Biosignatures of ancient microbial life are present across the igneous crust of the Fennoscandian shield

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Earth’s crust contains a substantial proportion of global biomass, hosting microbial life up to several kilometers depth. Yet, knowledge of the evolution and extent of life in this environment remains elusive and patchy. Here we present isotopic, molecular and morphological signatures for deep ancient life in vein mineral specimens from mines distributed across the Precambrian Fennoscandian shield. Stable carbon isotopic signatures of calcite indicate microbial methanogenesis. In addition, sulfur isotope variability in pyrite, supported by stable carbon isotopic signatures of methyl-branched fatty acids, suggest subsequent bacterial sulfate reduction. Carbonate geochronology constrains the timing of these processes to the Cenozoic. We suggest that signatures of an ancient deep biosphere and long-term microbial activity are present throughout this shield. We suggest that microbes may have been active in the continental igneous crust over geological timescales, and that subsurface investigations may be valuable in the search for extra-terrestrial life.
The deep biosphere realm includes sediments and both sedimentary and igneous-rock environments\textsuperscript{1-2}, and is estimated to represent one-tenth to one-third of all live biomass\textsuperscript{3-4}. Considering the vastness of this biosphere, it is reasonable to assume that the microbial processes strongly affect the energy cycles of our planet, including production and consumption of greenhouse gases\textsuperscript{5}, but the extent is not yet known. Although the understanding of deep biosphere metabolisms has evolved over the recent years, the observations are still patchy. For the continental crust, this scarcity is generally owing to the small number of deep boreholes, underground observatories, and mines that allow and enable research dedicated to the deep biosphere.

Precambrian crystalline rock makes up the largest volumes of the continental crust, accounting for >70% of the total continental surface area and >80% of crust at depths below 2 km\textsuperscript{6}. Environmental conditions in this cryptic habitat shift rapidly to anoxic within the upper tens of meters\textsuperscript{7}. The majority of microorganisms living in deep fracture water therefore maintain an anaerobic lifestyle in the absence of sunlight and scarcity of organic carbon sources\textsuperscript{8}. Metabolic pathways include sulfate reduction, fermentation, acetogenesis, methanogenesis, and methanotrophy\textsuperscript{10-14}.

The deep biosphere is believed to host some of the most ancient evolutionary lineages\textsuperscript{2-15} and has been proposed to have held the majority of Earth’s live biomass prior to plant colonization of land ~400 Ma ago\textsuperscript{16}. This suggests that deep environments, including ocean floors, have dominated on Earth for most of life’s history. However, in similarity with live communities, knowledge of the extent and nature of ancient microbial activity in the deep continental crust is still scarce. Studies that address deep ancient life in the igneous continental crust by combining either isotopic, morphological, or molecular biosignatures with geochemistry only exist from a few Fennoscandian shield sites (Fig. 1, yellow symbols). These studies revealed deep microbial activity dating back to the Devonian, marked by isotopic signatures of vein minerals, as well as morphological support for ancient microbial activity\textsuperscript{17-23}. Morphological reports of putative prokaryotes and/or eukaryotes exist from basalt vesicles in South Africa\textsuperscript{34}, USA\textsuperscript{25}, Japan\textsuperscript{26}, and mines in Germany\textsuperscript{27}. From the oceanic igneous crust, examples include fossilized fungal communities at seamounts in the Pacific and Atlantic oceans\textsuperscript{28,29} and molecular biosignatures in carbonates of the Lost City hydrothermal field at the Iberia margin\textsuperscript{30}. In the Fennoscandian shield, the morphological evidence of ancient subsurface microbial life include chitin-bearing partly preserved and partly mineralized fungal hyphae\textsuperscript{31}, and putative prokaryotic cells preserved within calcite veins\textsuperscript{17}. It still remains unclear whether the biosignatures from the few sites described so far are anomalous or represent the bulk of the habitable continental igneous crust over geologic timescales.

Here we assess how widespread biosignatures of deep ancient life are in the continental igneous crust of the Fennoscandian shield by analyzing low-temperature mineral specimens containing euherdal calcite and pyrite in fractures and cavities, frequently in spatial relation to solid bitumen (altered crude oil, e.g., through devolatilization or biodegradation, ref. 32 and references therein), collected from 33 abandoned mines (as deep as 1400 m) in Paleoproterozoic rocks across the shield, dominantly in the Swedish part (Fig. 1, close up of the most frequently sampled Bergslagen area in Supplementary Fig. 1). A multi-method approach is applied, involving high spatial resolution analytical transects for both U-Pb geochronology and stable isotopes of carbon, oxygen, strontium, and sulfur across mineral grains. Additional isotope determinations are made on the bulk sample scale and within single crystals via ion imaging. The molecular composition of solid bitumen and biomarkers from the mineral coatings are determined, including the stable carbon isotope composition of specific organic compounds.

