to be considered when assessing a potential precipitation or dissolution effect on carbonate minerals (Visscher & others, 1998; Dupraz & Visscher, 2005). In this, it is critical to combine actual *in situ* activity measurements at a micrometer scale with fabrics (Visscher, Reid, & Bebout,, 2000) and, when possible, metagenomic information (Mobberley, Khodadad, & Foster, 2013; Mobberley & others, 2015; Louyakis & others, 2017; Wong, Ahmed-Cox, & Burns, 2016; Preisner, Fichot, & Norman, 2016). Multiple analytical techniques are needed to determine the microbial origin of carbonate minerals. For example, the use of stable isotopes ($\delta^{13}C$) alone can easily lead to misinterpretations due to the extremely high and variable rates of photosynthesis, changing ratio of autotrophic versus heterotrophic carbon cycling or changes in surrounding chemical parameters such as salinity that influence inorganic carbon speciation (Arp & others, 2012; Chagas & others, 2016).

Precipitation of minerals associated with mats is merely the onset of lithification. Complete lithification of microbial mats yields microbialites and may involve physicochemical processes, such as evaporation, or influx of alkaline water, in addition to the microbial metabolisms described (Dupraz & others, 2009). Although widely varied in shape and origin, ooids, peloids, and oncolites can possibly be used as structural biomarkers (Chafetz, 1986; Riding 1991; Diaz & others, 2017), but further work is needed. Lithification can either be microbially induced (when the microbial mat community increases the alkalinity through the combined metabolisms) or microbially influenced (when environmentally-driven processes change the alkalinity). In both scenarios, the extracellular polymer matrix

acts as a nucleation site (Dupraz & others, 2009).

MICROBIALITE MORPHOLOGIES THROUGH TIME

Stromatolites formed in the early Paleoarchean (3.6–3.2 Ga; Allwood & others, 2006), but a biotic origin of the earliest formations is still being debated (Grotzinger & Knoll, 1999). During the Late Mesoarchean-Early Neoarchean (2.94–2.5 Ga), the fossil record shows more diverse stromatolite morphologies that were biogenic (Bosak, Knoll & Petroff, 2013; Lepot, 2020). For example, aragonite spheroids associated with organic globules were present in 2.72-billion-year-old Archean stromatolites (Lepot & others, 2008). Thrombolites appeared later, after approximately 2.3–1.9 billion years ago (Barlow & others, 2016; Kah & Grotzinger, 1992).

Several mechanisms for transition from a laminated (stromatolitic) to a clotted (thrombolitic) fabric have been proposed: 1) the dominance of coccoid cyanobacteria relative to filamentous morphologies (Moore & Burne, 1994); 2) a typical fan-shaped distribution of cyanobacterial filament in open marine thrombolites in the Bahamas (Planavsky & others, 2009), consecutively modified by early diagenesis with secondary cement deposition (Planavsky & Ginsburg, 2009); 3) infaunal boring (Tarhan & others, 2013); 4) disruption of a laminated fabric by animals and/or eukaryotic macrophytes (Walter & Heys, 1985); or 5) grazing by reticulopodia (Bernhard & others, 2013). A combination of the above mechanisms may be at play. Interestingly, modern thrombolites in open marine (Bahamas), hypersaline bay (Hamelin Pool, Western Australia), hypersaline (Lake Clifton, Western Australia, Great Salt Lake, USA, Lake Alchichica, Mexico) and freshwater (Green Lake, New York, USA) lacustrine environments all have a stromatolitic crust overlying a thrombolitic fabric. This transition from a laminated surface to a clotted fabric at depth observed in modern settings suggests that a revision of the macroscale terminology of microbialites is needed.

Also worth noting, diagenetic overprinting of mineral precipitates can significantly alter and sometimes erase the micro- to macrostructure and mineral composition (Freytet & Verrecchia, 1998; Pace & others, 2018).

MICROBIAL MAT TAPHONOMY

Mats preserve more than just layers of carbonate or silicate minerals. They may contain microfossils or chemical remnants of cellular structures, such as membrane lipids or rare earth elements (Awramik & Barghoorn, 1977; Walter & Awramik, 1979; Oehler & others, 2009; Benzerara & Menguy, 2009; Pawlowska, Butterfield, & Brocks, 2012; Knoll, 2016; Javaux & Lepot, 2018). Their preserved morphology may also include biological imprints and sediment features like ripples, roll-up structures, and cracks because of the stabilizing influence of cyanobacterial filaments on sediment structure (Noffke & Awramik, 2013).

