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Invited Review

The fossil record of igneous rock

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ABSTRACT

A growing awareness of life in deep igneous crust expands our appreciation for life's distribution in the upper geosphere through time and space, and extends the known inhabitable realm of Earth and possibly beyond. For most of life's history, until plants colonized land in the Ordovician, the deep biosphere was the largest reservoir of living biomass. This suggests that deep crustal habitats played an important role in the evolution and development of the biosphere. Paradoxically, the paleo-perspective of deep life has been largely neglected in the exploration of the deep biosphere as well as in paleontology as a whole. Here, we review the collective understanding of the fossil record in igneous crust with the aim to highlight a rising research field with great potential for substantial findings and progress in the near future. We include new results that emphasize the importance of direct or indirect dating of fossils and introduction of new techniques into the field. Currently, an incoherent record of morphological fossils- and chemofossils stretching from present to ~2.4 Ga implies the presence of an abundant and rich, yet largely unexplored, fossil record. Further investigations of deep paleo-environments will most certainly result in substantial insights into the distribution and development of biospheres throughout life's history, the early evolution of prokaryotes and eukaryotes, and Earth's early biogeochemical cycles. We emphasize the fossil record of igneous rock to give it the same status as the fossil record in sedimentary rocks, and to implement fossil investigations as standard procedures in future international drilling campaigns.

1. Introduction

The fossil record - our window into life's history on Earth - is almost exclusively based on findings in sedimentary rocks. This is to be expected, since sediment deposits can be said to represent the most extensive graveyards on Earth. Terrestrial and oceanic sediments are intimately linked to life processes and record a vast habitat encompassing most branches of life on our planet. Yet there are extensive habitats and reservoirs of living biomass on Earth not preserved in sedimentary deposits. In recent years, the exploration of the deep biosphere in crystalline basement of continents and oceanic crust has produced a growing awareness of life extending downwards, into deep igneous rock (Pedersen et al., 1997, 2008; Heim, 2011; Orcutt et al., 2011; Ivarsson et al., 2015a; Suzuki et al., 2020). Here, a cryptic biosphere excluded from solar energy and predominantly based on chemoautotrophy and heterotrophy resides within the interconnected network of open pore space, which provides conduits for both ground water flow and migrating microorganisms.

Our understanding of the deep biosphere has come a long way in the last 30 years, much owing to the establishment of large-scale underground facilities for i.e. nuclear waste repository research, or the establishment of international deep drilling programs such as ICDP and DCDP, later ODP and IODP (Table 1). A number of reviews cover both the continental and the oceanic deep realm, the latter with emphasis on sediments (Orcutt et al., 2011; Heim, 2011; Colwell and D'Hondt, 2013; McMahon and Ivarsson, 2019). Abundance and diversity is usually described as high, and both prokaryotes and eukaryotes including fungi, protists and nematodes have been identified (Orcutt et al., 2011; Heim, 2011; Colwell and D'Hondt, 2013; Sohlberg et al., 2015; Borgonie et al., 2015; Lopez-Fernandez et al., 2018a). The deep biosphere has extended the known limits for life with respect to extreme conditions such as temperature and pH, but also physically, with evidence gathering for the presence of life at depths down to ~5 km in continental settings, and ~ 1 km beneath seafloor (Lin et al., 2006; Pedersen et al., 2008; Orcutt et al., 2011; Purkamo et al., 2020). However, despite these results, the extension of deep life through time has surprisingly been overlooked.

The deep biosphere represents one-tenth to one-third of all live biomass today while the rest is predominantly land plants (McMahon and Parnell, 2018; Magnabosco et al., 2018; Bar-On et al., 2018). Prior

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Table 1
Explanations for abbreviations in the text (Golubic et al., 1981; Ivarsson et al., 2015a; Marlow et al., 2015).

Abbreviation/term	Explanation
DSDP	Deep Sea Drilling Project 1966–1983.
ODP	Ocean Drilling Program 1983–2003.
IODP	Integrated Ocean Drilling Program 2003–2013.
IODP 2013-	International Ocean Discovery Program 2013-
ICDP	International Continental Scientific Drilling Program 1996-
Endoliths	Rock dwelling organisms.
Euendoliths	Microorganisms that actively penetrate rock interiors and create habitable cavities.
Chasmoendoliths	Microorganisms that invade pre-existent fissures and cracks.
Cryptoendoliths	Microorganisms that invade pre-existent structural cavities.
Autoendoliths	Microorganisms that construct the structures in which they reside.

to the colonization of land in the Ordovician the situation was the opposite, and it has been put forward that most of Earth's live biomass (~80%) was to be found in the deep biosphere (McMahon and Parnell, 2018). This suggests that deep environments have played a crucial role in the early evolution of both prokaryotes and eukaryotes, and that the deep biosphere dominated life on Earth for most of life's history. Paradoxically, the fossil record of the deep biosphere has more or less been ignored, and a coherent fossil record similar to what has been established in sedimentary rocks is currently lacking. Paleontological material from mostly igneous oceanic crust, but also from continental crust, displays an incoherent record of fossils of mainly fungi (Ivarsson et al., 2015a, 2018a; Drake et al., 2017a) accompanied by geochemical data of prokaryotic activity from the present back to about 410 Ma (Drake et al., 2017b) (Fig. 1). Rare reports of much older deep biosphere fossils suggest life to be present in igneous oceanic crust already at 2.4 Ga (Bengtson et al., 2017), and sulfur isotope signatures in pyrite suggest life in subseafloor basalt to have been present already at 3.45 Ga (Aoyama and Ueno, 2018). Exploration of fossils in deep igneous provinces is in its early stages, and our intention with this review is to put the spotlight on the fossil record in igneous rock and to inspire further research. It is important to distinguish the fundamental differences in occurrence, preservation, diagenetic processes, and assessment of age between fossils in sedimentary and igneous rock. Traditional concepts such as stratigraphy and taphonomy developed for studying fossils in sedimentary rocks cannot be applied to fossils in igneous rock without substantial modifications. To some extent, new approaches and strategies need to be developed to match the physical and chemical challenges of igneous rock. Another fundamental aspect that needs to be accounted for is the difference between the deep biosphere of the igneous crust and the deep biosphere in sediments. Whereas the latter is based on heterotrophy and exploitation of refractory organic matter, the former is based to a much greater extent on autotrophy (Magnabosco et al., 2018; Bar-On et al., 2018). This makes the fossil record of the igneous crust unique within paleobiology and highly significant to understand past life (Onstott et al., 2019; Ivarsson et al., 2019a; McMahon and Ivarsson, 2019). We are convinced that deep igneous settings can provide crucial clues to the early evolution, past and present biogeochemical cycles, and abundance and diversity of life on Earth but also beyond. For example, the subsurface has been put forward in strategies related to upcoming missions with the aim to search for traces of life on Mars and icy moons (Onstott et al., 2019; Ivarsson et al., 2019a).

Fig. 1. Map showing locations world-wide, both continental and marine, where fossils from igneous rock has been reported. Key references for each location: 1,2) Ivarsson et al., 2013a. 3) Ivarsson et al., 2012, Bengtson et al., 2014. 4) Schumann et al., 2004. 5) Cavalazzi et al., 2011. 6) Ivarsson et al., 2011b. 7) Ivarsson et al., 2018b. 8) Ivarsson et al., 2015d. 9) Bengtson et al., 2017. 10) Peckmann et al., 2008. 11) Eickmann et al., 2009. 12,13) Reitner et al., 2006. 14,15) Drake et al., 2017b. 16) Drake et al., 2019. 17) Ivarsson et al., 2013b. 18) Lindgren et al., 2010. 19) Sakakibara et al., 2014. 20) Thorseth et al., 2003. 21) Carlsson et al., 2019. 22) Klein et al., 2015. 23) McKinley et al., 2000a, 2000b.

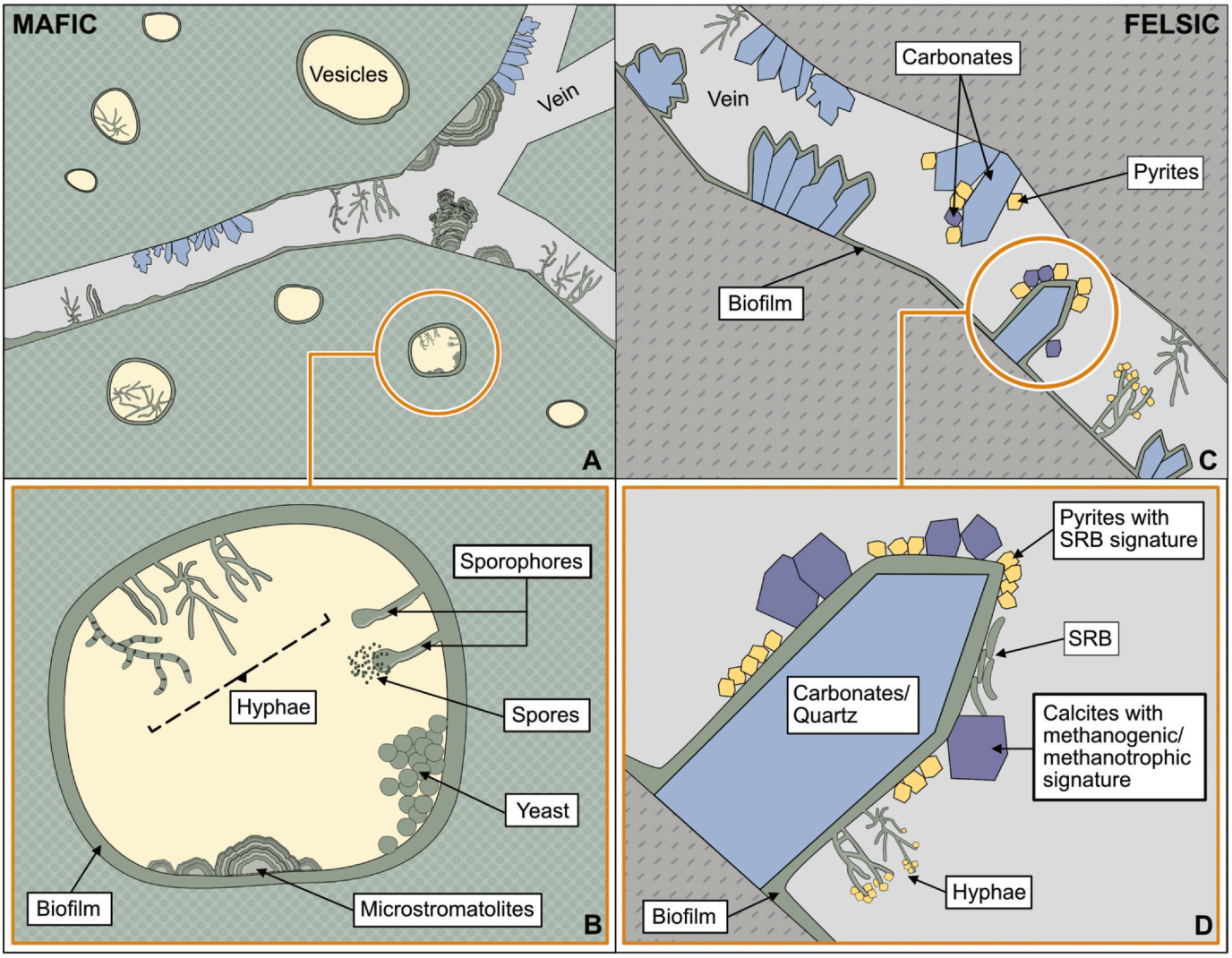

Fig. 2. Schematic figure visualizing habitats and colonies in subsurface igneous crust. A) An overview of habitats and fossilized endoliths in mafic rocks including veins and vesicles. B) Detailed figure of a vesicle with endoliths. C) Overview of habitats in felsic rock including fossils and chemofossils. D) Close-up of an euhedral crystal with associated fossils and chemofossils. SRB – sulfate reducing bacteria.

1.1. Colonization of igneous rock

The deep biosphere comprises ecosystems in both oceanic and continental settings beneath the rhizosphere or the bioturbated zone. These settings include sediments, sedimentary rocks and igneous rocks. In this review, we will focus on the latter. The habitable depth in the bedrock is limited by space (rock porosity), the availability of water, pressure and the maximum viable temperature depending on the local geothermal gradient (Oger and Jebbar, 2010; Heim, 2011). Microorganisms engaged in a rock-dwelling lifestyle are dependent on open pore space for migration and colonization. Igneous rocks contain various amounts of pore space in the form of fissures, cracks or vesicles, depending on composition, origin, age and geological history of the rock. Such pores often form inter-connected networks of pore space, which allows for ground water flow as well as microbial migration and colonization (Fig. 2). The oceanic crust, for instance, consists of an upper layer characterized by extensive fracturing, about 10% porosity, and with permeability ranging from 10^{-12} to 10^{-15} m^2 (Bach and Edwards, 2004; Orcutt et al., 2011). Fractures created by tension release or quick cooling occur with varying size and frequency, as do vesicles, as a result of pressure release during magma extrusion. About 60% of the oceanic crust is hydrologically active, and the total fluid volume corresponds to 2% of the total ocean (Orcutt et al., 2011), which makes the oceanic igneous crust the largest aquifer system on Earth (Fisher and Becker, 2000; Orcutt et al., 2011). The continental crystalline bedrock is generally of low porosity (at most a couple of percent). Groundwater flow as well as microbial activity are mainly restricted to interconnected networks of fractures, formed and re-activated episodically by tectonic forces (e.g., extension, compression), pressure release close to the ground surface, and cooling (Drake et al., 2009). With time, the pore space is filled by secondary mineralizations, which thwart, or eventually even stop, fluid flow and, thus, microbial migration.

