The complex role of microbial metabolic activity in fossilization

Kathrin Janssen1†*, Bastian Mähler2†*, Jes Rust2, Gabriele Bierbaum1 and Victoria E. McCoy3

1Institute of Medical Microbiology, Immunology and Parasitology, Medical Faculty, Rheinische Friedrich-Wilhelms Universität, 53127, Bonn, Germany
2Paleontology Section, Institute of Geosciences, Rheinische Friedrich-Wilhelms Universität Bonn, 53115, Bonn, Germany
3Department of Geosciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, 53211, U.S.A.

ABSTRACT

Bacteria play an important role in the fossilization of soft tissues; their metabolic activities drive the destruction of the tissues and also strongly influence mineralization. Some environmental conditions, such as anoxia, cold temperatures, and high salinity, are considered widely to promote fossilization by modulating bacterial activity. However, bacteria are extremely diverse, and have developed metabolic adaptations to a wide range of stressful conditions. Therefore, the influence of the environment on bacterial activity, and of their metabolic activity on fossilization, is complex. A number of examples illustrate that simple, general assumptions about the role of bacteria in soft tissue fossilization cannot explain all preservational pathways: (i) experimental results show that soft tissues of cnidaria decay less in oxic than anoxic conditions, and in the fossil record are found more commonly in fossil sites deposited under oxic conditions rather than anoxic environments; (ii) siderite concretions, which often entomb soft tissue fossils, precipitate due to a complex mixture of sulfate- and iron reduction by some bacterial species, running counter to original theories that iron reduction is the primary driver of siderite concretion growth; (iii) arthropod brains, now widely accepted to be preserved in many Cambrian fossil sites, are one of the first structures to decay in taphonomic experiments, indicating that their fossilization processes are complex and influenced by bacterial activity. In order to expand our understanding of the complex process of bacterially driven soft tissue fossilization, more research needs to be done, on fossils themselves and in taphonomic experiments, to determine how the complex variation in microbial metabolic activity influences decay and mineralization.

Key words: bacteria, fossil, experimental taphonomy, biofilm, biominalization, microbial mat, pyritization, decomposition, fossilization

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* Authors for correspondence: (Tel.: +4922828716821; E-mail: s5kajans@uni-bonn.de); (Tel.: +49228739338; E-mail: bastian.mahler@uni-bonn.de)
†Authors contributed equally to this work.
I. INTRODUCTION

Fossilization of soft tissue is a complex interplay of destruction through decay and preservation through mineralization (Briggs, 2003). Bacteria are the primary drivers of decay (Raff et al., 2008), although tissue autolysis, which includes decay processes driven by endogenous enzymes (Penning, 2006) like proteases, lipases and amylases, starts rapidly post mortem and can also influence the preservation and destruction of tissues (Krause & Jachau, 2002). Microorganisms such as bacteria outnumber body cells and are ubiquitous on the organism during its lifetime; they continue to be active on and in the carcass from the moment of death until it is destroyed through decay or until it enters the fossil record. Far from always being passive onlookers, bacteria play an essential role in some fossilization processes. Rapid heterotrophic decay of soft tissue may lead to the release of high concentrations of ions, from the carcass or the surrounding environment, which can stabilize the remaining tissues or influence authigenic mineralization. Therefore, bacterial activity can lead to excellent preservation, if the mineralization or stabilization rates remain higher than tissue decomposition rates (Allison, 1988; Briggs, 1995; Carpenter, 2005; Gehling, 1999; McNamara et al., 2009; Naimark et al., 2016a, 2016b, 2016c; Petrovich, 2001; Sagemann et al., 1999; Wilson & Butterfield, 2014). Other pathways of soft tissue preservation, which are less reliant upon bacterial activity, include altered preservation of recalcitrant tissues composed of resistant biomolecules such as melanin, or tissue polymerization as can occur for arthropod and leaf cuticle (Briggs, 1999; Briggs & Summons, 2014; Goldstein et al., 2004; Gupta et al., 2006; McNamara et al., 2016; Riley, 1997; Schweitzer, 2011).

For this reason, theoretical models and experimental investigations into the effect of bacteria on soft tissue fossilization tend to focus on the role of environmental conditions that either inhibit bacterial activity or promote specific metabolic pathways that encourage mineral precipitation. To provide a few examples:

Successful fossilization is often correlated with anoxic conditions due, for example, to rapid burial (Parry et al., 2017). Without oxygen, decay processes driven by aerobic bacteria are inhibited and the anaerobic metabolic pathways dominate. These are often more efficient in mineral precipitation than aerobic metabolic pathways and mineral precipitation can contribute to soft tissue preservation (Briggs & Kear, 1993a, 1993b, 1993c; Gostling, Dong & Donoghue, 2009; Martin, Briggs & Parkes, 2003).

In the Eocene, the Green River Formation in North America, famous for its diverse biota of exceptionally preserved fossils, was deposited in an environment in which fresh water alternated with highly alkaline and hyper saline waters (Surdam & Stanley, 1979). Today, the Green River Formation hosts the largest concentrations of trona, a mineral that indicates a pH of >11, in the world (Gäb et al., 2020). This combination of high salinity and high pH might have contributed to the preservation of fossils, particularly fish, found in that location. In taphonomic experiments on fish, it was found that the extreme environmental conditions in the Green River depositional environment could reduce decay and promote mineralization (Gäb et al., 2020).

For the most part, bacterially mediated soft tissue fossilization can be explained through adverse environmental conditions that limit decay and guide bacterial metabolic activity towards one of a few pathways that control the mechanism of tissue stabilization or replacement by authigenic minerals. However, there is evidence that some specific fossilization processes are driven by a small and specific subset of microbial metabolic activities (see Section IV). This highlights the importance of a detailed analysis of microbial interactions and their role in fossilization.

The influence of microbial activity on fossilization processes is still being investigated. To date, a number of experimental studies on the direct influence of bacteria on mineralization and preservation have been conducted, but they are mainly limited to marine settings [Briggs & Kear, 1993a, 1993b, 1993c; Butler et al., 2015; Eagan et al., 2017; Hof & Briggs, 1997; Martin, Briggs & Parkes, 2005; Naimark et al., 2018a; Raff et al., 2008; Raff et al., 2014; Sagemann et al., 1999; but see e.g. Naimark et al., 2016b, Peterson, Lenczewski & Scherer, 2010 and Mähler et al., 2020 for non-marine investigations]. Experimental studies can give insights into the initial and probably the most important steps in decay and mineralization processes that pave the way to fossilization (Briggs & McMahon, 2016; Newman et al., 2019; Sagemann et al., 1999).