We show that biosignatures of ancient microbial life are omnipresent in the shield, confirming that previous scattered observations are not anomalous. Our multi-proxy study confirms that ancient microbial methanogenesis and sulfate reduction have been ubiquitous over geological time and temporal shifts between these dominating microbial metabolisms are determined. The documented preservation of biosignatures in mineral veins over geological timescales and establishment of the timing of these microbial processes sheds light not only on the evolution and extension of deep life over time on Earth but also has important implications for astrobiological exploration strategies\textsuperscript{33}.

**Results**

**Carbonate carbon system.** Euhedral calcite in the cavities and veins occurs in several generations as shown by zonation detected in crystal cross-sections (Fig. 2b, f) and secondary ion mass spectrometry (SIMS) analytical transects (Fig. 2c, g, summary in Supplementary Data 1, full data in Supplementary Data 2-4). In samples from Kallmora and Grängesberg, the zonation is particularly well established. The oldest calcite phase is of blocky habit and is intergrown with bitumen, and is thus related to bitumen emplacement in the fracture system (Figs. 2a and 3a, b). This calcite generation has relatively low $\delta^{13}$O values ($−18 \text{ to } -11\%_{o}$) and $\delta^{13}$C values of 0 to $2\%_{o}$. At Kallmora, the first phase after the bitumen precipitation is euherdal calcite with heavy $\delta^{13}$C values ($+5.5 \text{ to } +10.2\%_{o}$). Overgrowth I, OGI and with $\delta^{14}$O values of around $−4 \text{ to } 0.5\%_{o}$. In Kallmora sample \#18910193, this generation occupies the whole crystals (Fig. 2c), whereas in Kallmora \#19334861 and \#18910078, this phase is followed by several overgrowths: OGI: with positive $\delta^{13}$C (+1.2 to +10.2$\%_{o}$, Figs. 2g and 3e) and lighter $\delta^{14}$O (−8.4 to −7.3$\%_{o}$, similar for following generations); OGI: a thin zone with moderately negative $\delta^{13}$C values (−7.9 to −3.7$\%_{o}$) followed by micro-crystalline porous calcite overgrowths IV and V, with more negative $\delta^{13}$C values (−15.8 to −12.3$\%_{o}$). Aggregates of euherdal, cubic to tabular pyrite, are intergrown with the calcite OGI–OGV, which partly to completely covers the pyrite aggregates (Fig. 3c).

**Carbonate geochronology.** The growth zonation of the Kallmora samples described above was targeted for high spatial resolution U-Pb geochronology using laser ablation inductively coupled MS (LA–ICP-MS), in the same crystals that were targeted for SIMS microanalysis for C and O isotopes. OGI yielded an age of 51.1 ± 2.3 Ma (Fig. 2d and Supplementary Data 5 and 6) in sample \#18910193, where it makes up the whole crystals. Sample \#18910078 also features OGI, but although the two spots yielded overlapping ages of 34.9 ± 2.0 Ma and 30.0 ± 6.6 Ma, respectively (Figs. 2h and 3f, g). Growth zone OGI was too thin for a single age determination, but spots that overlap this zone in \#18910078 and \#19334681 are ca. 23 Ma and ca. 26 Ma, respectively, confirming persistent younging trends of each growth zone across these samples. Twenty-three spots for OGV–OGV yielded ages of
ranging from 18.9 to 14.3 Ma for sample #19334681, with no discernible sub-populations; the data imply that the growth zoning is higher resolution than the laser sampling spot (Fig. 3g and Supplementary Fig. 3). For sample #18910078, a similar age span is present for OGIV–OGV (21.2 to 13.6 Ma), but two groups with intercepts at 15 ± 2.9 and 13.7 ± 0.6 Ma are more clearly discernible for the main crystallization phases for this sample (Fig. 2h).