The extent to which microorganisms of deep ecosystems are introduced into, vs. originated in, this biotope is far from understood. There is definitely an exchange with the surficial biosphere, and microorganisms are, to a considerable extent, transported by seawater flows in the oceanic realm and by percolation of rain or surface run-off in the terrestrial realm. However, the deeper communities reach, the more endemic and adaptive they become to their environment (Hubalek et al., 2016; Wu et al., 2016; Lau et al., 2016). Circulating groundwater and fluids in fractures mediate migration of microorganisms to vast areas, both vertically and horizontally. Single cells and spores are passively transported by fluid currents while flagellated prokaryotes and micro-eukaryotes are motile and mechanically advance through the aquatic system (Orcutt et al., 2011; Colwell and

D'Hondt, 2013; Borgonie et al., 2015). Fungal hyphae, whose physiology enables spatial exploration, migrate along fracture walls from one void to another as nutrient availability fluctuates (Ivarsson et al., 2020). The igneous crust is habitable as long as open pore space is available to the microorganisms, and fluid flow is active. The oceanic crust is considered to be hydrologically active about 10–20 Ma after formation (Staudigel et al., 1981). After that, secondary mineralization fill pore space and fluid flow successively decrease and eventually cease. However, the igneous oceanic crust is known to be inhabited by microorganisms more than 100 Ma after its formation (Suzuki et al., 2020), thus the time interval of 10–20 Ma is too narrow and endolithic colonization is active long after. The situation in continental igneous crust is similar to the oceanic crust in terms of secondary mineralization and filling of pore space with the exception of longer time spans. Continental igneous crust is not subducted and recycled on a ~ 100 Ma cycle as oceanic crust is, thus the longevity of habitable pore space is more extensive. Even if fractures are filled by secondary mineralization, re-activation of fractures by tectonic forces can enable episodes of colonization hundreds of millions of years apart (Tillberg et al., 2019; Drake et al., 2019). Thus, in principle, colonization of deep igneous rock is possible for most of its durability, as long as the geochemical conditions are within the limits for life.

According to observations of the fossil record, colonization of subsurface habitats normally begins with the initial establishment of a biofilm, either by prokaryotes or fungi, on the surface at the interface between open pore spaces and host rock/secondary minerals. Microorganisms need a solid substrate, like a mineral surface, for anchoring and further organization and growth of microbial communities (Costerton et al., 1995). This holds true for most type of environments. The establishment of a biofilm provides microorganisms an opportunity to control their environment with respect to intrinsic parameters, like metabolism and community organization, but also, importantly, extrinsic parameters including nutrient availability, pH and free oxygen availability (Costerton et al., 1995; Decho, 2000). The interface between pore space and host rock, where circulating fluids meet reduced minerals, provides a local environment characterized by a redox chemistry favorable for lithoautotrophs, who constitute the chemosynthetic base of deep ecosystems (Stevens and Mc Kinley, 1995). Depending on the type of organism responsible for biofilm production, the appearance and morphology of the remaining structure may vary. Prokaryotes usually form multiple biofilms preserved as microstromatolites, which are characterized by alternating layers of goethite and hematite or iron oxides (oxy-hydroxides)/clays (Bengtson et al., 2014; Ivarsson et al., 2015b, 2019a). Microstromatolites can also be formed by laminated manganese oxides (Ivarsson et al., 2015c). The overall interpretation is that these kind of cryptic-growing microstromatolites are formed by iron- or manganese-oxidizing bacteria (Bengtson et al., 2014; Ivarsson et al., 2015a, 2015b, 2015c), but morphogenesis may be more complex, with other organisms, such as fungi involved (Heim et al., 2017). Many *Frutexites*-type microstromatolites also show conspicuous branching patterns and have been described from a number of cryptoendolithic settings, ranging from shallower marine to hydrothermal hot springs (Walter and Awramik, 1979; Mamet and Préat, 2006), as well as the deep crust (i.e., Bengtson et al., 2014; Ivarsson et al., 2019a).

Fungi, on the other hand, usually produce simple, unlaminated biofilms whose main function is to cover as large an area as possible, normally filling entire vesicle walls with basaltic rock matrices. Fungal films have also been observed to grow over, cover or in other ways interact with prokaryotic microstromatolites (Ivarsson et al., 2015b). Whether this type of interactions occur for trophic or spatial reasons is not yet fully established (Ivarsson et al., 2015b).

From the initial fungal biofilms, sporophores and hyphae protrude in a perpendicular or semi-perpendicular direction out into the open voids (Ivarsson et al., 2013a, 2015a, 2015b; Bengtson et al., 2014) where they often form entangled and complex mycelia characterized by frequent branching and anastomosing between branches. It has been noted that the diameter of the hyphae increases as they advance into the open pore space from the initial biofilm, from a few micrometers to 30–40 μm in the centre of the mycelium (Bengtson et al., 2014). Fungal mycelium can also be used by prokaryotes as substrate for growth including single cells attached on and between hyphae as well as microstromatolites (the mineralized product of prokaryotic activity) sometimes growing on hyphae (Bengtson et al., 2014).

As colonies age, they begin to fill the entire pore space and eventually become mineralized by clays and/or iron oxides (Ivarsson et al., 2015a; Sallstedt et al., 2019). Microbial growth and subsequent mineralization is thus a major factor in the secondary filling of voids along with abiotic precipitation of zeolites and carbonates.

Microbial communities are dependent on, and spatially controlled by, the physical nature of their habitats, host rock chemistry and secondary mineralizations together shape the habitable space. However, microorganisms also interact with their ambient environment by active bio-mediated weathering as well as mineralization. Fungal hyphae have been reported to dissolve and penetrate secondary minerals like zeolites and carbonates, forming cavities or tunnel-like structures (Bengtson et al., 2014; Ivarsson et al., 2015b; Drake et al., 2017a). Growth of microstromatolites has also been shown to dissolve carbonates, a result of iron-oxide formation which lowers pH and may cause passive dissolution of carbonates in contact with stromatolite surfaces (Bengtson et al., 2014).

Microbial activity also induces the formation of minerals such as carbonates, oxides, sulfides and sulfates (Ivarsson et al., 2015a, 2015b, 2015c; Drake et al., 2015a, 2017a, 2017b, 2018b). Fossilization of microbial consortia results in the precipitation of iron oxides and clay minerals that eventually fill voids to various degrees, a similar process to that of ageing fungal mycelia. Fossils can also, to various degrees, function as nuclei for mineral growth. Fossil hyphae can act as substrate for zeolite growth (Carlsson et al., 2019). It is reasonable to assume that microbially mediated rock-weathering can mobilize elements like Si, Al, Fe, Mn, and Mg, and stimulate the formation of zeolites, clays, or even oxides. Fungi are known from soils to form oxalates on their hyphae (i.e. Gadd, 2010). Even though oxalates have not yet been observed in subsurface environments, this appears to be a viable process.

2. Fossil record in igneous crust

The fossil record in igneous rock consists of three types of fossil remains: 1) ichnofossils (trace fossils), which are the results of biological pitting and dissolution of volcanic glass and minerals, 2) fossilized microorganisms, which represent body fossils of microbes, and 3) geochemical signatures of life (chemofossils), which are either isotopic fractionation of mostly C and S preserved in the mineral record, preserved organic molecules (biomarkers), and/or their compound-specific isotope value, indicative of certain groups of microorganisms, i.e. methanogens or sulfate reducing microorganisms (usually bacteria but can also be archaea, e.g. Birkeland, 2005). Ichnofossils have been extensively covered in previous reviews (Staudigel et al., 2008; McLoughlin et al., 2009, 2010) and will therefore be left out here, where focus will be put on body fossils and chemofossils. We point out, however, that the interpretation of filamentous structures in Archean volcanic glass as ancient ichnofossils (Banerjee et al., 2006, 2007) is ambiguous and have lately come under critical scrutiny and may turn out to be non-biogenic (Grosch and McLoughlin, 2014).

A majority of fossils described from the deep biosphere have been mineralized in situ, within open pore space in contact with circulating hydrothermal fluids, represented today by vein-filling secondary mineralization. Body fossils are either enclosed in these vein-filling minerals (often represented by carbonates and zeolites), or they occur in unfilled, still open pore space (Fig. 3).

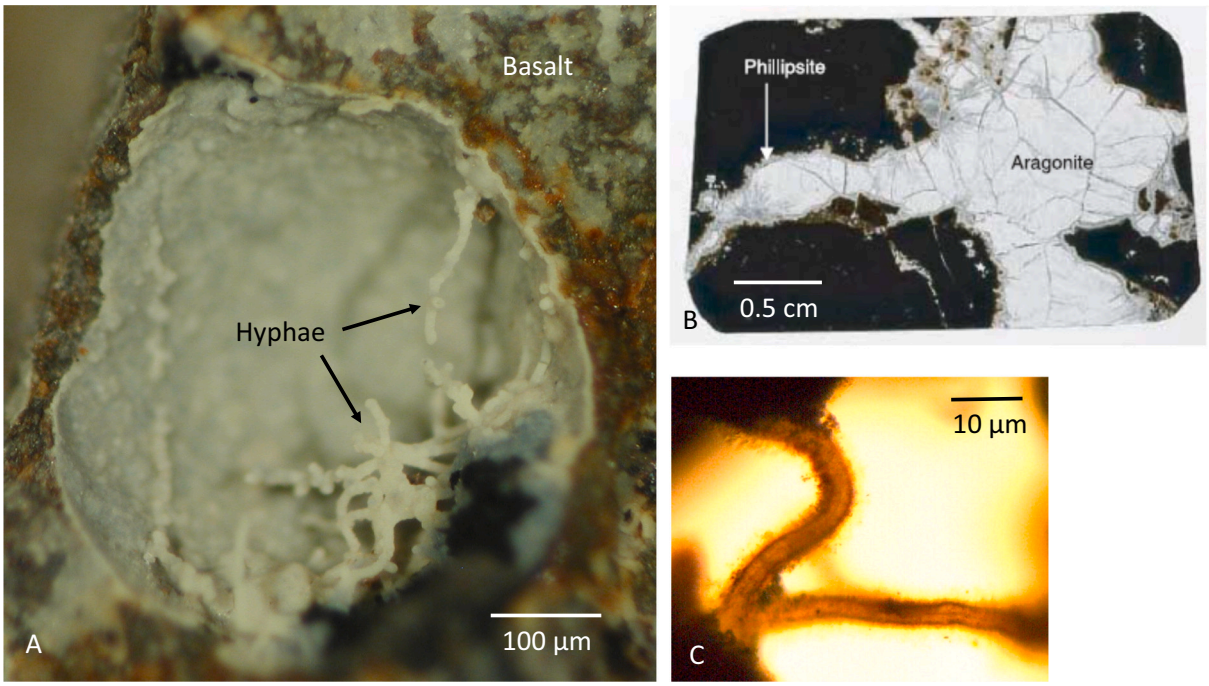

Fig. 3. Microphotographs showing A) an open vug with fossilized fungal hyphae in vesicular basalt from Detroit Seamount, Pacific Ocean, B) a thin section of a calcite vein in basalt from Koko Seamount, Pacific Ocean, and C) fossilized fungal hyphae preserved in calcite from Detroit Seamount.

2.1. Fossil record in igneous oceanic crust

The recognition of fossilized microorganisms in igneous oceanic crust is a recent discovery, and a direct consequence of drilling the crust during the ODP and IODP campaigns. Occasional observations of fossilized filamentous microorganisms in subseafloor basalts were reported almost two decades ago without making any substantial impact in the scientific community (Stephen et al., 2003; Reitner et al., 2006). Interestingly, they were interpreted as fungal hyphae based only on size and appearance. Schumann et al. (2004) described fungal hyphae forming a mycelium-like network in amygdules from the North Pacific Eocene oceanic crust. The interpretation of fungi was based on the presence of branching, septa and central pores. Similar filamentous fossils were subsequently described from subseafloor basalts (Ivarsson, 2006; Ivarsson et al., 2008a, 2008b, 2008c, 2009; Cavalazzi et al., 2011), and ophiolites (Peckmann et al., 2008; Eickmann et al., 2009; Carlsson et al., 2019), but lack of diagnostic morphological characters left the interpretation at "fossilized microorganisms". With the introduction of synchrotron-based X-ray tomography, which enabled detailed and highly resolved three-dimensional visualizations of internal morphologies and community structure supported by positive staining of chitin, a fungal interpretation of most of the filamentous fossils became plausible (Ivarsson et al., 2012, 2013a, 2016a; Bengtson et al., 2014). Fungal characteristics like hyphae, repetitive septa, hyphal tips, anastomoses between branches forming mycelium, and reproduction structures like sporophores, ascocarps and spores gave robust support to a fungal affinity of the filaments (Ivarsson, 2012; Ivarsson et al., 2012, 2013a, 2016a, 2018a; Bengtson et al., 2014) (Fig. 4). Subsequently, additional morphological and growth characteristics for fungal endoliths were identified, including a central strand and the initial formation of a biofilm along vein walls from which mycelium-forming hyphae and sporophores protrude (Ivarsson et al., 2012, 2015a; Bengtson et al., 2014). The presence of various reproduction structures indicated that the fungal communities were sustainable and indigenous to the endolithic habitats, and not the result of passive ingress of fungi from seawater (Ivarsson, 2012; Bengtson et al., 2014; Ivarsson et al., 2015a). Additional to hyphae, yeast-like growth stages have been described, either as separate cells or, more commonly, forming irregular clusters of cells (Ivarsson et al., 2013a, 2015a, 2015b). Transitions between yeast and hyphae (dimorphism) are also observed.

The taxonomical affinity of fossil fungi is usually difficult to assess, but Ivarsson et al. (2012) interpreted the majority of Eocene fungi from the Emperor Seamounts as Dikarya based on morphological traits such as repetitive septa. Furthermore, the observation of zygospores among Quaternary fungi from the Vesteris Seamount, Greenland basin, suggested the presence of Zygomycetes (Ivarsson et al., 2015d). Thus, the major fungal subkingdoms appear to be present in the oceanic crust.