Both intrinsic (gastrointestinal/integumental flora) and extrinsic (environment) bacteria have an impact on degradation and conservation processes (Bickel & Tang, 2010; Butler et al., 2015). Butler et al. (2015) identified endogenous gut microbes as the key organisms for the preservation of soft tissue under reducing conditions, through 35 days of microscopic observation of decaying arthropods in a laboratory setting. They also suggested that reducing conditions are essential for the preservation of soft tissue, because autolysis must be inhibited (Butler et al., 2015). Eagan et al. (2017) argued that different organisms harbour distinct endogenous floras which can lead to preservation – mainly through the formation of biofilms (see Section III for a discussion of
how these influence preservation) – or decay. Many bacteria are motile (they move using helical filaments with rotary motors, called flagella or retractable pili; Moens & Vanderleyden, 1996) and hence can colonize decaying matter in their surroundings. These different interpretations of the role of the gut biota versus endogenous biota in fossilization are not contradictory, but rather suggest a complex interaction between intrinsic and extrinsic microbes in fossilization, so that each microflora differs in importance depending on the details of the situation.

Mineralization induced by bacterial species or microbial communities strongly influences a number of preservation processes, for example, pyritization, phosphatization, and other processes of fossilization through authigenic mineralization. Wilby, Briggs & Riou (1996) and Jauvion et al. (2009) indicated that even if they exhibit the same general metabolic control. Most commonly, microbial metabolic activity and the resulting biochemical changes develop a local environment conducive to the precipitation of minerals. For example, the hydrolysis of urea by bacterial ureases leads to the preservation of internal structures as pyrite or phosphate minerals (e.g. digestive glands and muscle tissue), noting specifically that this may explain the fossilization processes in the La Voulte-sur-Rhône Lagerstätte in France (Jauvion et al., 2020). This was also experimentally demonstrated and discussed for the soft-tissue preservation of Miocene Frogs from Libros in Spain (McNamara et al., 2009). Some investigations into the mineralization properties of specific bacterial species in vitro (Boquet, Boronat & Ramos-Cormenzana, 1973; Braissant et al., 2003; Daskalakis et al., 2013; Hammes et al., 2003; Hatayama & Saito, 2019; Kappler, Schink & Newman, 2003; Phoenix, Konhauser & Ferris, 2003; Stocks-Fischer, Galinat & Bang, 1999; Warthmann et al., 2000, 2005) and in vivo (Jones et al., 2004) indicated that even if they exhibit the same general categories of metabolic activity, their mineralization properties may differ.

Bacteria catalyse a wide variety of complex processes that considerably influence local (or in some cases widespread) environmental conditions that can lead to mineralization (Hirschler, Lucas & Hubert, 1990) and thereby to the preservation of the tissue. In addition, they produce several exoenzymes such as proteases (which function in hydrolysis of proteins), chitinases (hydrolysis of chitin) or lipases (hydrolysis of lipids), that contribute directly to decomposition of soft tissue. All these aspects underline that a deep understanding of bacterially mediated fossilization processes requires a study of the spectrum of bacterial adaptations to adverse habitats. Even in the most stressful conditions that severely inhibit the activity of most bacteria, some specifically adapted species can still live, metabolize and decay soft tissue.

Research into soft tissue fossilization is deeply interdisciplinary, involving palaeontological, geological and microbiological aspects. The goal of this review is to bring together and summarize the key relevant knowledge of these fields, with a focus on microbiology, to make this available to all researchers interested in this topic.

II. BACTERIAL ACTIVITY AND MINERALIZATION

The precipitation of authigenic minerals is often a pathway to the preservation of soft tissues. This can range from the cellular-scale replacement of soft tissues (e.g. Martill, 1990) to a mineralized concretion entombing the fossil (e.g. McCoy, 2014). The mineralization step of fossilization can be studied in laboratory experiments (Fig. 1), as mineralization can occur within days, month or years after death (Mahler et al., 2020; Li et al., 2013; Sagemann et al., 1999).

Mineralization has been intensively investigated over the last 100 years and encompasses a number of different processes. The two processes most relevant for a study of bacterially mediated fossilization are biologically induced mineralization (BIM) and mineralization of organic matter.

(1) Biologically induced mineralization

Biologically induced mineralization (BIM) is under metabolic control. Most commonly, microbial metabolic activity and the resulting biochemical changes develop a local environment conducive to the precipitation of minerals. For example, the hydrolysis of urea by bacterial ureases leads to

Fig 1. Calcite precipitation on decomposing crayfish (Cambarellus diminutus). (A) Telson of a decomposing crayfish on day 7 at 24°C with calcite clusters on the inner side of the carapace. Arrows indicate calcite clusters. Scale bar: 2.5 mm. (B) Bacteria on the mineralized carapace of a crayfish during decay on day 11 at 30°C (Mahler et al., 2020). b, bacteria; c, carapace; p, pulmose seta. Scale bar: 10 μm.
a pH increase, which may result in calcium carbonate precipitation (Dhami, Reddy & Mukherjee, 2014; Hammers et al., 2003; Mobley & Hausinger, 1989; Phillips et al., 2013; Stocks-Fischer et al., 1999; Zhu & Dittrich, 2016). Free calcium ions attach to the negatively charged bacterial cell envelope [in Gram-negative bacteria to lipopolysaccharides and lipids, and in Gram-positive bacteria mainly to teichoic acids (Wilson et al., 2001)] as well as to biofilm exopolysaccharides and extracellular DNA which – depending on the surrounding pH – can contain high proportions of functional acidic groups. When these polymers are degraded or re-organized, ions are set free, and minerals can precipitate (Arp, Reimer & Reitner, 2003; Dupraz & Visscher, 2005; Hartley et al., 1996). Silicification gives an example directly related to fossilization: in the Ordovician Fezonuata biota of Morocco, giant (>1 m) anomalocarid arthropods are fossilized within siliceous concretions, whose precipitation is explained by BIM (Gaines et al., 2012a). Shortly after the large anomalocarid carcasses had been buried, anaerobic sulfate reduction influenced the local chemical environment to be conducive to silica precipitation. Initially, sulfate reduction decreased the pH, resulting in the dissolution of volcanic ash and the release of silica into the porewater, but as decay proceeded, the pH subsequently rose, resulting in the precipitation of silica around the carcasses (Gaines et al., 2012a).