Preservation of prokaryotes as microfossils is not widespread, and their interpretation is challenging (Knoll, 2016; Schopf & others, 2017; Manning-Berg & others, 2019). However, a variety of chemical or molecular fossils aid in the interpretation of lithifying and non-lithifying mats (lipids, such as hopanes, isoprenoids, etc.; Didyk & others, 1978; Allen & others 2010; Briggs & Summons, 2014; Pagès & others, 2014; Knoll, 2016; Hickman-Lewis, Cavalazzi, & others, 2020). Lipids are excellent biomarkers specific to groups of bacteria and archaea, both in modern (Jahnke & others, 2008) and in fossil microbial mats (Summons & Walter, 1990). These complex branched and/or cyclic hydrocarbons are resistant to microbial degradation during early diagenesis. Thus, membrane-associated polar lipid

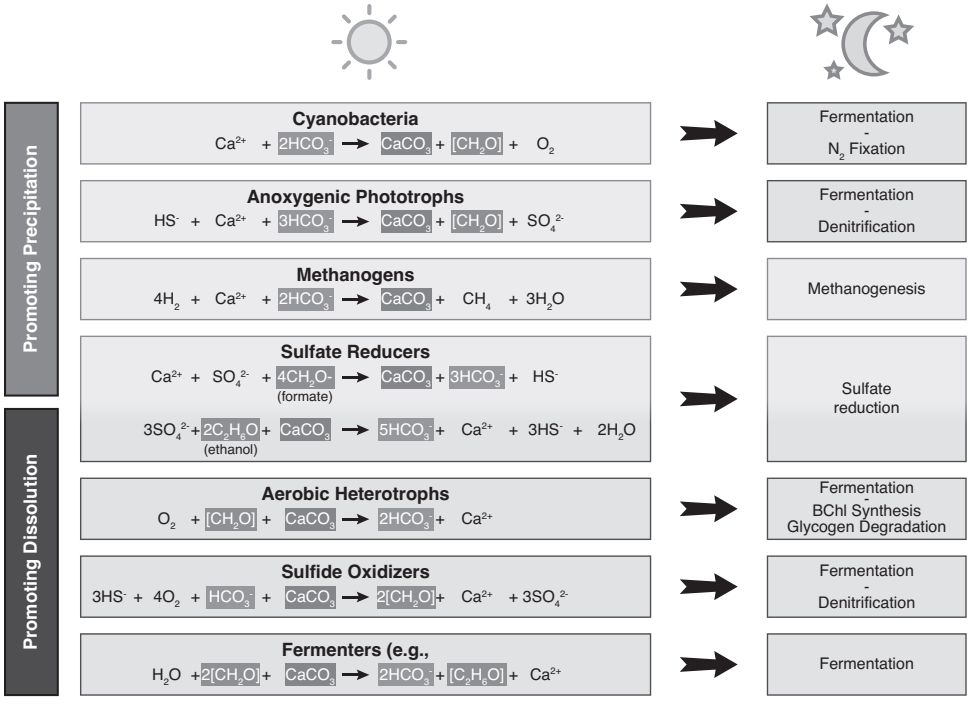

FIG. 8. Key metabolic reactions in a modern photosynthetic microbial mat combined with the precipitation/dissolution of carbonates: $HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + H^+$ (for details, see VISSCHER & STOLZ, 2005; DUPRAZ & VISSCHER, 2005; and DUPRAZ & others, 2009). Reactions (in light gray-shaded boxes) promote precipitation of carbonates; reactions in darker-shaded boxes result in conditions favoring dissolution of carbonates. Note that depending on the electron donor type, sulfate reduction can either promote precipitation or dissolution (GALLAGHER & others, 2012). Photosynthetic microbial mats depend on light for carbon fixation, and several metabolic activities within guilds vary between light (boxes on the left) and dark (boxes on the right) conditions. The actual precipitation potential depends on the sum of actual metabolic activities for each group at each depth during a diel cycle.

biomarkers can be used to identify specific functional groups of microbes, notably those of phototrophs, sulfate reducers (having ester-bound lipids), and methanogens (with ether-linked lipids). Protists and other eukaryotes also contain specific lipids that can be used as diagnostic biomarkers (SUMMONS & WALTER, 1990; JAVAUX & LEPOT, 2018). However, prior to the onset of bioturbation in the Ediacaran (635–542 Ma) microbial mats may have formed a barrier to inclusion of planktonic biomass in sediments where heterotrophic activity fueled by cyanobacterial oxygen largely degraded the freshly deposited biomass (PAWLOWSKA, BUTTERFIELD, & BROCKS, 2012). Most Archean and Proterozoic samples that have been investigated with respect to lipid biomarkers originated in shallow sediments that were most likely colonized by microbial mats (GEHLING, 1999; EIGENBRODE, FREEMAN & SUMMONS, 2008; HICKMAN-LEWIS, & others, 2018). The presence and specific distribution of Rare Earth Elements (REE), i.e., specific REE anomalies, has also been used to interpret microbial mats and the paleoenvironmental conditions in which they developed (TAKAHASHI & others 2005; LAWRENCE & KAMBER 2006; CENSI & others, 2015; COFFEY & others, 2013; HICKMAN-LEWIS, GOURCEROL, & others, 2020). REE Yttrium (Y) and Thorium (Th) have been used to discriminate between trapping and binding and *in situ* precipitation of minerals in Precambrian stromatolites (CORKERON & others 2012),

but results can be ambiguous (Nutman & others, 2016; Allwood & others, 2018). High-resolution stable isotopes of carbon and nitrogen in organic remains and of sulfur in sulfide biominerals provide insight in ancient microbial mat metabolisms (Marin-Carbonne & others, 2018; Lepot & others, 2019).