The predominance of fungal remains in the basaltic subseafloor is surprising since prokaryotes have been expected to dominate the biota of these somewhat extreme environments (Orcutt et al., 2011), and by molecular methods shown to be present in numbers (Lever et al., 2013; Jungbluth et al., 2014; Jørgensen and Zhao, 2016; Suzuki et al., 2020). However, only few and far-in-between fossil findings of presumed prokaryotic nature have so far been accounted for (Fig. 5). For example, Thorseth et al. (2003) described coccoidal structures interpreted as fossilized endolithic microorganisms from basaltic glass alteration rims in dredged and drilled samples from the Australian Antarctic Discordance (ages ranging from 0 to 2.5 Ma, and 18 to 28 Ma, respectively). The coccoidal Mn-rich fossils were primarily associated with the porous outer zones of alteration rims bordering secondary zeolites and further resembled microbial structures found in the Arctic Ridges (i.e., Thorseth et al., 2001a, 2001b). Most of these fossils, or partly fossilized structures, were described from volcanic glass and zeolites and were nearly always associated with the presence of ichnofossils (trace fossils) (Thorseth et al., 2003; Ivarsson et al., 2008a; Staudigel et al., 2008). While a prokaryotic nature of the fossils is inferred mainly from their sizes and the presence of Mn-oxides, it is difficult to provide a closer taxonomic affiliation. This is indeed the problem with most fossils of microbial and specifically prokaryotic nature, which lack specific diagnostic features, including macro-structures inferred as the result of prokaryotic activity, such as ancient stromatolites (i.e., see biogenicity discussions in Hofmann et al. (1999), Cady et al. (2003), Awramik and Grey (2005)). Therefore, a combination of morphological and

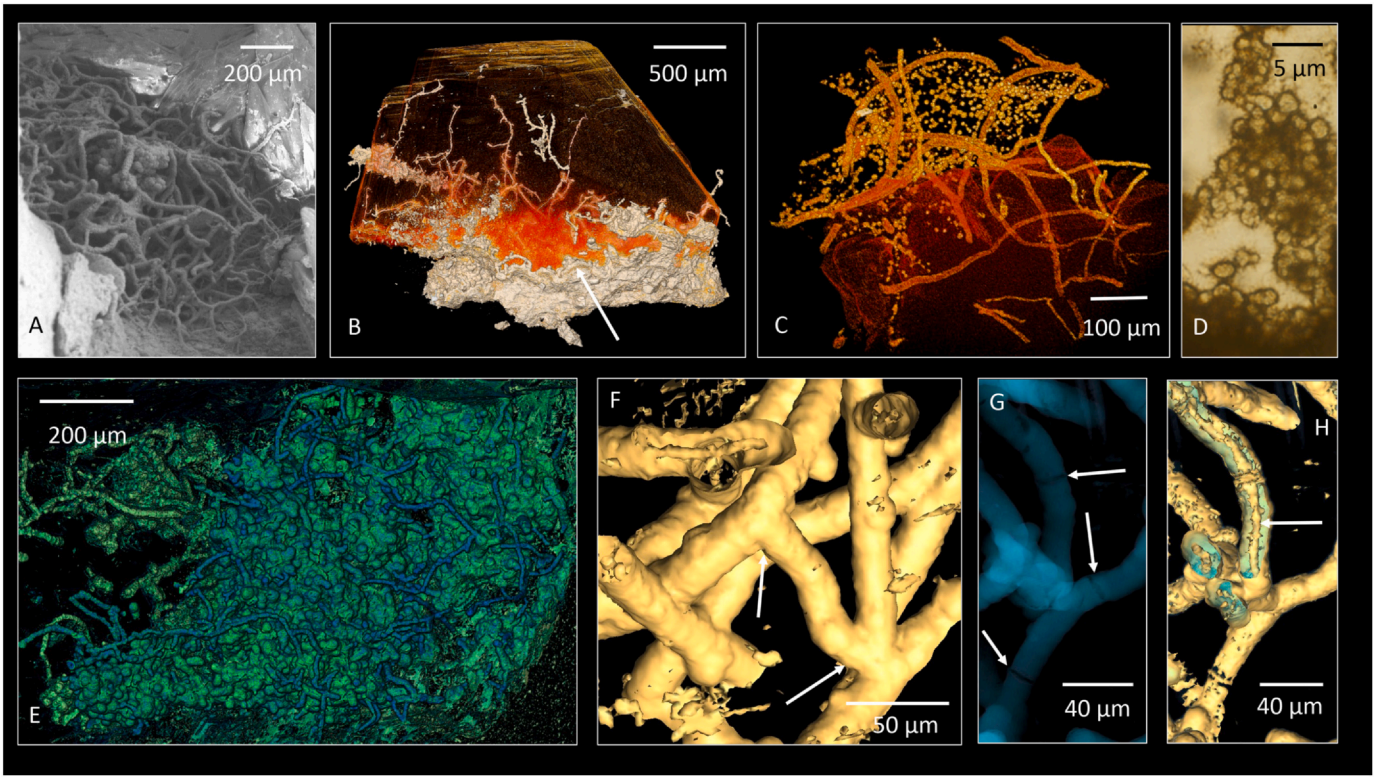

Fig. 4. A) ESEM image showing a fossilized fungal mycelium in an open vug in basalt from Koko Seamount, Pacific Ocean. B) Tomographic reconstruction (volume rendering) of fossilized fungal mycelium protruding from a vein wall in basalt and embedded in later zeolite growth. White arrow marks the basal film lining the host rock. C) Tomographic reconstruction of fossilized fungal mycelium partly in a carbonate crystal and partly in an open vug. In between hyphae cell-like structures occur as "pearls-on-strings" interpreted as remains of prokaryotes. D) A microphotograph of a thin section showing yeast cells in calcite. From Troodos ophiolite, Cyprus. E) Tomographic reconstruction of a fossilized fungal community with dimorphic yeast and hyphal growth stages as well as transitions in between both. Hyphae in blue, yeast in green. F) Tomographic reconstruction (isosurface) showing anastomosis (arrows) between branches in fossilized fungal hyphae. G) Tomographic reconstruction (voltex) showing fossilized hyphae with repetitive septa. H) Tomographic reconstruction (isosurface and voltex) of G showing septa and central strand in hyphae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

geochemical traits together with an evaluation of biogenicity is recommended in order to classify microbial fossils here on Earth, or from potential future extra-terrestrial material (Cady et al., 2003; Ivarsson et al., 2019a). Other putative prokaryotic fossils described from basaltic rock (e.g., Ivarsson et al., 2011a) include micrometer-sized flexible filaments occurring in close association with chromite grains, suggested to have played a role in chromite oxidation. In addition, samples from Koko seamount, part of the Pacific Ocean Emperor Seamounts-chain drilled during the IODP program, contain abundant filamentous fossils of very varying diameters. Most of these appears to be of fungal affinity, but it is also possible that some of the thinnest ones could represent bacterial filamentous fossils (Ivarsson et al., 2008a, 2008b, 2008c, 2011a).

Other prokaryotes from the deep igneous crust of the Koko seamount resemble strings of cells with an ultra-texture similar to the sulfur-oxidizing archaea *Pyrodictium*, suspended as sheaths draped over fungal hyphae (Bengtson et al., 2014). These cob web-like sheaths were identified as part of a consortium containing fungal hyphae and branching cauliflower-like microstromatolites. The microstromatolites are characterized by laminated iron oxides and are morphologically similar to *Frutexites* (Bengtson et al., 2014; Ivarsson et al., 2015b). The Koko seamount microstromatolites had either grown directly on top of fungal hyphae, or they had been lining the vein walls and become mineralized as complex laminated iron- or manganese oxides (Bengtson et al., 2014; Ivarsson et al., 2015a, 2015b, 2015c). Curiously, along with their presence in the subseafloor crust, *Frutexites*-like microstromatolites have also been described from a number of different settings including more shallow marine settings and hydrothermal hotsprings (e.g., Walter and Awramik, 1979; Mamet and Préat, 2006). While the exact morphogenesis of the laminated and branching *Frutexites* remains unclear, several studies suggest that they could be the result of bacterial processes and interactions with the surrounding environment, for example by Fe-or Mn-oxidizing bacteria (Böhm and Brachert, 1993; Cavalazzi et al., 2007; Bengtson et al., 2014; Ivarsson et al., 2015a, 2015b, 2015c; Heim et al., 2017). The branching nature of *Frutexites* is also similar to that of bacterially produced shrubs described by Chafetz and Guidry (1999) and could make up the biotic endmember of a range of similar structures, some of which are believed to result from abiotic mineral precipitation. It is, however, likely that *Frutexites* morphogenesis is often the result of mainly biotic processes related to microbial metabolism and the adsorbing properties of microbial extracellular polymeric substances (EPS).

The presence of eukaryotes in relation to extant *Frutexites*-forming biofilms from Äspö Hard Rock Laboratory, Sweden, has also been reported, and while their role in the accretion of *Frutexites* is unclear, they may play a part in the build-up of laminated Fe-oxide microstromatolites, suggesting the cooperation of a complex *Frutexites*-forming biota (Heim et al., 2017). Recently, Ivarsson et al. (2019a) reported the presence of unusual *Frutexites* with an alternating banding consisting of clay and Fe-oxides (see Fig. 4 in Ivarsson et al., 2019a), which differs slightly from that of the more common Fe—Fe banded versions (e.g., see Bengtson et al., 2014). The association between putative microbes and clay, however, is not a new phenomenon. In fact, clay authigenesis seems to play a ubiquitous part in biofilm formation and preservation, in the deep crust as well as in the shallower surface realm (e.g., Ferris et al., 1986, 1987; Konhauser and Urrutia, 1999). In

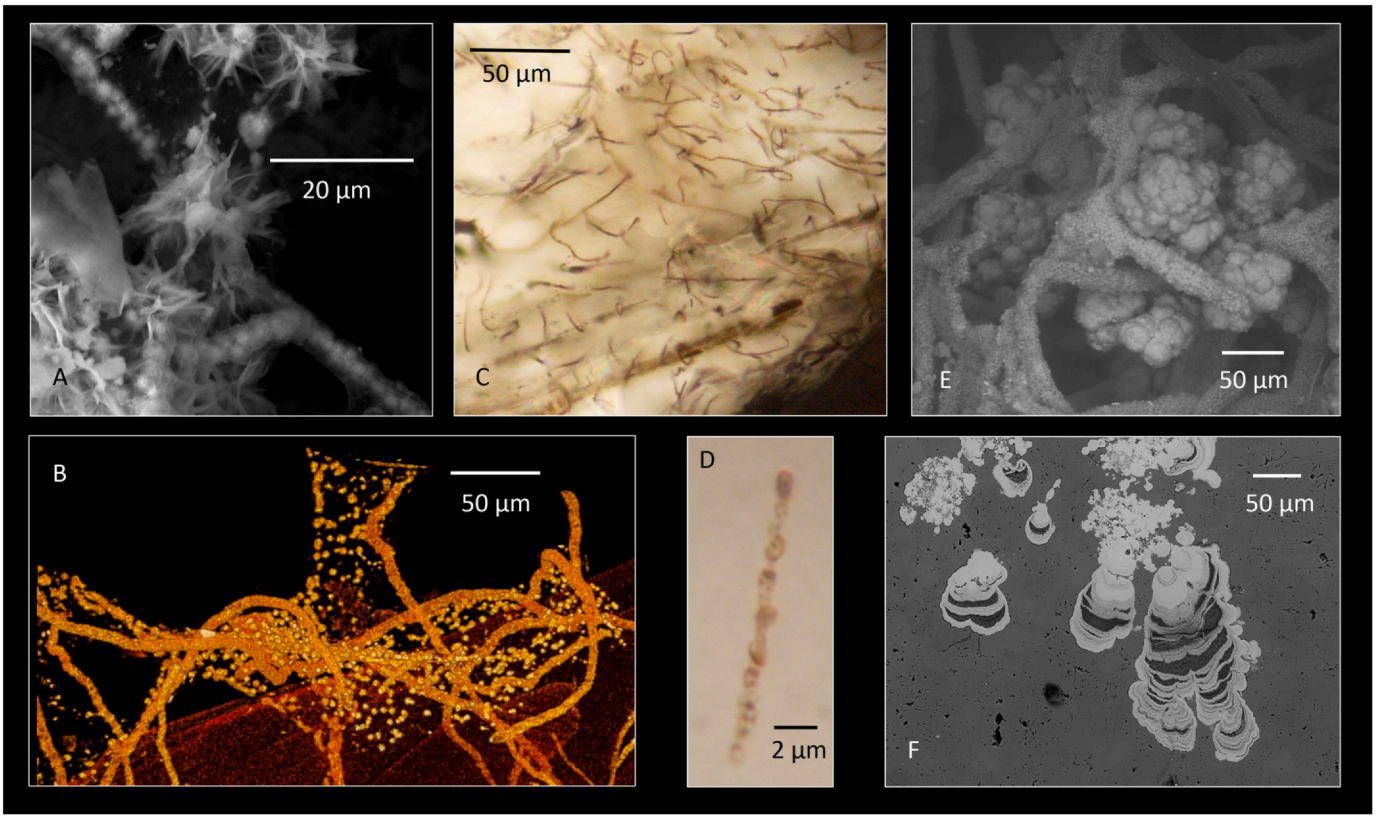

Fig. 5. Images of prokaryotic fossils from Emperor Seamounts, Pacific Ocean. A) ESEM image of cells on a string attached in between fungal hyphae forming a cob web. B) Tomographic reconstruction of cell-like structures suspended in a cob web-like fashion in between fungal hyphae. C) Filamentous structures embedded in carbonates in a thin section. D) Close up of a filament from C showing segmentation. E) ESEM image of cauliflower-like *Frutexites* that has grown on a fungal mycelium (now fossilized). F) Cross section of *Frutexites* in a thin section showing the distinct layered internal build-up.

the deep igneous crust of both land (Drake et al., 2017a, 2017b) and oceans (e.g., Ivarsson et al. 2013a, Ivarsson et al., 2015a, 2015b, 2015c; Bengtson et al., 2014), fungal biofilms in particular seem to be preserved with authigenic clay minerals suggesting an important link between the biological cell and clay species such as Al, Si, Fe, and Mg. To evaluate the effect of host rock geochemistry on the precipitating clay minerals associated with fossil microbial biofilms, Sallstedt et al. (2019) investigated several sites differing in ambient redox degree, and suggested that swelling smectites of montmorillonite type dominate the fossil related clay mineralogy at both oxic seamounts and deep anoxic continental granite environments. This, in turn, suggests that life's presence at depth in the crust has a greater effect than previously known on the geochemistry of secondary alteration products like clay minerals and that biology, in fact, may play a large role in the precipitation of authigenic secondary minerals in the igneous subsurface (Sallstedt et al., 2019).