(2) Biologically induced mineralization together with organic matter

For fossilization, typically BIM works together with organic matter, including extracellular biopolymers or by-products of the metabolic processes of microorganisms (Dupraz et al., 2009; Weiner & Dove, 2003; Wilmeth et al., 2018). The importance of an organic substrate in mineralization, especially as a site of nucleation, is highlighted in the process of silicification. Silicification requires large amounts of dissolved silica, typically sourced from volcanism or siliclastic organisms (Butts & Briggs, 2011; Butts, 2007, 2014; Channing & Edwards, 2009; Farrel, 2014; Farrell, 2013). Soft tissues from dead marine organisms like Desulfovibrio are able to reduce sulfate to hydrogen sulfide (SH–) and elemental sulfur. Iron monosulfide and elemental sulfur can react directly to pyrite or form an intermediate stage (e.g. mackinawite; greigite), which reacts further with iron oxides to form iron monosulfide and elemental sulfur. Iron monosulfide and elemental sulfur can react directly to pyrite or form an intermediate stage (e.g. mackinawite; greigite), which reacts further with sulfur to form pyrite (Berner, 1970, 1984; Canfield & Raiswell, 1991). The amount of pyrite depends on the amount of available organic matter, the pH (a pH < 6 leads to the formation of marcasite and a pH > 6 to pyrite) and the availability of iron oxides or dissolved iron (Canfield &
Raiswell, 1991). Theoretical and experimental models also suggest that the porosity and the lability of the decaying organic material influences pyritization (Grimes et al., 2001; Stockdale, Davison & Zhang, 2010), by influencing chemical gradients within the tissue and, thereby variation in nucleation (Gabbott et al., 2004; Grimes et al., 2002).

Some of the most commonly precipitated BIM minerals are carbonates (Lin et al., 2018), whose precipitation is typically induced by bacterial metabolism (Boquet et al., 1973). In some cases, the precipitated carbonate replaces or infills voids in decaying soft tissue, such as in the Jurassic La Voulte-sur-Rhône fossil site, in which soft tissues are preserved in a variety of minerals including carbonates (Jauvion et al., 2020; Wilby et al., 1996). In Burgess Shale type-fossilization (typically in Cambrian biotas), the precipitation of authigenic carbonate cements caps the fossiliferous beds which decreases permeability, limits oxidant diffusion, and therefore changes decay processes (Gaines et al., 2012a, 2012b; Gaines & Droser, 2005). Most commonly, though, precipitated carbonate minerals entomb the decaying carcass in a carbonate concretion (Baird et al., 1986; Martill, 1990; McCoy, 2014; McCoy, Young & Briggs, 2015a; Orr et al., 2000; Park & Downing, 2001; Wilby et al., 1996). Most anaerobic decay processes create a local chemical environment conducive to carbonate precipitation, for example through the production of bicarbonate ions (Allison & Pye, 1994; Coleman & Raiswell, 1993; Gaines & Vorhies, 2016; McCoy, 2014; Raiswell, 1971; Raiswell & Fisher, 2000; Wilson & Brett, 2013). Taphonomic experiments have successfully resulted in the precipitation of carbonate cement on decaying carcasses or in sediment around decaying tissue, although not to the extent of growing a full, solid concretion (Briggs et al., 1993; McCoy, Young & Briggs, 2015a; Pye et al., 1990). As with pyrite and phosphate minerals, organic matter can also act as a nucleus for carbonate precipitation (Giuffre et al., 2013).

III. BIOFILMS AND MICROBIAL MATS

In many environmental settings, bacterial cells not only exist as free-floating single cells (a planktonic lifestyle) but also bind to surfaces with other bacterial cells to form communities called biofilms (a sessile lifestyle). The most characteristic and representative sessile bacteria in marine settings are in the families Alteromonadaceae, Vibrionaceae and Bacteroidetes (Dang & Lovell, 2016). In some cases, biofilms have been found to be composed of only one species, but more commonly they consist of several different bacterial species (O’Toole, Kaplan & Kolter, 2000; Sutherland, 2001b). The formation of a biofilm by a bacterial strain is carefully regulated (Busscher, Cowan & van der Mei, 1992; Pratt & Kolter, 1998). After adhesion on a surface, bacteria multiply to a threshold density and then induce expression of the biosynthesis genes that control the synthesis of an extracellular polymeric substance (EPS); this consists mostly of polysaccharides, but also contains proteins, lipids and extracellular DNA (Fig. 3). The extracellular matrix is necessary for a sessile lifestyle (Flemming & Wingender, 2010; O’Toole et al., 2000) because it enables long-term adhesion to a surface, functions as a protective barrier against chemicals, grazing organisms from the surrounding medium, and dehydration, and also provides a perfect environment for interactions among different microorganisms (Decho, 2000; Flemming & Wingender, 2010; Mah & O’Toole, 2001; Roberson & Firestone, 1992).

A microbial mat is a particularly complex type of biofilm, with a complex interplay between various microorganisms (Bolhuis & Stal, 2011; Cantrell & Duval-Perez, 2012; Freydet & Verrecchia, 1998). A microbial mat is formed when...
A microbial community is layered on the basis of abiotic environmental gradients such as water, light and oxygen (Bonilla-Rosso et al., 2012; Stolz, 2000; Vasconcelos et al., 2006). In some cases, species of the domains Archaea and Eukarya are also involved in the formation of a mat (Bolhuis, Fillinger & Stal, 2013; Casamayor et al., 2002; Robertson et al., 2009). Within the stratification of the mat, physical gradients such as oxygen saturation, pH, osmolarity and redox potential influence microbial activity (Arp et al., 2012; Stewart & Franklin, 2008; Visscher & van Gemerden, 1991). The individual layers of a microbial mat perform specific tasks (production of storage materials, N- and C-fixation, fermentation, sulfate reduction, vitamin synthesis), therefore they exhibit various metabolic pathways, and there are interactions between the layers (Kim et al., 2015; Saghai et al., 2017; Stolz, 2000). With a few exceptions (Bolhuis et al., 2013; Freytet & Verrecchia, 1998; Stal, van Gemerden & Krumbein, 1985) extant microbial mats are primarily located in extreme settings, such as hypersaline (Braissant et al., 2009; Guerrero & de Wit, 1992; Ionescu et al., 2015; Schneider et al., 2013), alkaline (Arp et al., 2003), high-temperature (Klatt et al., 2013) or Antarctic lakes (Ohutsuka et al., 2006), where the amount of biofilm-grazing metazoans is limited due to the harsh environment (Briggs, 2003; Gall, 2001).