Precipitation of minerals alone does not guarantee lithification of the mat. Some mats may lithify (Tice & Lowe, 2004; Duda & others, 2016), but the majority do not and are not preserved or easily recognized in the rock record. Certain surface features of soft mats are found in the fossil record (Hagadorn & Bottjer, 1997; Schieber, 1999; Gerdes, 2007; Noffke, 2008; Noffke & others 2001, 2013; Noffke & Awramik, 2013). So-called microbially-induced sedimentary structures (MISS), typically siliciclastic in composition, capture the sediment dynamics in shallow-marine, lacustrine, and riverine settings. Some MISS date to the Archean, such as the 2.9 Ga Pongola Supergroup in South Africa and the 3.5 Ga Dresser Formation in Western Australia. These fossilized surface structures resemble contemporary intertidal ripple marks and erosion pockets, polygonal crack features, gas domes, pinnacles and pustular mat fabrics (Gerdes, 2007; Harwood & Sumner, 2011; Visscher & others, 2010; McMahon, Davies, & Went, 2017; Morris & others, 2020). For more details, see p. 71–90). Also, a large number of preserved siliciclastic mats and MISS images are in an atlas of microbial mat features (Schieber & others 2007). Particularly well-preserved microbial mats from the Bhander Formation, Vindhyan Supergroup, India, have features that typically only occur in siliciclastic rocks (ripples, wrinkle marks, cracks) also preserved in carbonates (Sarkar & others, 2016).

Microbial mats stabilize sediments through their combined network of filamentous microorganisms and sticky, somewhat plastic EPS (Noffke, Decho, & Stoodley, 2013; Decho & Gutierrez, 2017). This matrix facilitates preservation of both vertebrates and invertebrates, which occurs by molding morphological features (Iniesto & others, 2016). Typically, successful fossilization occurs during rapid burial in non-cohesive sediments, but the slow incorporation in the mat matrix provides a similarly efficient alternative in low-depositional environments. Soft tissue, such as skin and scales, dating back to the Neoproterozoic, are particularly well preserved in microbial mats (Darroch & others, 2012). The cohesiveness of microbial mats prevents erosion and, consequently, many fossilized mats from the Ediacaran, Triassic, and Cretaceous that were thriving in hot and humid conditions hold excellent examples of fossils, including *Dickinsonia* sp., *Parvancorina* sp., and burrowing *Yichnus* sp. (Gehling, 1999; Chen & others, 2013; Xiao & others, 2019). Other fossils of interest include dinosaur tracks, tetrapods, crustaceans, fish, and possibly plants (Iniesto & others, 2018).

Thick, moist mats have the best preservation potential, sometimes showing exquisite detail (Marty, Strasser, & Meyer, 2009), biostabilizing, and preventing reworking by wind or currents (Bouougri & Porada, 2012. Cuadrado, Perillo, & Vitale, 2014; Kvale & others, 1995). A hot climate and high evaporation rates may further enhance the preservation potential of mats, supported by increased microbially-influenced organo-mineralization and elevated saturation indices (Nemati & Voowdouw, 2003).

CONCLUSION

Life began during the Archean eon, potentially as prebiotic gels associated with minerals. These transitioned to life that may have formed primitive microbial mats (Trevors, 2011) 3.7–3.4 billion years ago, either in fluvial, shallow tidal, or hot spring environments. Some of these mats may have lithified, forming the first stromatolites preserved in the fossil record. Although microbial mats have undoubtedly evolved through geological time in response to changing environmental conditions, their presence throughout the past 3.5 billion years

makes contemporary mats a potential analog for the past. The appearance of oxygenic photosynthesis and potential to fix atmospheric nitrogen—important milestones in the evolution and diversification of life—is ascribed to microbial mats. Early eukaryotes may have even emerged in these systems via endosymbiosis. The cohesive properties of these laminated organosedimentary ecosystems are most often based on filamentous microorganisms and exopolymeric substances.

Microbial mats are consummate survivors unequaled in Earth's history, and they possess an astonishing array of adaptive metabolisms enabling them to thrive in the harshest of environments. Their preserved remains, billions of years old, provide insight into the origins of life on Earth. The study of living mats is key to understanding these remains and how processes of mineral precipitation may have led to their preservation. Microbial mat ecosystems have played a pivotal role in the preservation of soft and hard tissue and provide a wealth of diverse trace and chemical fossils, making these benthic biofilms crucial paleontological phenomena.

In addition to having many other roles, microbial mats and microbialites are critical biogeomorphological agents on coastlines that in the near future will aid in the protection of nearshore environments (Morris & others, 2020), as they have done from the onset of life on our planet. Their long presence in geologic time makes microbial mats potential targets for the search for evidence of extraterrestrial life (Dupraz & Visscher, 2005).

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