The igneous crust thus seems to harbour a far more complex biota than previously known, with intriguing evidence for the presence of fungus-like organisms as far back as 2.4 Ga (Bengtson et al., 2017), and several findings which also indicate the presence of potential prokaryotes. There are unresolved questions concerning the ecological relationships between eukaryotes and prokaryotes in the deep crust. For example, in cases where microstromatolites and strings of cells have been found to use fungal mycelia as a framework for growth, a symbiotic-like relationship between the engaging partners has been inferred (Bengtson et al., 2014). Eukaryotes are probably dependent on available prokaryotic biomass for colonization of otherwise oligotrophic environment; thus the fungi are favoured by a partnership with prokaryotes. It has, for instance, been shown that fungi may grow on what has been interpreted as prokaryotic biofilms and microstromatolites, in a seemingly grazing manner (Ivarsson et al., 2015b).

Fungal hyphae have also been shown to weather, penetrate and bore into secondary minerals like carbonates and zeolites, indicating active bio-weathering and mobilization of elements (Bengtson et al., 2014; Ivarsson et al., 2015b; Drake et al., 2017a). Thus fungi are most likely an active geobiological agent not fully accounted for in subseafloor environments. As described in the previous paragraph, most filamentous and fungal fossils, including yeasts, are preserved by clay minerals, but occasionally they may also contain a central strand of iron oxides. Fossil prokaryotic remains usually reflect the biological affinity, i.e. remains of iron oxidizers are preserved as iron oxides, while remains of manganese oxidizers consist of manganese oxides (Ivarsson et al., 2015c). Prokaryotic activity can also leave behind mineralizations with isotopic signatures characteristic for methane consumption and sulfur cycling (Lever et al., 2013).

In addition to the mineralogical composition of fossils in deep igneous rocks, organic matter has also been detected and to some degree characterized within many fossils. Poorly crystalline carbonaceous matter is most commonly detected by Raman spectroscopy, indicating a slight degree of maturation and decomposition (Ivarsson et al., 2015b, 2015c, 2015d). More complex organic compounds like fatty acids and lipids have been detected by ToF-SIMS (Ivarsson et al., 2008b). However, the characterization of biomarkers has not yet been successful enough to use as a discriminating factor to distinguish between groups like eukaryotes or prokaryotes. An exception is chitin, which was detected by staining with WGA-FITC under fluorescence microscopy and thus could be used as an indicator for the presence of fungi (Ivarsson et al., 2012, 2019b; Bonneville et al., 2020).

The fossil record in modern oceanic crust extends from the present to about 80 Ma (Ivarsson et al., 2015a), and in ophiolites, to the Devonian (~400 Ma) (Peckmann et al., 2008; Eickmann et al., 2009). There are no reports in older rocks, so far, of endolithic fossils, with one

exception: Bengtson et al. (2017) described fungal-like hyphae from carbonate-filled vesicles in basaltic lava from the Ongeluk Formation, South Africa, with an age of 2.4 Ga (Gumsley et al., 2017). The fossils resemble in appearance and morphology Paleozoic fossils described by Peckmann et al. (2008) and Eickmann et al. (2009), respectively. Fossils from all three locations are also chloritized, thus they have experienced slight metamorphic overprinting. The Ongeluk host rock was radiometrically dated to about 2.4 Ga, and based on the time of chloritization it was concluded that the fossils could not be younger than 2.06 Ga. Interestingly, this dating aspect highlights one issue with fossils in igneous rock. In sedimentary rocks, the dating is easier and more precise since fossils are correlated to the stratigraphy. In igneous crust however, a similar stratigraphy is absent and dating of fossils has to rely on other criteria. The age of the host rock, usually determined by radiometric dating, gives a maximum age that cannot unreservedly be used as the precise age of colonization and subsequent fossilization of the organisms, which occurs in vesicles. In general, oceanic crust is hydrologically active about 10–20 Ma after its formation (Staudigel et al., 1981). After that, compaction and formation of secondary minerals cease the circulation of fluids, and most open pore space is sealed. In samples from seamounts formed by hotspot volcanism, fluid inclusions in carbonates that enclose fossils have been used to show that the mineralizations were a result of hydrothermal fluids, and thus the colonization and fossilization must have occurred while the hotspot and its associated hydrothermal activity still was active (Ivarsson et al., 2009).

Thus, dating of fossils is difficult in oceanic igneous rock. The Ongeluk fossils (Bengtson et al., 2017) would have been an even more noteworthy claim if the fossils could have been better constrained with respect to time. Here we present new results of stable C isotopes, and trace element compositions of the calcites that enclose the fossils. The C isotope compositions in the SIMS spot analyses show a narrow range (-10.8 ± 0.7‰ V-PDB, $n = 102$) and a normal distribution that suggest precipitation at a single event (Fig. 6). The REE + Y patterns are (Fig. 7, $n = 22$), when PAAS-normalized, similar to modern seawater (Deng et al., 2017) with a characteristic LREE-depletion compared to the other REEs (but without the negative Ce-anomaly of modern water) and even more so to Archaean stromatolitic carbonates (Van Kranendonk et al., 2003) with similar LREE-depletion, and positive Eu- and Y-anomalies. In contrast, mid-ocean ridge basalts show much larger degree of LREE to HREE depletion (Fig. 7). These data suggest a marine origin and that the fossils were enclosed in carbonate while the rock was exposed to circulating seawater. A marine origin is supported by high Mg-concentrations of the calcite (4300 ± 1700 ppm, Suppl. Data 1), as high Mg is characteristic for abiotic marine carbonates (e.g., Berg et al., 2019). There are also very high Fe concentrations in the calcites (18,000 ± 5000 ppm, Suppl. Data 1) in line with precipitation from an anoxic "ferruginous-ocean" typical for this period of the Proterozoic eon (Song et al., 2007). There is also a strong positive correlation between Fe and V in the calcite ($R^2 = 0.93$, Suppl. Data 1) underlining the strong redox control of the Fe uptake (Tribovillard et al., 2006). Owing to plate tectonics the oceanic crust is rarely older than 90 Ma, thus the C isotope and trace element data puts the Ongeluk fossils in a geological context constrained by a few tenths of millions of years after the formation of the basalt, with the reservation that Phanerozoic plate tectonics and the relative recency of the crust in the modern ocean may not be entirely applied to the Paleoproterozoic.

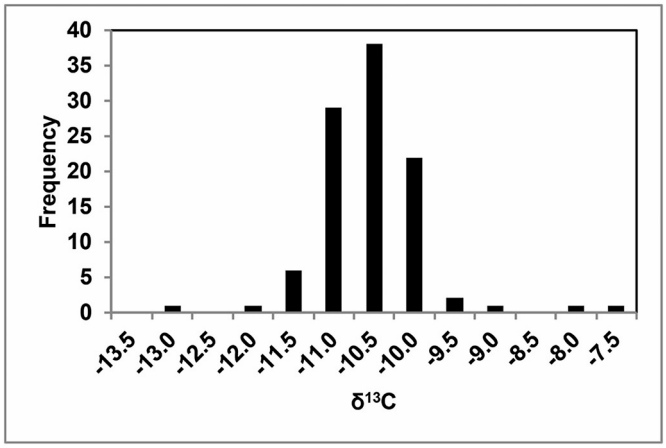

Fig. 6. Histogram of $\delta^{13}C$ (SIMS, $n = 105$) values (vs V-PBB) of calcite in vesicles from Ongeluk.

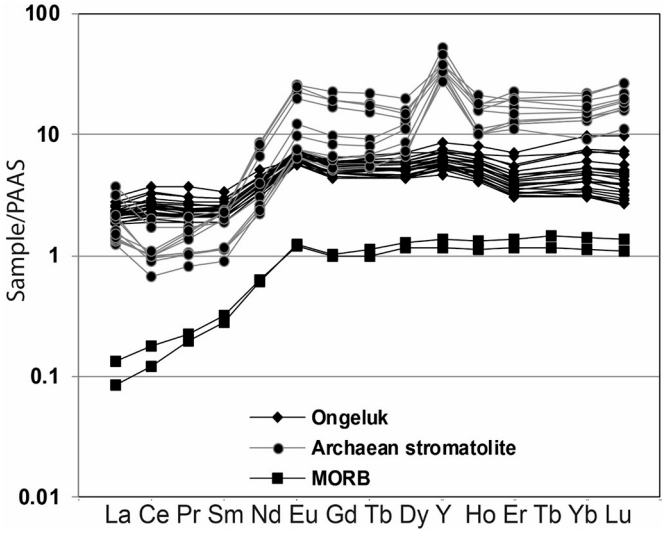

Fig. 7. PAAS-normalized REE-patterns of calcite in vesicles from Ongeluk, together with values of global MORB (Mid-Ocean Ridge Basalt) average and median (Arevalo and McDonough, 2010) and of 3.45 Ga old stromatolitic carbonates from the Pilbara Craton (Van Kranendonk et al., 2003).

We can also present new U–Pb dating of the calcite enclosing the fossils, which are in line with previous arguments (Bengtson et al., 2017), and the new C-isotope and trace element data presented here. U–Pb geochronology of the Ongeluk vesicular carbonate give very large error (2472 ± 310 Ma, Fig. 8, Suppl. Data 2), which is generally due to unfavourably high common lead concentrations compared to radiogenic lead. Nevertheless, this age is within the expected Proterozoic interval and is indicial evidence together with other lines of evidence, including trace element concentrations for a ~ 2.4 Ga precipitation. This means that mycelium-forming fungus-like microorganisms were engaged in endolithic lifestyle in the oceanic crust at this Proterozoic time interval.

The findings accounted for above concern fossils within mafic basaltic rocks of the oceanic crust. In contrast, remains of microorganisms in ultra-mafic rocks are rare, although such rocks have commonly been in the spotlight regarding microbial life and exotic geochemistry favorable for chemoautotrophs. However, a few studies are to be found in the literature. Ménez et al. (2012) reported high concentrations of organic matter intimately associated with serpentine-hosted hydrogarnets recovered from the Mid-Atlantic Ridge. The organic matter included aliphatic and aromatic compounds and functional groups such as amides, usually associated with biopolymers such as proteins, lipids and nucleic acids. The authors concluded that the organic matter was a result from past microbial activity within the serpentinized oceanic crust, potentially supported by the by-products of serpentinization. Klein et al. (2015) reported dense microbial colonies fossilized in brucite-calcite veins in Cretaceous serpentinites that were strongly enriched in organic carbon (up to 0.5 wt% of the total carbon) but depleted in ^{13}C ($\delta^{13}C_{TOC} = -19.4$‰). They also detected a combination of bacterial diether lipid biomarkers, archaeol, and archaeal tetraethers

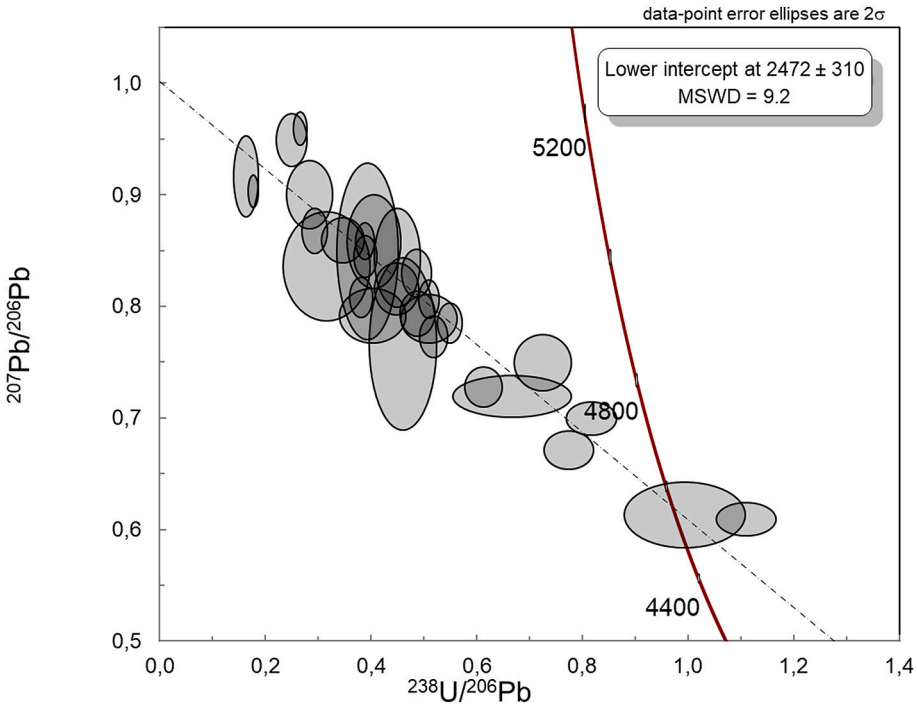

Fig. 8. New Laser-Ablation Inductively Coupled Mass Spectrometry-derived U-Pb-geochronology of Ongeluk calcite embedding the fungus-like fossils. The obtained age of 2472 Ma has a large error ± 310 Ma and a large mean squared weighted deviation (9.2), generally owing to high common lead contents compared to radiogenic lead. The U concentrations were also relatively low as well (~0.01–0.10 ppm, n = 32, 2 rejected spots).

analogous to those found in carbonate chimneys at the active Lost City hydrothermal field. Ivarsson et al. (2008b) observed filamentous microfossils in aragonite-filled veins in ultramafic rocks from the Mid-Atlantic Ridge (MAR), similar in nature to the filamentous fossils previously described from subseafloor basalts. However, lack of morphological traits made it difficult to constrain the biological affinity of the fossils. Alt et al. (2007) had previously reported high sulfide-S (up to ~3000 ppm), and negative $\delta^{34}S$ (to −32.1%) indicative of microbial reduction of seawater sulfate from whole rock analysis of peridotites and gabbros.

2.2. Fossil record in continental igneous crust

2.2.1. Fossils

The record of fossilized microorganisms in the crystalline continental crust is not as extensive as that from the oceanic crust. This may be due to the fact that fossilized remains are better preserved in mineralized basaltic voids and micro-cavities than in open fractures of granites, which feature continuous flow of groundwater that may alter and flush away the fragile fossilized biofilms. Most of the studies for deep life in the crystalline continental crust have utilized infrastructures aimed for other purposes, such as deep mines and deep exploratory boreholes for nuclear waste repository siting.