**1. Mineralization of biofilms/microbial mats and fossilization**

Complete lithification of a microbial mat is a long process that occurs over the course of a few months to a few years. Abiotic factors such as calcium carbonate equilibrium in the surrounding sea water as well as biotic factors like microbial activity or diurnal changes in pH (also influenced by metabolic products) influence the lithification process (Vasconcelos et al., 2006; Wilmeth et al., 2018). A few studies have determined that carbon fixation, which is mainly performed by photoautotrophic bacteria in the upper layer of a microbial mat, can prevent lithification (Schuler, Havig & Hamilton, 2017; Wilmeth et al., 2018). The morphology and mineralogy of precipitates is influenced by the composition of the EPS matrix, which is mainly driven by the structure of the microbial community (Braissant et al., 2003). Additionally, the EPS matrix controls the mineralization processes through the fixation of cations from the surrounding medium and seems to be one of the most important factors controlling preservation through carbonate precipitation (Dupraz et al., 2009).

The best-studied mineralized biofilm is dental calculus. In dental plaque, a biofilm which contains a variety of Gram-positive and Gram-negative bacteria (Jin & Yip, 2002; White, 1997), microorganisms adhere to the protein layer on the enamel surface and synthesize a biofilm matrix in which calcium and phosphate is absorbed from the saliva. Additionally, acidic conditions in the biofilm caused by the microbial degradation of sugars lead to a demineralization of the tooth enamel. In this way, high concentrations of ions are accumulated in the biofilm matrix which acts as a barrier to ion exchange (Braissant et al., 2009; Sutherland, 2001a) and this supersaturation can result in the precipitation of calcium phosphate, the fundament of dental calculus (Marsh, 2006; Nancollas & Johnson, 1994). In intensive care units in hospitals, crystalline biofilms are also known to pose a problem in urinary catheters (Stickler, 2008).

Biofilms and microbial mats have also been shown to play an important role in fossilization. A dead organism in an aquatic system can quickly be enveloped by a microbial mat, which can influence degradation, disarticulation and
decay processes as well as protect against predators and bioturbation (Briggs, 2003; Gehling, 1999; Iniesto et al., 2016). During the envelopment of an organism by a biofilm, the conditions around the carcass will change due to microbial activity (pH change, oxygen loss/anoxia, high concentrations of sulfur compounds) and consequently mineralization of the biofilm can occur. This will often lead to fossilization and preservation of the carcass, commonly as a detailed mould or cast.

Well-preserved Ediacaran fossils, commonly found as casts and moulds, putatively derive from bacterial activity in microbial mats. The ‘death mask’ model, first described by Gehling (1999), was built upon the understanding that microbial mats were widely distributed on the sediment during the Ediacaran. By contrast, starting in the Cambrian, the ‘agricornic revolution’ and ‘substrate revolution’, based on an expansion of bioturbating organisms, resulted in the widespread loss of seafloor matgrounds (e.g. Seilacher & Pfüger, 1994; Bottjer, Hagadorn & Dornbos, 2000; Mángano & Buatois, 2017). In the Ediacaran, when microbial mats were ubiquitous on the seafloor, dead carcasses on the sediment were quickly overgrown by microbial mats which resulted in a scaling of the body and, through initial microbial decay, in a decrease in oxygen saturation. Afterwards, anaerobic sulfate-reducing bacteria produced hydrogen sulfide which reacted with dissolved Fe²⁺ from the sediment to form pyrite (FeS²) (Droser, Gehling & Jenkyns, 2006; Gehling, 1999; Gehling et al., 2005). A carcass, which was surrounded by the sediment, was rapidly stabilized by the combination of a microbial mat and authigenic pyrite precipitation (Gehling, 1999; Kenchington, Wilby & Street, 2015). Tarhan et al. (2016) contradicted the common theory of pyrite fossilization and stated that silicification due to high concentrations of silica in ancient oceans had more influence on the excellent preservation, whereas other studies showed that silica layers are formed after pyritization (Lafflamme et al., 2011; Liu et al., 2020; Tarhan et al., 2016).

Experimental studies have also investigated the impact of biofilm formation (including microbial mats) on preservation and fossilization processes, as well as on the formation of pseudomorphs to replicate soft tissue (e.g. Butler et al., 2013; Darroch et al., 2012; Iniesto et al., 2013, 2016, 2017, 2018; Raff et al., 2008, 2013; Raff et al., 2014; Raaff & Raaff, 2014). In the experiments performed by Iniesto et al. (2013, 2016, 2017) muscle fibres, bone marrow and skin structures in frogs and fish were preserved after placing the animals on a microbial mat for a duration of 3 years. The midbrain (tectum) of a frog, decayed on a microbial mat, was replaced by calcium carbonate crystals after 1.5 years (Iniesto et al., 2017). In addition to calcium carbonate, microbial mats also triggered the formation of a thin crystalline Mg-silicate phase (Iniesto et al., 2015). Darroch et al. (2012) and Gibson, Schillbauer & Darroch (2018) detected the formation of pyrite when diaploblastic (Condylactis gigantea) and triploblastic (Dolabella auricularia, Galleria mellonella) organisms decayed on sediment in freshwater inoculated with microbial mats, especially when Fe²⁺ ions were added to the system.

The generation of pseudomorphs by microbes can stabilize a carcass and protect it from destruction (Butler et al., 2015; Raaff et al., 2013). In a study performed by Raaff et al. (2013), various seawater bacteria were characterized regarding their ability to form pseudomorphs or to degrade soft tissue. In addition to the observation that the interactions of microbes play an important role in the preservation or the decay of a carcass, they showed that preservation by pseudomorph formation is not limited to single-celled organisms (Raaff et al., 2013). Eagan et al. (2017) also isolated bacterial strains from decaying shrimp (Artemia sinica) larvae and sea urchin (Heliocidaris erythrogramma) embryos and tested their behaviour on soft tissue individually and in combination. Whereas Marinobacter sp. formed pseudomorphs and stabilized the carcass, Bacillus sp. destroyed soft tissue. If both species were incubated together in the solution, the pseudomorph formation of Marinobacter sp. outcompeted the decay ability of Bacillus sp. (Eagan et al., 2017).