In the crystalline continental crust, fossilized microorganisms and biofilms occur in deep igneous rocks of both magmatic and volcanic type (e.g., basalts). In the latter, they occur mostly in mineralized cavities (vesicles) of basalts (Sakakibara et al., 2014) much like fossils from the oceanic crustal ophiolites. With a few exceptions (see e.g., Sakakibara et al., 2014) most findings reported from basalt concluded that the host rock originally formed in the oceanic crustal realm (Peckmann et al., 2008; Eickmann et al., 2009; Bengtson et al., 2017; Carlsson et al., 2019), and in the following discussion we therefore instead focus on findings from magmatic rocks, such as granitoids (with some exceptions), which both formed and were colonized within the continental crustal realm.

Life in general thrives where there is liquid water present and where there are gradients that enable substrate and nutrient transport and fluid mixing, and the deep subsurface is no exception (Pedersen et al., 2014). In contrast to the porous media in sediments, crystalline rocks are dominated by fracture-controlled advective flow (Kieft et al., 2018). These hydrological discontinuities will control colonization of the deep subsurface and point to where fossilized life should be searched for – in fractures and veins. Hence, evidence of fossilized lifeforms in granite is much patchier than that in sedimentary rocks and even compared to vesicular basalt. Findings of ancient life in deep fractures and veins can be divided into either morphological evidence of the fossilized microorganisms and/or in the form of other diagnostic signatures left behind following microbial activity. These chemofossils include isotopic signatures or preserved organic compounds (biomarkers) within minerals. Reports of chemofossils are far more common in the literature than in situ findings of mineralized microorganisms, and we will start to describe these in situ findings and continue with the isotopic and biomarker records together with recent chronological constraints gained by applying high spatial resolution techniques to minerals formed following microbial activity.

Fossilized remains of microorganisms in granitoid fractures can be divided, based on their type of preservation, into 1) diagnostic organic molecules, sometimes associated with preserved morphological fossils, 2) completely mineralized remnants with little or no organics preserved, and 3) casts remaining as voids within minerals.

Fossil assemblages from the deep biosphere are typically filaments mineralized by oxides, silicates, and clay minerals (Hofmann and Farmer, 2000). The filaments have diameters of less than 10 µm and can be several millimeters in length. In cavities in calcite veins of unknown age at 207 m depth in Paleoproterozoic diorites in Sweden, carbonaceous microfossils of colonial (ovo)coccoids cells of 1 µm size have been reported (Pedersen et al., 1997). The calcite had low $\delta^{13}C$ values (down to −46.5‰$_{PDB}$), suggesting anaerobic oxidation of methane in the granite fractures (Drake et al., 2015a). Assemblages of ~1 µm sized coccoidal and rod-shaped structures have been reported from deep mineral veins from the Columbia River basalts (continental flood basalts < 10 Myr old), Washington, USA (McKinley et al., 2000a, 2000b). Heim et al. (2012) described a fracture mineral succession in Proterozoic diorite at 450 m depth at Äspö HRL, Sweden, with a thin dark amorphous layer lining the boundary between secondary fluorite and calcite. The amorphous layer had corrosion marks and branched tubular structures. ToF-SIMS imaging revealed abundant, partly functionalized organic moieties, for example, $C_xH_y^+$, C_xHyN^+, $C_xH^yO^+$

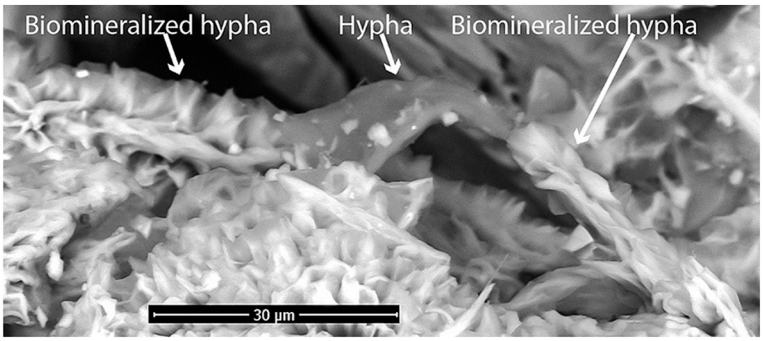

Fig. 9. An ESEM image of a hyphae, partly carbonaceous, partly mineralized by clay. Note the distinct transition from carbonaceous to mineralized.

interpreted to represent the remains of a microbial biofilm that was established much later than the initial cooling of the Precambrian host rock, but the age remains elusive.

Fossilized fungal hyphae have, in similar fashion to those in the oceanic crust, been reported from the crystalline continental bedrock of Sweden and Germany (Fig. 9). Fossilized fungal hyphae forming mycelium-like networks with possible terminal conidial cells or chlamydospores were described from the Triberg granite, and from a uranium mine at Krunkelbach, both in Germany (Reitner et al., 2006).

Fossilized hyphal mycelia with frequent branching and anastomosis between branches occur in granite from the Lockne impact crater, Sweden (Ivarsson et al., 2013b). The hyphae occur in drill core samples, in vesicles related to hydrothermal veins. These veins and fossils were previously interpreted as impact related. Recent high spatial resolution Rb/Sr dating of the hydrothermal mineral assemblage has, however, revealed a much younger age of the mineralization (357 ± 7 Ma) compared to the impact (458 Ma) (Tillberg et al., 2019). This means that the fungal colonization took place at least 100 Myr after the impact event, thus long after the impact-induced hydrothermal activity ceased. Similar, but slightly older Paleozoic ages (396 ± 7 Ma) have been reported from low-temperature mineral assemblages in open fractures carrying curved nonseptate and branched fossil fungal hyphae at 400 m depth in Paleoproterozoic granitic gneisses at Forsmark, Sweden (Drake et al., 2018a). Empty filamentous casts of microorganisms have been detected in a quartz-feldspar-calcite vein dated to 355 ± 15 Ma, at 300 m depth in Forsmark (Drake et al., 2017b). In a cavity in a Paleoproterozoic quartz vein at 740 m depth at Laxemar, Sweden, fungal hyphae that formed extensive mycelia covering zeolites and carbonates have been detected (Drake and Ivarsson, 2018; Drake et al., 2017b). The hyphae are of unknown age and were partly mineralized as clays (Sallstedt et al., 2019), partly as carbonaceous material like the Lockne fossils (Ivarsson et al., 2013b). Associated pyrites were analyzed by secondary ion mass spectrometry and were shown to have isotope signatures (significantly ^{34}S-depleted; δ^{34}S down to −53.3‰) specific for microbial sulfate reduction (MSR). The spatial relationship between the fungi and the pyrite indicates that the fungi and the sulfate reducers occurred in a symbiotic-like relationship. It was thus suggested that the fungi were anaerobic fungi equipped with hydrogenosomes instead of mitochondria, forming H_2 during their respiration that could fuel sulfate reducers (Ivarsson et al., 2016b; Drake and Ivarsson, 2018). In turn, the biomass of the sulfate reducers and anticipated preexisting microbial biofilms inferred by the isotopic composition of calcite, served as a source of carbohydrates for the fungal metabolism - a symbiosis similar to the anaerobic rumen consortia of facultative anaerobic fungi and methanogens.

The fossilized findings of fungi are in line with investigations of modern communities in fracture waters. In exploration for fungi in boreholes and open fractures at the Äspö HRL, Sweden, five yeasts, three yeast-like fungi, and seventeen filamentous fungal strains were isolated at depths between 201 and 448 m (Ekendahl et al., 2003), and more recent metatranscriptome studies confirm the presence of fungi (Lopez-Fernandez et al., 2018a). In Finland a high fungal diversity was detected in deep bedrock fracture aquifers at 300–800 m depth at Olkiluoto (Sohlberg et al., 2015), at 600 m depth in Archaean rocks at Romuvaara (Purkamo et al., 2018), and at Pyhäsalmi mine (Bomberg et al., 2019). Here, most of the observed fungal sequences belonged to the phylum Ascomycota. Also in deep fracture waters of South African mines, fungi were detected among other eukaryotes such as Sar (a clade of protists), Platyhelminthes, Nematoda, Rotifera, Annelida, and Arthropoda (Borgonie et al., 2015). The identified fungal species all belong to Ascomycota or Basidiomycota.

2.2.2. Isotopic and biomarker evidence and timing

As stated above, morphological evidence of fossilized microorganisms appears quite rarely in the studies carried out so far in the deep fractured crystalline bedrock. Evidence for ancient microbial activity is instead provided by diagnostic isotope signatures preserved within minerals formed in association with the microbial activity, and by preserved organic remains within the mineral coatings (Drake et al., 2015a, 2019). Isotopic diagnostic tools include C and S isotopes, which can be used to trace microbial methanogenesis, methane oxidation and sulfate reduction. Microbial methane is usually ^{13}C-depleted compared to other carbon compounds (Whiticar, 1999). As a consequence of the fractionation occurring during methanogenesis, which discriminates against ^{13}C, the residual CO_2 becomes ^{13}C-rich (Milkov and Etiope, 2018). Subsequent involvement of the residual C into precipitating carbonate minerals is therefore a useful diagnostic C-isotope tracer for methanogenesis. During anaerobic oxidation of methane (AOM) by a consortium of methanotrophic archaea and sulfate-reducing bacteria, the significant ^{13}C-depletion is inherited from the source methane and can subsequently be detected in the δ^{13}C signature of authigenic carbonate (Peckmann and Thiel, 2004). At the same time sulfide is formed by reduction of sulfate and after reaction of the produced bicarbonate and sulfide with dissolved Ca^{2+} and Fe^{2+}, carbonate (mainly calcite) and sulfide (pyrite) minerals precipitate, respectively (Borowski et al., 2013; Drake et al., 2013). The diagnostic $\delta^{13}C_{calcite}$ signature is one of the most widely used tools to distinguish ancient and modern AOM, but moderate $\delta^{13}C_{calcite}$ excursion can also be used to infer other carbon sources, such as organoclastic utilization. In addition, pyrite formed during MSR basically inherits the S isotopic composition of the hydrogen sulfide (with only small fraction (Böttcher et al., 1998)) and because the MSR metabolism produces hydrogen sulfide strongly depleted in ^{34}S, $\delta^{34}S_{pyrite}$ serves as a marker for MSR, and high spatio-temporal variation in $\delta^{34}S_{pyrite}$ can reveal reservoir effects in more stagnant systems (Lin et al., 2016). If the minerals formed in the fractures have favorable composition and have remained in closed systems they can be directly dated, e.g. by high spatial resolution techniques (e.g. Rb/Sr (Tillberg et al., 2020), U–Pb (Roberts et al., 2020)), which are particularly important to apply because crystals typically show zonation due to multiple growth stages.

Isotopic investigations of fracture coatings and veins in the bedrock of Sweden and Finland, were until recently focused on conventional

bulk sample analysis of C and O isotopes. Bulk analyses of coatings from Stripa mine (Clauer et al., 1989), Äspö (Tullborg et al., 1999), Laxemar (Drake et al., 2012, 2014; Drake and Tullborg, 2009), and Forsmark (Tullborg et al., 2008) in Sweden, and Olkiluoto in Finland (Sahlstedt et al., 2010) have all presented moderate to large ^{13}C-depletions that suggest microbial activity in situ.

More recent utilization of secondary ion mass spectrometry (SIMS) analyses to mineral coatings in granite fractures have documented large isotopic heterogeneity within individual crystals and a significant variability overall, both for the S and C isotope systems. From the Forsmark and Laxemar areas calcite with significantly ^{13}C-depleted (δ^{13}C down to −125‰$_{PDB}$) and ^{13}C-enriched (δ^{13}C up to +36.5‰$_{PDB}$) values have been documented in fractures and veins at depths down to 800 m, and have been assigned to AOM and methanogenesis, respectively (Drake et al., 2015a, 2017b). Rb−Sr and U−Pb dating of two of the generations of these fracture mineral assemblages revealed 355–410 Ma and 173 ± 8 Ma (Forsmark) ages, respectively (Drake et al., 2017b). For the Götemar area, similar C isotopes to those at Forsmark and Laxemar were found in the fracture calcites, but dating was unsuccessful. We have re-visited the samples in a new analytical session and can present a Jurassic age (160 ± 3.3/5.5 Ma, Fig. 10A, methods follow those described in Drake et al., 2017b, and is given in supplementary data 3, together with the raw data) overlapping with that in Forsmark for a ^{13}C-depleted calcite (δ^{13}C down to −50‰$_{PDB}$). This indicates a regional Jurassic Fennoscandian fracture reactivation event with associated AOM activity in the deep bedrock. δ^{34}S compositions of pyrite from the same Fennoscandian granite fracture systems, show substantial variability, −54 to +132‰$_{CDT}$ coupled to MSR (Drake et al., 2013, 2015a, 2018a, 2018b). For the most ^{34}S-rich pyrite sample, a Rb/Sr-dating of co-genetic calcite and a clay mineral lining that is a presumed fossilized biofilm (Fig. 11) gave an age of 393 ± 15 Ma (Drake et al., 2018b).

In the granitic rocks at Laxemar, late-stage vein-filling calcite formed at low temperatures (< 50 °C, anticipated age < 10 Ma, no direct dating) by MSR–AOM paleoactivity as shown by δ^{13}C values as low as −125‰$_{PDB}$ and preserved diagnostic lipid biomarkers (Drake et al., 2015a). We can now present new U−Pb timing constraints for this AOM-related calcite generation (Fig. 9B-D, from 538 m depth), confirming the anticipated late Miocene age although with relatively large error (± 2 Ma). Relatively young microorganism-related calcite and pyrite have also been reported from the Devonian meteorite impact crater at Siljan, Sweden (Drake et al., 2019). At depths down to 642 m in the fractured granitoid rocks of the crater rim, δ^{13}C values of secondary calcite showed values of −53 to +22‰$_{PDB}$ consistent with methane formation and utilization. Furthermore, $\delta^{34}S_{pyrite}$ values of −42 to +78‰$_{CDT}$ were detected, consistent with MSR, which was also

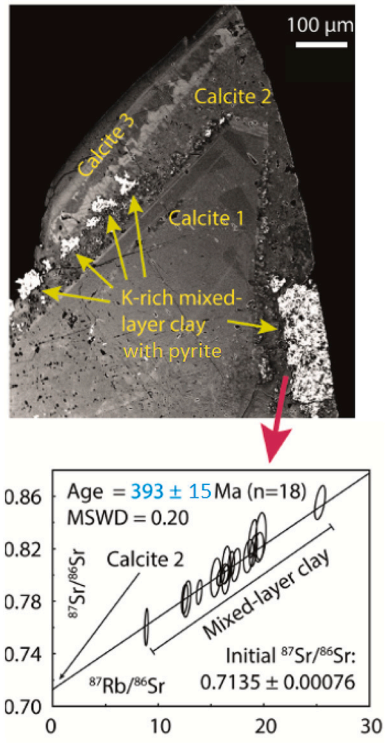

Fig. 11. Calcite and clay minerals in a sample from Laxemar Sweden (drill core KLX14A: 80 m depth) used for in situ Rb−Sr dating (figure modified from Drake et al., 2018b). The clay mineral coating is anticipated mineralized biofilm and the pyrite of this sample showed extremely ^{34}S-enriched values (δ^{34}S up to +132‰ V-CDT).

inferred from lipid biomarkers of the mineral coatings dated by U−Pb carbonate dating to ages in the range of 80 ± 5 to 22 ± 3 Ma. In the Caledonian nappes of western Sweden, deep micro-karst intersected by the COSC-1 borehole hosted secondary assemblages of zeolite, ^{34}S-depleted, MSR-related pyrite and calcite, of which the latter yielded relatively young U−Pb ages of 9.6 ± 1.3 Ma and 2.5 ± 0.2 Ma (Drake et al., 2020). Also in the deep fractured Paleoproterozoic rocks at Olkiluoto, Finland, there have been reports of substantial isotopic variability for $\delta^{13}C_{calcite}$ (−53.8 to +31.6‰$_{PDB}$) (Sahlstedt et al., 2016) and $\delta^{34}S_{pyrite}$ (−50 to +82‰$_{CDT}$) (Sahlstedt et al., 2013), but ages of these minerals remain elusive. Taken together, the isotopic variability for both S and C in pyrite and calcite, respectively, is larger in the igneous continental crust fracture habitats than reported from any other

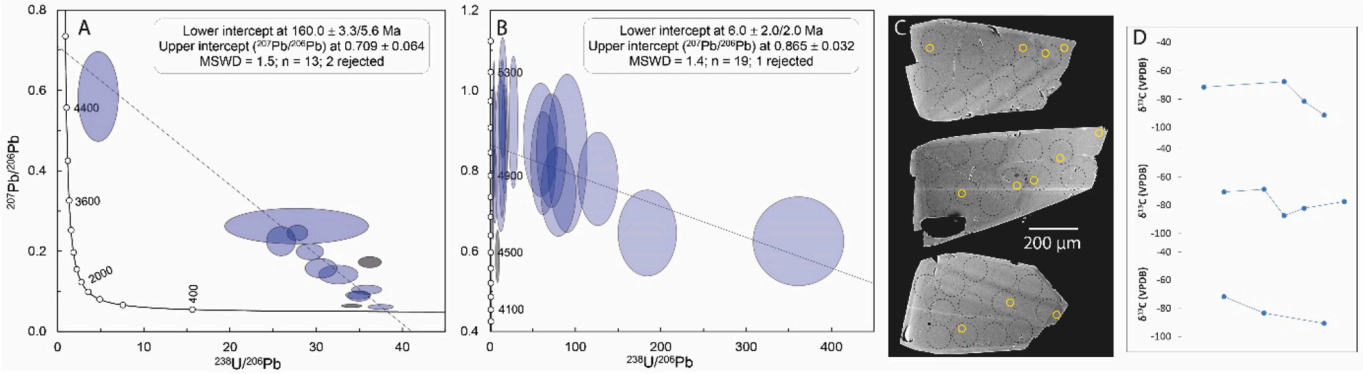

Fig. 10. New U−Pb geochronology determinations of AOM-related calcite from A) Götemar, Sweden (23 m depth, see Drake et al., 2017b for C-isotope data), and b) Laxemar, Sweden (sample from Drake et al., 2015a). C shows spot placement in relation to U−Pb spots (large spheres) in b, d) shows C-isotope data (from Drake et al., 2015a) representing the small yellow circles in C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

environment on Earth. Possible explanations for this large isotope variability include – in addition to the isolated semi-closed nature of these deep systems - for the sulfur isotopes, that rates of metabolism, like MSR rates, are very slow under the oligotrophic conditions prevailing. Slow rates of MSR have been shown to result in large fractionation of the sulfur isotopes (Leavitt et al., 2013), particularly at high sulfate concentrations (Bradley et al., 2016). Isotope enrichments of more than 70‰ have indeed been indicated at pyrite formed during MSR in sulfate rich waters at −450 m depth at Äspö, Sweden (Drake et al., 2015b), which can explain the very low $\delta^{34}S$ values observed for ancient pyrite. Successive precipitation of such ^{34}S-depleted pyrite due to MSR along the groundwater flow path can eventually lead to the extremely heavy $\delta^{34}S$ values in pyrite precipitated in more distant parts of the fracture reservoir undergoing Rayleigh isotope fractionation (Drake et al., 2018b). For the large variability of the C isotopes, explanations also include slow metabolisms that result in large fractionations, but also the pathway of methanogenesis. Methanogenesis occurring through the carbonate reduction pathway processes produces a ^{13}C-depleted methane and a strongly ^{13}C-rich residual CO_2 (Meister and Reyes, 2019). Calcite formed from the latter becomes isotopically heavy, particularly if there are very low concentrations of other C sources that can dilute the methane-related signature, which can explain the heavy $\delta^{13}C$ values of > > +20‰ in these crystalline rock fractures (Drake et al., 2019). The origin of extremely ^{13}C-depleted calcite may similarly be explained by almost exclusive incorporation into calcite of ^{13}C-poor C originating from anaerobic oxidation of a very ^{13}C-depleted microbial methane (Drake et al., 2015a). In these carbon poor settings the original isotopic signature of C originating from AOM is thus not significantly diluted by other C sources in the groundwater prior to incorporation into calcite. This is in contrast to in marine settings where dilution of porewater DOC and DIC usually makes seep carbonates isotopically heavy compared to the source methane, although the AOM step involves fractionation resulting in a lighter product (Peckmann and Thiel, 2004). Back-flux of the AOM reaction can be an alternative explanation (Yoshinaga et al., 2014).

2.2.3. A morphological atlas of fossils in igneous rock

For diagnostic purposes of micro-fossils in igneous crust the following charts (Figs. 12 and 13), and associated tables (Tables 2,3) were assembled. All described morphological features are observed in open pore space or in thin sections prepared from veins and vesicles filled with secondary minerals.

The prokaryotic fossils are predominantly mineralized by iron- or manganese oxides, and all fungal fossils are mineralized by clays of smectite or montmorillonite type. The central strand of hyphae is mineralized by iron oxides, while spores can be mineralized by manganese oxides.

3. Fossilization

Fossilization in general is influenced by the geochemical conditions of the environment, and subsurface fossils are no exception. The similarity in mineralization and fossilization between geologically and geochemically quite disparate settings such as mafic, ultra-mafic and felsic granite provinces suggests a complex view influenced by the present biogeochemistry (Ivarsson et al., 2015a, 2015b, 2015c, 2015d, 2018a, 2018b; Drake et al., 2017b, 2018a; Sallstedt et al., 2019). The chemical composition of the host rocks influences the chemical composition of the circulating fluids, which in turn gives rise to fossilization. The chemical composition of fluids in mafic,- compared to felsic, host rocks as well as the overall geochemical regime, like pH and pO_2, vary quite dramatically, which should be reflected in the taphonomy and fossilization. However, subsurface fossilization appears quite homogenous and universal across various environments suggesting other forces in action.

Fossilization is normally a step-wise process where organic matter is degraded and replaced by minerals until the organism is more or less completely mineralized and fossilized (Fig. 14). Depending on the preservation and diagenesis, various amounts of organic molecules can remain. Fungal fossils in deep environments are exclusively fossilized by montmorillonite-type clays and minor amounts of Fe-oxides (Ivarsson et al., 2015a, 2015b, 2015c; Sallstedt et al., 2019). Fungal clay authigenesis appears to be consistent through all morphological growth structures, environments and ages (Sallstedt et al., 2019). However, this is not unique to subsurface environments only. Clay authigenesis seems to play a ubiquitous part in biofilm formation and preservation in the shallower surface realm as well (e.g., Ferris et al., 1986, 1987; Konhauser and Urrutia, 1999; Gadd, 2010). The extracellular organic matrix probably has a large impact on what chemical species are attracted to the organic matter of the organisms and subsequently the type of clay that precipitates around microbial cells and EPS (Sallstedt et al., 2019). There is a known connection between organic matter and the adsorption of metals and subsequent clay mineralization, which can be observed in many types of aqueous environments (Konhauser and Urrutia, 1999; Gadd, 2010). Montmorillonite clays of the smectite group are layered minerals with high aspect ratios formed of two silica tetrahedral sheets with one octahedral sheet in between (TOT structure) (Feuillie et al., 2013). In addition, the edges of the platelets display broken-end hydroxyls sites, the protonation of which evolves as a function of pH. However, parameters like solution composition, solute:sorbent ratios and ionic strength also influence the process and surface complexation (Fein et al., 2001). Because of their crystallography and small unit size, smectite minerals are among the most reactive minerals in subsurface environments.

In the deep igneous crust of both land (Drake et al., 2017a, 2017b) and oceans (e.g., Ivarsson et al., 2013a, 2015a, 2015b, 2015c; Bengtson et al., 2014), fungal biofilms in particular seem to be preserved with authigenic clay minerals suggesting an important link between the biological cell and the clay species defined by proportions of cations such as Al, Si, Fe, and Mg. Put simply, the negatively charged carbonaceous matter attracts the positively charged Si, Al, Mg, Fe (and minor Na, and Ca) cations of the fluids. Initial adsorption of cations on the carbonaceous matter produces nuclei and sparks subsequent mineral growth and clay mineralization (Drake et al., 2017b). The result is complete mineralization by clay minerals. In larger hyphae or growth structures, Fe oxide can be a minor constituent next to clay minerals. Mineralization normally starts at the centre with a fully mineralized Fe-oxide dominated central strand (hypha) or core (yeast) followed by clay-mineral dominated margins. To evaluate the effect of host rock geochemistry on the precipitating clay minerals associated with fossil microbial biofilms, Sallstedt et al. (2019) investigated several sites differing in ambient redox degree, and suggested that swelling smectites of montmorillonite type dominate the fossil related clay mineralogy at both oxic seamounts and deep anoxic continental granite environments.

Even though the mineral composition between fossils in mafic and felsic systems does not vary much, the degree of mineralization does. In oceanic mafic systems all fungal fossils are mineralized, and it has been suggested that the mineralization starts while the organisms are alive (Ivarsson et al., 2013a, 2013b). In felsic systems, both mineralized and non-mineralized carbonaceous fungal fossils are observed. It seems as if the conditions in felsic systems favour preservation of carbonaceous fossils and, contrary to mafic systems, the mineralization of fungi takes place post mortem. The presence of partly mineralized hyphae (Fig. 8) as well as carbonaceous and mineralized hyphae next to each other and in the same mycelia suggests fossils can be preserved as carbonaceous matter for a long time and that mineralization occurs long after death depending on the geochemical regime (Drake et al., 2017b).

In contrast to subsurface eukaryotic fossils, prokaryotic fossils and traces seems to reflect the metabolic activity of the organisms rather than secondary mineralizations and abiotic clay authigenesis. In the oceanic crust, prokaryotic morphological remains such as cells or