IV. CASE STUDIES

Soft tissue fossilization is often explained by very broad categorical descriptions of microbial metabolic activity. For example, sulfate reduction promotes pyritization (Farrell, 2014), and methanogenesis promotes carbonate concretion growth (Bojanowski & Clarkson, 2012; Cotroneo et al., 2016; Irwin, Curtis & Coleman, 1977; Raiswell, 1987). Or anoxic conditions allow the preservation of soft tissue whileoxic conditions allow a high microbial decay rate and therefore the destruction of soft tissue (Briggs & Kear, 1993a, 1993c; Gostling et al., 2009; Martin et al., 2003). Here we present three case studies in which initial simple theories of fossilization, often based on broad categories of microbial activity, have given way to a more complex understanding of how microbial metabolic activity influences fossilization. Further research into other open questions of soft tissue fossilization may reveal similarly complex stories.

(1) Cnidarians and oxic fossilization

It is widely assumed that anaerobic conditions can lead to better preservation. This anoxia can be a pre-occurring condition, for example through burial, or a side-effect of decay processes (Briggs & Kear, 1993a, 1993c; Gostling et al., 2009; Martin et al., 2003). The anaerobic conditions inhibit scavengers and degrading aerobic bacteria. Anoxia also promotes various anaerobic metabolic pathways, which are often relevant for biologically induced mineralization. However, a detailed survey of the cnidian medusa fossil record (encompassing scyphozoan, hydrozoan, and cubozoan medusae) found that the majority of these fossils are found in shallow, coastal fossil sites, and that they are rare in deeper, oxygen-poor sites, suggesting that medusa fossilization may be more likely in oxic than in anoxic...
environments (Young & Hagadorn, 2010). However, note that more recently it has been shown that Lagerstätten, such as the Qingjiang Biota from the Cambrian of China (Fu et al., 2019), have well-preserved cnidarian fossils across a wide range of environmental conditions, sediment compositions and geochemical circumstances (Young & Hagadorn, 2020). Experimental work suggested that the above conclusion regarding better fossilization in oxic environments is also applicable to non-medusae cnidaria. Hancy & Antcliffe (2020) experimentally investigated the degradation patterns of the cnidarian anemone Actinia equina, in the polyp rather than medusa form, and found that diagnostic cnidarian characteristics, for example, the hypostome, degraded as fast or faster under anoxic conditions than under oxic conditions. Cnidarians (with the exception of mineralized forms such as corals) are among the softest and most labile organisms and their preferential preservation and inhibited decay is often associated with oxic conditions (Hancy & Antcliffe, 2020).

Preserved medusae can occur as remnant organic residue, with tissues replaced by early diagenetic minerals, or as moulds/casts whose formation was facilitated by sediment cementation and microbial mats, but almost all medusa fossils show evidence of decay (Young & Hagadorn, 2020), indicating that bacterial activity, and its influence on decay and mineral precipitation, plays a critical role in medusa fossilization. Previous studies have suggested that unique microbial communities, both on cnidarian carcasses and developed in the surrounding sediment through microbial decay, might be important for cnidarian fossilization (Titelman et al., 2006; Young & Hagadorn, 2020). The underlying reasons for this unusual pattern of cnidarian fossilization could involve some combination of decreased decay in oxic conditions, increased decay in anoxic conditions, and decreased mineralization in anoxic conditions (relative to bilaterians, which are common in anoxic fossil sites and also commonly the focus of taphonomic experiments), which in turn depend upon the metabolic pathways of internal and external microbiota around a decaying cnidarian. To date, there is only minimal research about the differences between the microbial colonization of cnidarians and bilaterians, but even these preliminary results suggest intriguing possibilities.

In taphonomic experiments, bilaterians commonly degrade from the inside out, with gut microbes playing an important role in the decay and loss of soft internal characters (Butler et al., 2015; Bickel & Tang, 2010). However, to counterbalance the rapid gut biota-driven internal decay of bilaterian tissues, it has also been suggested that gut biota rapidly escape the gut after death and form internal biofilms that eventually provide a template for, and induce, mineralization, thereby preserving soft tissue (Butler et al., 2015). By contrast, experimental decay of cnidian tissues indicated the opposite pattern of outside-in degradation, suggesting that the biota of the gastrovascular cavity does not play a major role in cnidian degradation (Hancy & Antcliffe, 2020). Moreover, if this biota in cnidarians is not vigorously decaying the soft tissues, they are most likely also not strongly promoting the precipitation of minerals. Cnidarians, unlike bilaterians, do not develop a gastrointestinal tract, but have a single orifice for their gastrovascular cavity, which has significant implications for internal microbial colonization, and further supports the hypothesis that cnidaria and bilateria have distinct gut biotas with different effects on decay and fossilization (Hancy & Antcliffe, 2020).

In the absence of a strongly decaying internal bacterial community, cnidian fossilization is most likely more controlled by external bacteria. The surface microbiota of different cnidarians is species specific and depends on geography, environmental conditions and the developmental stage of the organism, although it is not yet fully known which of these parameters shapes the community most (Carlos, Torres & Ottoboni, 2013; Franzenburg et al., 2013; Fraune et al., 2010; Fraune & Bosch, 2007; Mortfeld et al., 2016). Interestingly, some studies identified various Pseudoalteromonas species in association with the mucus or specific tissue, such as tentacles, of cnidaria (Romanenko et al., 2017; Schuett & Doepke, 2010; Stabili et al., 2018). These play a prominent role in the production of various antimicrobial substances or toxins, and have also been shown to exhibit variable decay and mineralization behaviour on soft tissue depending on oxygen availability. Under aerobic conditions the bacterium Pseudoalteromonas tunicata forms pseudomorphs surrounding marine embryos, whereas anaerobic conditions result in the destruction and decay of the internal structures of the cells (Raff et al., 2014). Moreover, many cnidaria are sessile marine organisms that are rarely capable of locomotion, so they secrete mucus (viscoelastic adhesive gel) to reduce water loss, enable adsorption to surfaces and also due to various antimicrobial molecules – to protect against bacterial pathogens (Guarnieri et al., 2018; Mariottini & Grice, 2016; Stabili et al., 2015). Thereby, the cnidian host is able actively to select beneficial bacterial partners and drive the microbial community composition (Franzenburg et al., 2013). Titelman et al. (2006) noted that the growth of some bacteria was variously inhibited or promoted on dead jellyfish, which may be due to these surface effects.