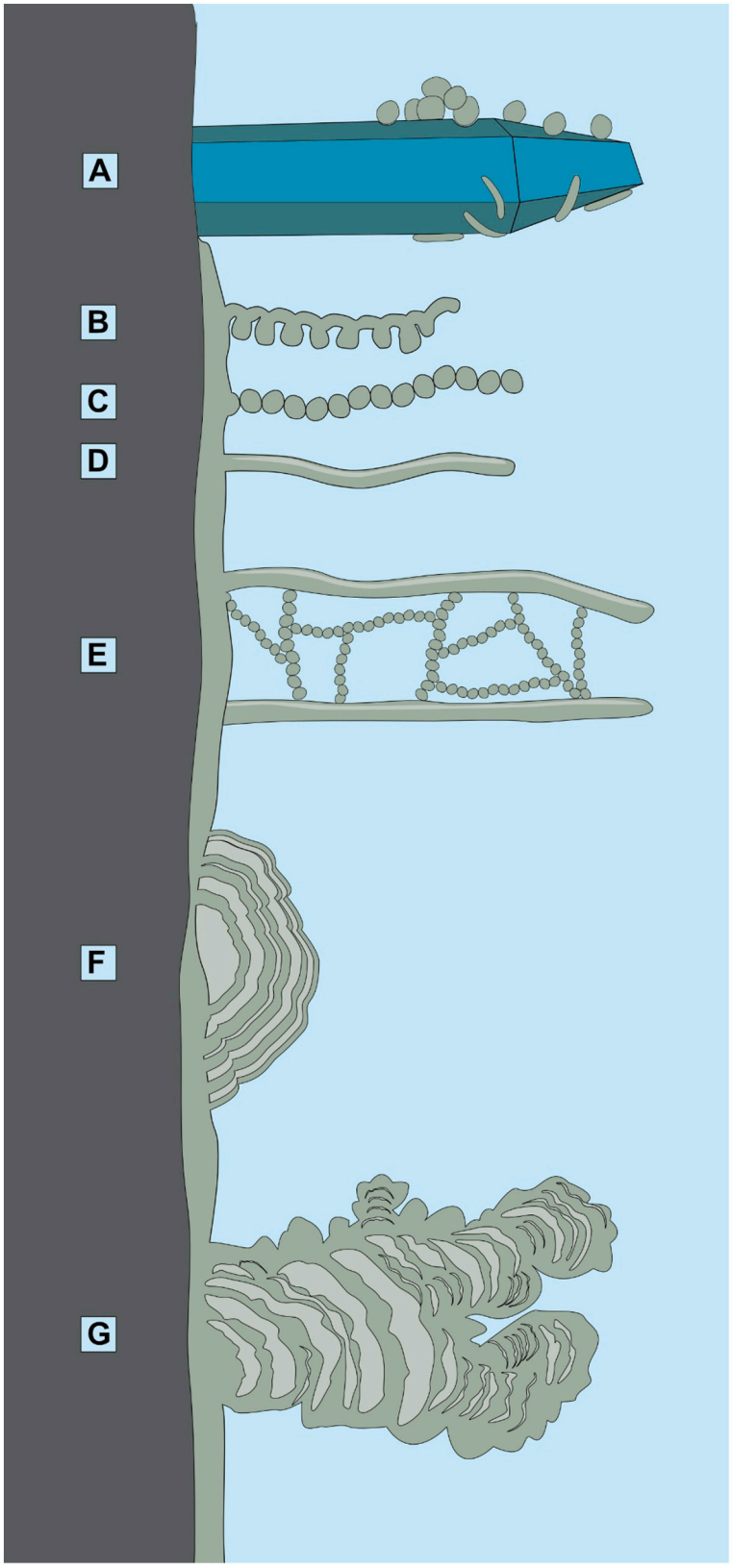

Fig. 12. Chart for prokaryotic morphologies. A) An euhedral crystal with single cellular structures on, both coccoidal and rod-shaped. Note how some have etched the surface leaving a negative pit in the mineral. B) Twisted filament. C) Cells in a row forming a filament. D) Straight, curvi-linear filament without any characteristic morphologies. E) Cells attached as strings suspended in between fungal hyphae. The prokaryotic cells use the fungal mycelium as framework for growth and numerous parallel strings can form cob web like sheaths between hyphae. F) Microstromatolite. Laminated mound-like build ups formed by cyclic growth. The layers usually consist of different iron oxides like goethite-hematite, or iron oxides-clays. Layered manganese oxides have also been reported. G) *Frutexites*. Complex microstromatolites with branching and outgrowths.

microstromatolites are mineralized by Fe- or Mn oxides, presumably as a result of their metabolism. Most prokaryotic fossils have been interpreted to be involved in Fe- and/or Mn-oxidation, and encrusted by the products of their metabolic activity; initial precipitation and encrusting of Fe- and/or Mn oxides, followed by complete mineralization (Thorseth et al., 2003; Bengtson et al., 2014; Ivarsson et al., 2015a, 2015b, 2015c).

The difference between prokaryotic and eukaryotic fossilization can be seen as a result of their separate ecological role and position in the food chain. The autotrophic and heterotrophic prokaryotes are primary

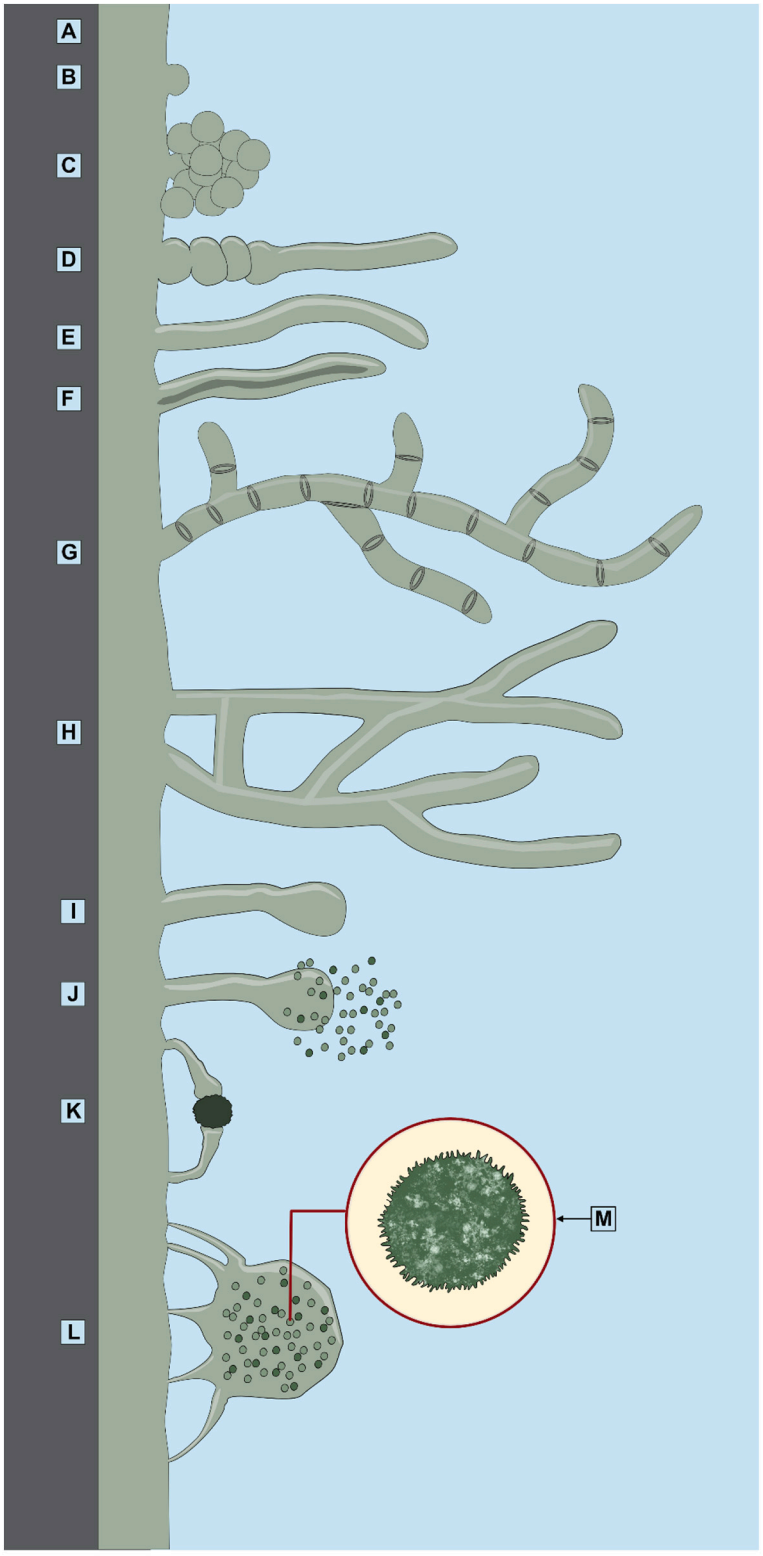

Fig. 13. Chart for eukaryotic (fungal) morphologies. A) Basal biofilm. B) Yeast cell. Numerous yeast cells can sometimes give the basal biofilm an irregular appearance. C) Numerous yeast cells forming an agglomerate. Hundreds of cells can form "tower"-like mounds perpendicular to the vein/vesicle wall. D) Dimorphic growth. Transitions between yeast cells and hypha. E) Unseptated hyphae. F) Hyphae with a central strand. G) Branched and septated hyphae. H) Hyphae with anastomosis between branches forming a mycelium. Branching is either as T- or Y-junctions. I) Sporophore. J) Sporophore releasing spores. Can be seen in sporophores embedded in carbonates. K) Zygote. Last stage of zygospore formation. L) Asci, fruit body of sac-fungi. The sac is filled with spores and the fruit body is attached by hyphae to the host rock. M) Spores released from the asci. Note the rugged surface attachement.

Table 2
Morphological characteristics for prokaryotic fossils and biomineralizations.

Cell	Coccoidal and rod-shaped cells, 1–5 μm in diameter, and normally attached to minerals. Sometimes etching, leaving a negative pit.
Filament	Curvi-linear, straight, or twisted simple or occasional branched filaments with diameters of a few μm and lengths up to 20 μm. Sometimes filaments are built up of cells ("pearls-on-a-string").
Microstromatolite	Laminated structures of iron- or manganese-oxides that can range from 20 to hundreds of μm in size.
Frutexites	Cauliflower-like, branching microstromatolites with similar size range as the normal microstromatolites, but with a more extensive length.

Table 3
Morphological characteristics for eukaryotic fossils.

Biofilm	(~10–20 μm in thickness) a basal film laid down directly on the host rock, secondary mineral or microstromatolites in an open pore space. The biofilm can either be smooth and acellular or be cellular with varied content of (yeast) cells or hyphae. Usually, hyphae, sporophores or yeast cells protrude from the film.
Hypha	5–50 μm in diameter, from tens of micrometers to millimeters in length. In general shorter when occurring as single compared to hyphae in a mycelium where beginning and end can be difficult to distinguish. With increasing diameter a central strand is usually seen, sometimes making up half of the hyphal diameter. Repetitive septa are not uncommon, usually with about 10 μm space between septa. Branching common and, in mycelia, anastomosis between branches.
Yeast	(~10–20 μm in diameter) single cells usually in assemblages that can form irregular tower-like structures perpendicular to a surface. Differentiated from spores by the smoother surface and the closeness between cells. Yeast cells in assemblages are always in contact with each other while spores occur separated from each other, having been released in a "cloud".
Reproduction structures	Reproduction structures: sporophores - hyphae, normally growing directly from the basal film, endings with a terminal swelling (10–30 μm in diameter). Fruit bodies – sacs (50–200 μm in diameter) either filled with spores or empty (Ivarsson, 2012). Spores – circular single cells (5–10 mm in diameter) with a ragged surface. Occur in random assemblages, sometimes in conjunction with sporophores or sac-like fruit bodies.

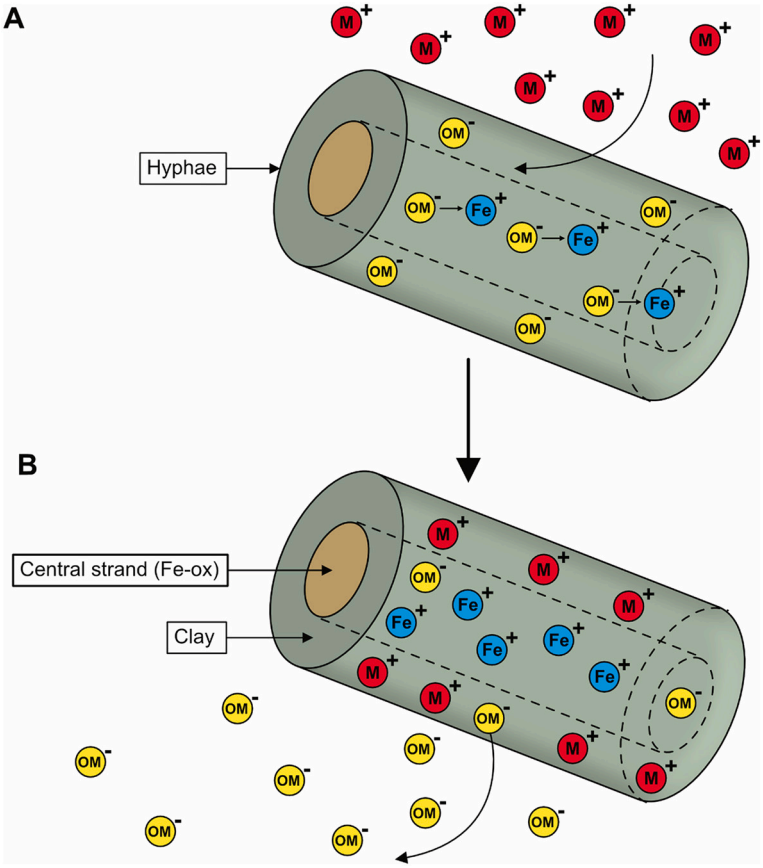

Fig. 14. Schematic figures showing the mineralization and fossilization of microorganisms. Exchange of negatively charged organic matter (OM) with positively charged metal ions (M) including Si, Al, Mg, Fe, resulting in replacement of OM by clay. Positively charged iron (Fe) is replacing OM in the central strand.

producers in utilizing the chemical redox potential of the rocks and gases, while fixing carbon from primarily CO_2 and HCO_3^-. The fungal eukaryotes consume the prokaryotes and their metabolic products are basically CO_2, and thus not easily mineralized species. However, their abundant production of biofilms and EPS as well as production of negatively charged biomass mediate secondary clay formation. The similarities in mineralizations across different subsurface environments and geochemical regimes suggests that life in the deep oligotrophic crust probably has a far greater effect on the cycling of chemical species, such as Fe, Al, Si and Mg than what has previously been established, and may in fact be responsible for a great fraction of secondary clay minerals in the deep subsurface (Sallstedt et al., 2019).

4. Deep collaboration

The dominance of fungal fossils over prokaryotic fossils in the deep subseafloor is probably due to preservational advantages of fungi and difficulties in searching for and interpreting the prokaryotic fossil record (Ivarsson et al. 2018). Whatever reason, the abundance of fungal fossils in close association with prokaryotic remains has led to unforeseen insights into the community structure of deep microbial colonies. In the oceanic crust, prokaryotes have been seen to take advantage of the fungal mycelia as a framework for growth. Strings of single cells forming cob web-like sheets in between fungal hyphae with an ultrastructure similar to the sulfur-oxidizing archaea *Pyrodictium* have been reported (Bengtson et al., 2014). Microstromatolitic *Frutexites* have in a similar way been shown to grow on fungal hyphae, thus forming communities of at least three preserved and identifiable organisms

intimately connected to each other. Eukaryotes are probably dependent on available prokaryotic biomass for colonization of otherwise oligotrophic environments, thus the fungi are favoured by an interaction with prokaryotes. For instance, it has been shown that fungi overgrow prokaryotic biofilms/microstromatolites, more or less grazing the prokaryotes (Ivarsson et al., 2015a, 2015b, 2015c).