Bacteria exhibit broad spectra of oxygen tolerance, varying from strictly aerobic to strictly anaerobic bacteria that need, respectively, oxic or anoxic conditions for growth. Under anaerobic conditions, for example, sulfate-reducing microorganisms can cause decay just as efficiently as aerobic bacteria, but require more substrate to gain the same amount of energy (Allison, 1988; Canfield, 1994). Anaerobic Closstridia belong to the fastest growing bacteria with generation times below 10 min for Clostridium perfringens (Labbe & Huang, 1995) and produce several degradative enzymes (Fourie et al., 2020). In wastewater treatment plants, anaerobic hydrolytic bacteria are the most efficient degraders for solubilizing complex substrates to provide soluble products for several heterotrophic metabolizers (Gerardi, 2006). These results initially suggested the possibility that there is no difference in soft tissue decay rate under aerobic or anaerobic conditions (Allison, 1988; Briggs & Kear, 1994; Kidwell & Baumiller, 1990). Further studies found that
an anaerobic environment alone did not retard tissue decay sufficiently for fossilization and that the addition of reducing agents to stop autolysis was required for effective preservation (Butler et al., 2015; Hippler et al., 2011). Kichell & Baumiller (1990) could not detect any difference in decay rate of the echinoid Arbacia punctulata under oxic or anoxic conditions. Several other studies showed only minor evidence that an anaerobic environment leads to better preservation of soft tissue (Bartley, 1996; Briggs & Kear, 1994).

(2) Siderite concretions

The precipitation of siderite concretions \([\text{Fe}[\text{CO}_3]]\) often leads to fossilization, for example, in the Carboniferous Mazon Creek biota (Baird et al., 1986; McCoy, 2014). Generally speaking, siderite concretion growth around a decaying organism happens in sulfate-limited environments, where bacterial metabolism primarily occurs through microbial iron reduction and methanogenesis (Cotroneo et al., 2016; Lin et al., 2020). Based on this, the theoretical expectation is that iron-reducing bacteria such as Geobacter or Shewanella would be the primary drivers of siderite precipitation (Richter, Schicklberger & Gescher, 2012), and pure cultures of both of these species have induced the precipitation of siderite in laboratory settings (Lin et al., 2020; Mortimer et al., 2011; Zachara et al., 1998). Studies of the Norfolk Marshes, where siderite concretions are currently growing, found that the bacterial community is dominated by bacteria that typically reduce sulfate, specifically Desulfovibrio and Desulfobacter, and there is no evidence of iron-reducing bacteria (Coleman et al., 1993). Although the concretion microbiota is dominated by Desulfovibrio, the surrounding mud is inhabited by Desulfobacter. Coleman et al. (1993) assumed, based on the amount of sulfate present and the amount of iron being reduced, that Desulfovibrio species are able to perform sulfate reduction as well as iron reduction. Further evidence for this comes from pure culture experiments with Desulfovibrio, which readily carried out iron reduction (Coleman et al., 1993), and precipitated siderite around a nucleus (Lin et al., 2020; Lovley et al., 1993). The dominant role of sulfate reducers over iron reducers in siderite precipitation is also supported by pH effects of these metabolic processes. Iron reduction alone raises the pH to a level that does not allow siderite precipitation (because calcite or aragonite is favoured above pH ~7.2), whereas even a small amount of sulfate reduction along with iron reduction can keep the pH within the range for siderite precipitation (Lin et al., 2020; Roberts et al., 2013; Soetaert et al., 2007; Zeng & Tice, 2014). These pH considerations suggest that siderite precipitation involves iron reduction coupled to a low/basal level of sulfate reduction (Lin et al., 2020), although this interplay is fragile as a high amount of hydrogen sulfide would prevent the formation of siderite and promote pyrite (FeS2) precipitation (Berner, 1981; Lin et al., 2020).

However, not all sulfate-reducing bacteria are capable of iron reduction; several Desulfobacter species (e.g. Desulfobacter curvatus, Desulfobacter postgatei), which dominated the surrounding mud microbiota in the Norfolk marshes, could not reduce Fe(III) oxide in experiments, and therefore, are unlikely to play a major role in siderite precipitation (Coleman et al., 1993; Lovley et al., 1993). Environmental conditions might influence whether a bacterium reduces sulfate or iron, or whether pyrite or siderite is the preferred precipitate (Coleman et al., 1993).

Although we are beginning to untangle the complicated role that microbial metabolic activity plays in siderite concretion formation, there are still mysteries. For example, experiments on concretion growth, both in the laboratory and in the Norfolk Marsh field setting, successfully resulted in the precipitation of siderite around decay-resistant tissues and inorganic materials, such as aragonite shells, wood, and fragments of metal (Allison & Pye, 1994; Lin et al., 2020; Pye et al., 1990). Experiments involving whole carcasses or labile tissues have not produced siderite precipitation (e.g. Allison & Pye, 1994; McCoy et al., 2015b). This contrasts with the fossil record in which many siderite concretions nucleate around soft-tissue organisms; for example, in the Essex fauna at the Mazon Creek fossil site, many of the siderite concretions nucleated around Essexella, a soft-bodied organism interpreted as a jellyfish (Baird et al., 1986; Clements, Purnell & Gabbott, 2019).

(3) Pyritization of arthropod brains

One of the most current examples of an incompletely understood fossilization process is the preservation of arthropod neural tissues in Cambrian arthropods (Parry et al., 2017; Purnell et al., 2018). It was initially assumed that nervous tissue, which decays quickly in taphonomic experiments (e.g. Sansom, Gabbott & Purnell, 2011), would not preserve in the fossil record. A series of studies reported on a preserved brain and optic lobes of the arthropod Fuxianhuia from the early Cambrian Chengjiang Lagerstatte, in southwest China, followed by a number of other similar reports from a range of Cambrian arthropod taxa and a variety of sites (Ma et al., 2012, 2015; Yang et al., 2013, 2016). These fossilized nervous tissue structures occur in sites with Burgess Shale-type preservation (Edgecombe, Ma & Strausfeld, 2015; Ortega-Hernández, Lerosey-Aubril & Pates, 2019), and range in composition from a carbon film, through partial pyritization, to complete pyritization (Ma et al., 2015). Burgess shale-type preservation typically results in carbonaceous compressions of labile tissues which are sometimes subsequently pyritized (Ma et al., 2015). The stabilization of labile tissues in Burgess Shale-type preservation is primarily due to interactions between sediment and soft tissue (Butterfield, 1990; Laflamme et al., 2011; Wilson & Butterfield, 2014). Fine-grained sediments, such as the clay minerals in many Burgess Shale-type fossil sites, strongly influence decay and preservation by preventing oxygen diffusion (Allison, 1988; Naimark et al., 2016a, 2016b). In association with bacterial EPS, fine-grained sediment can form a cast around carcasses (Martin et al., 2005). The sediment also strongly influences microbial activity by releasing ions. Slightly acidic conditions lead to a release of cations such as Al3+ or Fe3+ out of the sediment that can (1) inhibit bacterial growth due to the toxicity of metal ions (McMahon et al., 2016;
Morrison, Misra & Williams, 2016; Williams et al., 2011) and (2) prevent decay of proteins because of a tanning effect where Al5+ ions form insoluble complexes with peptide residues (Naimark et al., 2016a,b, 2018a, 2018b; Newman et al., 2019; Wilson & Butterfield, 2014). Petrovich (2001) also proposed a stabilizing effect of Fe2+ ions bound to soft tissue and thereby an initiation of mineralization as a nucleation site (Petrovich, 2001). These various decay-inhibiting effects could explain the preservation of nervous tissue as carbonaceous films (Ma et al., 2013; Ortega-Hernández et al., 2019).

This release of Fe2+ ions may also contribute to the pyritization of some arthropod brains. For the pyritized neural tissue, it is assumed that the lipid-rich tissues of the central nervous system (CNS), were degraded into simple carbon chains (Ma et al., 2015). During this aerobic decay process triglycerides are hydrolytically split into glycerol and fatty acids and the sulfur-containing amino acids cysteine and methionine are degraded, whereby sulfur of the sulfate groups (SH−) is oxidised to sulfate (SO42−) or released as H2S deeper in the tissue (Schoenen, 2019). Afterwards, because of rapid burial in fine-grained sediment, oxygen diffusion is inhibited (Ma et al., 2015). Under such anaerobic conditions glycerol can be broken down further, whereas fatty acids stay in place as non-degradable substances (Schoenen, 2019).

Due to the presence of sulfate-reducing bacteria like Desulfovibrio, sulfate will be reduced to H2S (Schoenen, 2019), which then reacts with iron minerals or iron liberated from the partly decayed nervous tissue (Saleh et al., 2020) to form iron sulfide (FeS) and finally pyrite (Berner, 1970).

However, despite the theoretical understanding of how these brains preserve, this process has never been replicated experimentally. Rather, neural tissue typically degrades very rapidly in taphonomic experiments (Murdoch et al., 2014; Sansom, 2016). This suggests that the experimental models do not fully capture the process of brain preservation (Parry et al., 2017; Purnell et al., 2018). Interestingly, pyrite precipitation has been explored rarely through taphonomic experiments (Farrell, 2014). Only taphonomic experiments with plant material showed successful precipitation of pyrite crystals (Brock, Parkes & Briggs, 2006; Grimes et al., 2001), whereas in laboratory experiments with soft tissue only the precursor molecule FeS could be detected (Darroch et al., 2012; Gibson et al., 2018; Newman et al., 2019).

In vitro experiments with enrichment cultures of sulfate reducing bacteria isolated from a mine drainage seep resulted in precipitation of FeS and further diagenesis into FeS2 at the inner and outer cell envelope of the bacteria (Donald & Southam, 1999). Thiel et al. (2019) investigated a putative connection between bacterial pyrite formation and methanogenesis and describes the complex interplay and the obstacles that need to be overcome to analyse microbial mineralisation processes. Very specific and unique environmental conditions, such as the perfect balance between sulfate and iron concentrations, and microbial community compositions can lead to the excellent preservation of decay-prone nervous tissue and further experiments are required to elucidate them.

V. DEVELOPING A MORE DETAILED UNDERSTANDING OF MICROBIAL METABOLIC ACTIVITY DURING FOSSILIZATION

Microbial metabolic activity is extremely complex, and, at least in some situations, soft tissue fossilization is a delicate, microbial-driven process highly sensitive to small changes in metabolic activity. The case studies described above, as well as other examples not discussed here, suggest that specific metabolic pathways can play important roles in fossilization. So how can we determine which variations in microbial activity influence fossilization? There are two main approaches. First, interrogate the fossils directly, using a variety of analytical approaches, to determine what metabolic activity was actually taking place at the time of fossilization. Second, investigate (or at least measure) the influence of various metabolic pathways on decay and mineral precipitation in controlled environments through taphonomic experiments.

(1) Evidence from the fossil record

Geochemical analyses of fossils may provide information about ancient metabolic activity. For example, the molecular fossils of lipids (fatty acids, steroids), hydrocarbons and other organic compounds called ‘biomarkers’ may provide evidence of fossilization processes. These biomarkers are more resistant to degradation than peptides, carbohydrates or nucleic acids, and can be taxonomically specific for groups of organisms. Therefore, biomarkers that have undergone biological and chemical alteration processes (e.g. isomerization reactions) can provide interesting insights into the distribution of organisms, metabolic pathways and phylogeny of ancient microorganisms as well as ancient bio- and geochemical conditions (Brocks et al., 2003; Brocks & Pearson, 2005).

Lipid biomarkers were extracted from Ediacaran macrofossils and the detection of specific ancient steroids revealed a classification of the fossil Dickinsonia to the animal kingdom – which would be the earliest confirmed description of a metazoan animal in the fossil record (Bobrovskiy et al., 2018). Biomarkers also enable the analysis of different modes of biomeralization (Wiemann, Crawford & Briggs, 2020) or the elucidation of putative carbonate crust formation by Archaea via anaerobic oxidation of methane [Stadnitskaia et al., 2008; here, the method was combined with sequencing of fossil 16S ribosomal RNA (rRNA) gene sequences]. Metabolic pathways influencing soft tissue preservation also have been investigated via biomarker analysis. Melendez et al. (2013) showed evidence for the anaerobic recycling of crustacean organic matter through microbial sulfate reduction and photosynthesis (e.g. performed by the green sulfur bacteria Chlorobium) by the detection of organosulfur compounds.