In terrestrial deep settings fossilized fungal hyphae have been closely related to pyrite with S isotopic signatures consistent with MSR as well as with carbonates having C isotopic signatures for methanotrophic archaea (Drake et al., 2017b, 2018a; Tillberg et al., 2019), thus, an interaction between prokaryotes and fungi is established in the continental deep realm as well. The anoxic conditions in deep continental settings infer an anaerobic nature of this partnership. Anaerobic fungi producing H_2 in their respiration may support H_2-dependent prokaryotes like sulfate reducers and methanogens (Ivarsson et al., 2016a, 2016b; Ivarsson et al., 2019a, b; Drake and Ivarsson, 2018). In summary, interactions between prokaryotes and eukaryotes are key in the colonization of deep settings, at least for fungi. Fungi are heterotrophs and require available biomass for their metabolism, thus a prokaryotic presence is probably crucial for eukaryotic colonization of deep subseafloor and subsurface environments.

5. Future prospects and challenges

The study of the fossil record of deep life in igneous crusts is in its cradle, and major challenges wait ahead. The following are areas within which future challenges are being identified:

5.1. Prokaryotic portion of the fossil record

A major portion of the fossil record of deep igneous crust consists of fungal remains. This is most likely not a result of fungi dominating the deep communities but rather of fungi being more easily mineralized and fossilized than prokaryotes. Fungal cell walls are thicker and more complex, consisting of layers of, for instance, chitin and melanin, which enables preservation. Prokaryotic activity has so far best been recorded as chemofossils and detected by isotopic measurements or biomarkers in secondary mineralizations like carbonates and sulfides (Drake et al., 2013, 2015a, 2017a,b, 2018a, 2018b, 2019). Prokaryotic cells have been shown to be preserved as fossils (Pedersen et al., 1997; Thorseth et al., 2003; Ivarsson et al., 2011a; Bengtson et al., 2014) but more commonly as entire communities such as microstromatolites (Bengtson et al., 2014; Ivarsson et al., 2015a, 2015b, 2015c, 2015d, 2019a, 2019b). We are just about to understand what deep-biosphere prokaryotic activity leaves behind in the fossil record and we need to formulate strategies how to approach and interpret these fossil remains. By doing this we will get insight in the prokaryotic abundance and diversity, but also into metabolic pathways through time.

5.2. Analytical challenges

A challenge for the study of fossils in deep igneous crust is to enhance in situ analyses of individual fossils and to combine different sets of data to strengthen the interpretation and biological affinity of individual fossils, for example, detailed morphological studies could be better integrated with detailed biochemical investigations. As it stands now, most reports focus on either morphology and geological context or biochemical data, mainly because a certain set of analytical set-ups are restricted within the research groups, which usually are specialized towards one specific field.

5.3. Dating fossils in deep igneous crust

One issue with fossils of the deep igneous crust is timing, thus relating the fossils to a reliable age. The stratigraphic relationship between fossils and geological deposits found in sedimentary rocks do not, for obvious reasons, exist in igneous rocks. Instead, radiometric dating of the host rock (or adjacent units) or dating of fossils in overlying sediments are used as circumstantial dating. Association with such non-precise dating requires that the fossil itself can be constrained to a geological context with high precision, for instance related to secondary mineralization. Dating of secondary mineralizations closely associated with fossils and/or of minerals showing microorganism-affected isotope composition has been successful from deep fractures in the Fennoscandian shield (Drake et al., 2017b, 2018a, 2018b, 2019). In situ dating of fossils would be optimal, but remains a challenging task because the minerals responsible for fossilization of microorganisms have to be feasible for dating. Secondary minerals like carbonates, low-temperature feldspars and clay minerals closely related to fossils have been dated by Rb/Sr and/or U−Pb dating (Tillberg et al., 2019; Drake et al., 2019), and constrain fossils in time. Of particular interest is a clay-mineral-rich fracture coating that resembles a fossilized microbial mat and contains organic C according to Raman spectroscopy, which was dated by Rb/Sr in situ dating to 393 ± 15 Ma (Fig. 10). Theoretically, individual fossils would be possible to date with the Rb/Sr method if certain criteria are fulfilled, e.g. i) the Rb-content is high enough (K > ~1 wt% is a good starting point), and ii) the Sr-content at a minimum in the Rb-rich phases, and iii) the fossils are large enough to harbour 40–50 μm sized spots, iv) the fossils are formed at a single event, v) the fossils have not been significantly heated (that may reset the isotope system) or occurring in a reactivated fracture system (that may lead to preferential leaching or diffusion of the isotopes of interest), and vi) associated low-Rb carbonates that can be related to the fossils (Drake et al., 2018b, see Tillberg et al., 2020 for a review of the feasibility of the method). For low-temperature feldspars and illite, the $^{40}Ar/^{39}Ar$ dating method may be an alternative (Drake et al., 2009b; Sandström et al., 2009).

The U−Pb dating of the Ongeluk carbonates presented in this paper has large error of ~300 Ma, mainly owing to that common lead dominated over radiogenic lead, although U concentrations were reasonable for dating purposes. Nevertheless, this timing constraint serves the important purpose of assigning a Paleoproterozoic age to the fossils.

Titanite mineralized within putative ichnofossils in Archean ophiolites have been dated by direct U−Pb dating and gave an age of 2949 ± 250 Ma (Banerjee et al., 2007). This is the age of the mineralization of the infilling titanite and not the formation of the actual trace fossil, but it gives, at least, a minimum age of the fossil.

Currently, the fossil record of igneous crust stretches from the present to about 410 Ma, but with geographic discrepancies. Prior to ~410 Ma the record is very sparse. Fungal-like fossils in the Ongeluk formation of 2.4 Ga (Bengtson et al., 2017) reveal that the oceanic crust was already inhabited by hypha-forming microorganisms by this time, and thus highlight a 2 Ga gap in the fossil record.

5.4. Influence on global biogeochemical cycles

Considering that the deep biosphere currently is the second largest reservoir of living biomass on the planet and that is was the largest reservoir before plants colonized land (McMahon and Parnell, 2018), it is reasonable to assume that it has played a crucial role in global biogeochemical cycles during Earth's history. It has been shown that microorganisms of the deep biosphere involved in abundant bio-weathering of host rock and secondary minerals mobilize elements like H, C, N, O, P, S, Si, Al, Na, K, Ca, Mg, Fe, and Mn among others (Ivarsson et al. 2015a, 2015b, 2018). Eukaryotes in the fossil record have been shown to be involved in decomposition of marine organisms and organic matter that has been introduced in shallow basalts, as well as being engaged in degrading prokaryotic biomass. Thus, they are responsible for cycling of C and associated elements like H, N, P in both shallow and deep oceanic crust (Ivarsson et al., 2015a). Isotopic evidence has also been reported for methanogenesis and MSR at depth (Lever et al., 2013). Fluids dissipating at vents have signatures of

chemoautotrophy, thus C is microbially processed at depth (McCarthy et al., 2011). It is reasonable to assume substantial element substitution between the oceanic crust and seawater, as well as crystalline continental crust, groundwater and atmosphere. Microbial methane formation and oxidation have been reported from deep within the igneous continental crust (Simkus et al., 2016; Bomberg et al., 2015; Kietäväinen et al., 2017; Ino et al., 2018), and based on the observations reported so far, the process seems to be widespread and date back at least 410 Myr (Drake et al., 2015a, 2017b, 2019; Tillberg et al., 2019). However, the significance of this deep source and sink for methane on the global biogeochemical cycles remains to be deciphered.

Microbial fossilization by clays and iron/manganese oxides including passive microbial formation of zeolites and Fe-, Mn oxides immobilizes dissolved elements like Si, Al, Mg, Na, K, Fe, Mn, Ti from fluids. Microbial precipitation of carbonates and sulfides through AOM, methanogens, and MSR influence element substitution between atmosphere,-groundwater, and -crystalline rock.

Microbially mediated weathering of volcanic glass and basalts of oceanic crust on the early Earth has been proposed as a major driving force of oxidation of the reduced oceanic crust. This subsequently resulted in the formation of oxide minerals as well as rocks like granite with a larger content of oxidized minerals compared to mafic and ultramafic rocks, and eventually to the formation of micro-continents (Grosch and Hazen, 2015).

5.5. Comparing fossils to live species/communities

For more reliable morphological characterizations of fossils, comparisons with modern living forms are necessary (Thorseth et al., 2001a, 2001b, 2003; Orcutt et al., 2020). This involves both highly detailed morphological investigations of, for instance, certain growth features but also of the community structure as a whole including microorganism interrelationships and microbe mineral interactions. Isolation and speciation by molecular approaches of strains are also of importance to understand the diversity and species richness of endolithic communities; if expected species are known it is easier to relate morphologies of the fossil record to modern features. In subseafloor mafic crust, for instance, iron- and manganese oxidation are major metabolic pathways among prokaryotes, which corresponds well to what is seen in the fossil record (Orcutt et al., 2011; Ivarsson et al., 2015a, 2015b, 2015c, 2015d). However, a plethora of other prokaryotes exist in the oceanic crust involved in, e.g., nitrogen cycling, sulfur oxidation, and methane consumption, but because of uncharacteristic morphologies or the lack of metabolically induced biomineralization they are rarely preserved or identified as fossils. It is more feasible to identify the fossil remains of such microorganisms as chemofossils. In the continental deep biosphere, morphological fossils of prokaryotes have proven even harder to identify than in the oceanic crust, which is somehow of a paradox since molecular investigations indicate a predominance of prokaryotes over eukaryotes (Pedersen et al., 1997, 2008; Lopez-Fernandez et al., 2018a). Recent metatranscriptome data from boreholes in Äspö HRL show all three domains of life to be present but with a predominance of Bacteria (Lopez-Fernandez et al., 2018a). All domains show a diverse range of metabolic strategies carried out by multiple taxa, of which many group within newly described candidate phyla or can not be mapped to known branches on the tree of life. This suggests that a large portion of the active biota in the deep biosphere as yet unexplored, which opens important prospects for the assignment of biological affinity to fossils with unfamiliar morphologies. Microbial communities also differ between different groundwater regimes. A diverse metabolic range is seen in modern marine water that is fed by organic carbon from the surface, while old saline waters with residence times of thousands of years are more constrained to 16S rRNA gene sequences responsible for oxidizing hydrogen and fixing carbon dioxide (Lopez-Fernandez et al., 2018a, 2018b). Similar correlations between communities and particular hydrogeologic regimes have been seen in other environments like the Tau Tona gold mine in Witwatersrand Basin, South Africa, where the older, saline and reducing waters are dominated by *Firmicutes* (Magnabosco et al., 2016). It is also seen in the oligotrophic groundwaters of Äspö that non-viable cells are rapidly degraded and recycled into new biomass (Lopez-Fernandez et al., 2018b), which to some extent could explain the lack of a prokaryotic body fossil record. Furthermore, the abundance of nano-sized Archaea and Bacteria highlights the importance of typically ultra-small cells in the deep subsurface (Lopez-Fernandez et al., 2018b). A metagenome analysis of three deep subsurface water types revealed phylogenetically distinct microbial community subsets of both Bacteria and Archaea that either passed or were retained by a 0.22 μm filter (Wu et al., 2016). The estimated genome sizes of the populations were generally smaller than those of their phylogenetically closest relatives, suggesting that small dimensions along with a reduced genome size may be adaptations to oligotrophy. If nano-sized microbes are ever to be found in the fossil record they are probably below detection limit of today's instrumentation, but nevertheless important to keep in mind when investigating the fossil record of the igneous crust. Thus metagenomic and molecular analyses are an important source of information when interpreting fossils of the deep igneous realm. They can give hints of possible morphologies or chemofossils in certain systems but also possible metabolic pathways depending on the geochemical and hydraulic regime. For morphological comparisons independent isolation and culturing is unbeatable. With isolated strains it is possible to perform laboratory experiments based by inoculation of certain strains into sterile rock samples for colonization studies. The taxonomic usefulness of these techniques, however, deteriorates with age – comparisons between living and Archean/Proterozoic communities are, at best, valuable as analogues.

5.6. Establish the igneous crust as a part of Earth's fossil record

The establishment of the fossil record of the igneous crust as a relevant research field within paleobiology, geobiology, and oceanography is of prime significance. A number of current publications highlight the importance and previous neglect of this fossil record (Ivarsson et al., 2015a, 2015b, 2015c, 2015d, 2018a, 2018b, McMahon and Ivarsson, 2019; Onstott et al., 2019) but more work is needed to increase the awareness of the scientific community. More research is crucial to understand the full complexity of these fossils, and their global significance. The deep biosphere is currently the second largest reservoir of living biomass, and before plants colonized land the deep biosphere was the predominant reservoir for living biomass. The deep biosphere fossils thus played a significant role in the last 400 Ma of Earth's history but even more so prior to 400 Ma (McMahon and Parnell, 2018). Fossils from the deep biosphere hold a key to our understanding of early evolution on Earth with respect to both prokaryotes and eukaryotes, as well as early biogeochemical cycles.

Paleontological material should be emphasized during drilling in igneous crust as a complement to other analytical procedures. Initially, drilling campaigns should be devoted to deep biosphere fossil investigations to enhance our understanding of the deep paleo-record of life, but in the longer perspective the aim should be to establish fossil investigations as a standard analytical procedure in drilling campaigns like IODP or ICDP just as biostratigraphy is when sediments and sedimentary rocks are drilled and logged. If a simplified chart or atlas is used during logging of drill cores parameters like microbial vs. abiotic iron/manganese-oxidation would be distinguishable. Microbe-mediated weathering could easily be detected and indicate an increased weathering rate and mobilization of elements compared to samples where strictly abiotic processes prevailed. Presence of fungal fossils would indicate the presence of available organic matter at the time of mineralization. With more precise diagnostic methods microbial fossils could probably be used as indicators of redox chemistry, oxygen availability, elemental abundance of C, N, P, H, S, Fe, Mn as well as

bioessential trace elements, and determination of temperature regimes, just like mineralogical and geochemical analysis are today. The fossil record can add a whole new dimension to deep drilling investigations and provide data on past biogeochemical conditions including living conditions.

Declaration of Competing Interest

The authors declare no conflict of interest. The content of this manuscript is not under consideration for publication elsewhere. All authors have been involved in the work, approved the manuscript and are in agreement with its submission.

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