Inorganic geochemistry, specifically isotope analyses of mineralized fossils, may also reveal information about the metabolic pathways that influenced fossilization. Phosphatization, or the replacement of soft tissue by authigenic phosphate minerals, is widely thought to be a microbially mediated process, influenced at least partially by enzymatic hydrolysis of tissues (Briggs, 2003). Phosphatized fossils are known from the
Precambrian to the recent (Dornbos, 2011; Schiffbauer et al., 2014), and may record directly the activity of certain enzymes through geological time due to their specific isotope fractionation patterns. Inorganic orthophosphate (Pi), a necessary precursor for the phosphatization of soft tissues, can be produced from organic phosphate through enzymatic hydrolysis, in which the released Pi incorporates one oxygen atom from water and the other three from the organic phosphate substrate (Liang & Blake, 2006). Different enzymes result in different oxygen isotope fractionation between the ambient water and the incorporated oxygen atom, and thus the δ18O values of Pi can indicate what enzyme was involved in its production (Liang & Blake, 2006, 2009). This has been experimentally tested for cell-free enzymes, including eukaryotic and prokaryotic alkaline phosphatases, 5′-nucleotidase, deoxyribonuclease-1, and phosphodiesterase-1 (Liang & Blake, 2006, 2009). Similar enzyme-specific signals are also distinguishable from other sources of Pi, including microbial turnover, photo-oxidation, and recycling of Pi (Liang & Blake, 2006). The oxygen isotope value is evident in either the dissolved Pi or in phosphate minerals precipitated from the Pi (Liang & Blake, 2006, 2009). Therefore, an analysis of the oxygen isotope composition of phosphate minerals in the geological record that have been precipitated from Pi produced due to enzymatic hydrolysis of organic material may provide direct evidence of enzyme activity throughout geological time. The enzymes tested so far are highly conserved and active in archaea, bacteria and eukaryotes (Liang & Blake, 2006), suggesting that the same enzymes would also have been acting throughout geological time and, therefore, that the enzymes active in fossil isotope fractionation could be recognized through comparisons with living enzymes. Similar enzyme-specific fractionation may also be found in microbially mediated precipitation of other minerals.

(2) Evidence from taphonomic experiments

Taphonomic experiments, although already a valuable tool for understanding the general processes of fossilization (Briggs & McMahon, 2016; Purnell et al., 2018; Gabbott, Sansom & Purnell, 2021), have yet to examine in detail the specific metabolic processes that contribute to decay and mineralization. Metabolomics is the identification and quantification of metabolic products from pure or mixed populations of organisms (Dettmer, Aronov & Hammock, 2007), and environmental metabolomics is the application of metabolomics to determine metabolic responses to environmental changes (Bundy, Davey & Viant, 2009). The application of metabolomic techniques to classic taphonomic experiments could provide valuable information to advance our understanding of how metabolic processes influence fossilization. There is an extensive recent literature reviewing metabolomic techniques (e.g. Beale et al., 2018; Bundy et al., 2009; Dudzik et al., 2018; Smilde et al., 2010).

The goal of taphonomic experiments is to identify the conditions responsible for the preservation of organic remains and which microbiota is involved. Often single bacterial species or simple bacterial communities – composed of only a few species at most – are introduced into a relatively sterile environment, and their influence on fossilization is assessed (Raff et al., 2013; Raff et al., 2014). However, these simple microbiosa are unlikely to reflect a true fossilization environment. For some fossilization processes, such as in the case studies described above, it may not be possible to reduce the effects of microbial metabolic activity on fossilization to simple models. Rather, fossilization is more likely due to complex and structured biofilms and microbial mats. Various experiments have demonstrated that biofilms and microbial mats play an important role in fossilization (e.g. Iniesto et al., 2015, 2016, 2017, 2018), but these have involved the introduction of natural microbial mats into the taphonomic experiments, rather than controlling mat development as a variable. However, experimental research on oral biofilms (e.g. dental plaque) has resulted in a set of techniques to control biofilm growth in vitro carefully on a sub-millimetre scale while regulating a range of variables such as pH, temperature, fluid-flow rates, and microbial composition (Brown et al., 2019; Kim, Park & Chung, 2012). In these microfluidic devices growth and biofilm formation of selected bacteria can be monitored and controlled through laboratory techniques. Optimizing these techniques on a small scale, and applying them to set up a complex but controlled biofilm in a taphonomic experiment, and then monitoring the active metabolic processes with transcriptome analysis (McCarren et al., 2010), may enable a better understanding of fossilization processes. Additionally, enzyme activity can be measured to give insights into bacterial activity (Bickel & Tang, 2010).

Taphonomic experiments would also be useful to determine which geological proxies are best for investigating ancient metabolic activity. For example, enzyme-specific signatures in the δ18O composition of phosphate minerals have been identified in many different precipitation scenarios, but not yet in phosphate minerals precipitated during the replacement of soft tissue. Phosphate minerals are among the most common minerals precipitated in taphonomic experiments (Briggs et al., 1993), so it would be relatively easy to follow the methodology of Liang & Blake (2006, 2009) to assess enzyme-specific fractionation during experimental soft tissue fossilization. Similar experiments could also be used for other geochemical metabolic proxies and other mechanisms of fossilization.

VI. CONCLUSIONS

(1) Over many years of taphonomic investigations, including studies of soft tissue fossils, ancient fossilizing environments, and controlled laboratory experiments, palaeontologists have developed a deep appreciation
for the importance of bacteria in soft tissue fossilization, including a general model of how major categories of microbial metabolic activity influence the process of fossilization. In particular, there is a focus on the importance of ‘stressful’ environments, for example, low-oxygen, high-salinity, etc., that inhibit microbial metabolic activity and therefore mitigate its destructive effects on soft tissue. However, for every deeply stressful environment, there is a suite of bacteria that have conquered it, and that thrive under or even prefer these conditions while vigorously metabolizing soft tissue. Moreover, there is growing evidence that some fossils and fossilization processes cannot be explained by broad metabolic categories and stressful environmental conditions.

(2) Despite the general effects of anoxia, soft-bodied cnidarians decay less and fossilize most effectively in oxic rather than anoxic environments, and some evidence suggests that across all taxa anoxia does not uniformly inhibit decay.

(3) Siderite precipitation and siderite concretion growth – a common mechanism for preserving soft-tissue fossils – requires a delicate balance of iron reduction and sulfate reduction that has so far only been identified in a few bacterial species.

(4) Arthropod brains can fossilize, despite being lost very quickly in taphonomic experiments, due to complex interactions between tissues, microbes, and the environment.

(5) These examples, and others not listed herein, suggest that at least some fossilization processes may require very specific metabolic processes, which may correlate with specialized bacteria/biofilms/microbial mats living in specific environmental conditions. More extensive taphonomic experimentation to study and measure the effects of specific metabolic processes on decay and mineralization, combined with more investigation of geochemical proxies for metabolism in fossils, is critical for untangling the more complex role of microbial metabolic activity in fossilization.

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