A complementary relative timing indicator is the $^{87}$Sr over $^{86}$Sr ratio. LA-multi collector (MC)-ICP-MS transects for Sr isotopes were made in corresponding spots as for C and O in the calcite crystals. The Kallmora sample #18920193 shows similar and overlapping values in the range 0.7262 ± 0.0013 ($n=4$), suggesting formation at a single event. The #18910078 sample that features several overgrowths and the OGI has overlapping $^{87}$Sr/$^{86}$Sr (0.7247 ± 0.0026) with the corresponding growth zone in #18920193. The later zones have overlapping values (OGII–OGIII: 0.7257 ± 0.0028, $n=6$), although generally turning into lower values in the outermost growth zones (OGIV–OGV, 0.7183–0.7249). A Nygruvan, Norberg sample (#19090180), shows C and O, and $^{87}$Sr/$^{86}$Sr composition in agreement with OGI of the Kallmora samples (0.72759 ± 0.00070). Calcite with heavy δ$^{13}$C values in several mines have $^{87}$Sr/$^{86}$Sr in the same range (Riddarhyttan, Grängesberg/Grängesberg-Malingsbo, $n=6$, 0.72787 ± 0.00065) as Kallmora and Nygruvan in Norberg.

**Sulfur system.** Pyrite was analyzed for δ$^{34}$S in 25 samples from 19 mines and had highly variable values, with an overall range between −39 and +147‰. The largest range in an individual sample and also within a single crystal was 127‰. Superheavy pyrite δ$^{34}$S values were detected at several sites, e.g., Kallmora (up to +147‰), Grängesberg (+98‰), Gräsborg (+86‰), Ställberg (+81‰), and Storstreck (+50‰). Transects of SIMS micro-analyses dominantly show a trend from lighter values in the interior and successively heavier in the outer growth zones. A noteworthy feature is that samples with superheavy pyrite do not contain any extremely depleted values (lightest part being in the range −13 to +38‰). The isotopically most diverse pyrite was from Kallmora and shows evolution of δ$^{34}$S from around +20–50‰ in the crystal interior towards heavier values with growth up to the maximum detected values of around +147‰. SIMS ion images show that the δ$^{34}$S increase is not steady and straightforward. Instead, the δ$^{34}$S shows dips and spikes that are petrographically related to growth zones (Fig. 4 and Supplementary Fig. 4). The crystals are terminated by a zone of lighter pyrite that approaches similar values as the core (+30–50‰). Bulk sample values for these Kallmora samples were +81.4 ± 1.1‰ and +81.3 ± 1.0‰.

**Organic signatures from calcites and bitumen.** Calcite from Kallmora (#18920193 and #19334861) and Stråssa (#19210370) was analyzed for trapped organic molecules (via gas chromatography–mass spectrometry; GC-MS). Calcite dissolution released robust above blank concentrations of unsaturated regular (C$_{16:0}$–C$_{24:0}$) and methyl-branched ($i$-C$_{17:0}$, $ai$-C$_{17:0}$, and $i$-C$_{19:0}$) fatty acids, as well as monounsaturated regular fatty acids.
Morphology. Complex microbial morphologies occur in samples from six sites, at variable degree of preservation. In the sample from Lund (#19360094), an interconnected network of filamentous structures are observed in relation to bitumen (Fig. 6a, c–e). The filaments are between 10 and 20 µm in diameter and over 100 µm in length. They branch frequently and anastomoses between branches occur. The transition from a biofilm to an interconnected network suggests that the film represent a biofilm from which the filaments protrude. The filaments are almost completely mineralized by clays with calcite and pyrite, which indicate the filaments are indigenous and not modern contaminants. In a few other samples from, e.g., Dannemora (#e22202), mineralized or partly mineralized filaments are observed. They are usually associated with, and protrude from, a carbonaceous film on the mineral surfaces. However, a lack of diagnostic and complex morphology makes interpretation and conclusions on biological affinity scarce. In sample #19920262 from Grängesberg, >1 mm-long curvi-linear filaments with a diameter of ~10 µm are associated with globular bitumen (Fig. 6g, h). The filaments grow from a biofilm at a pyrite crystal surface and micro-crystalline pyrite occur on their surface. The filaments are partly carbonaceous with a similar but slightly higher back-scatter intensity compared to the bitumen spheres, but no diagnostic morphology can be observed. At Riddarhyttan (#20180313), the bitumen film has longitudinal corrosion features (Fig. 6b, f). They occur either as randomly, or irregularly distributed, longitudinal textures of similar ~10 µm diameters.

Discussion

Isotopic evidence for deep ancient life across the Fennoscandian Shield. Isotope records of carbon in calcite (δ13C: −29 to +27‰) and sulfur in pyrite (δ34S −39 and +147‰) show a substantial variability that suggests microbial influence in a majority of the mines (Supplementary Data 1). The Kallmora samples, in similarity with most of the studied sites, show an initial precipitation of solid bitumen and calcite. This site is used as the main example for detailed isotopic interpretations. The oldest blocky calcite type has relatively low δ18O values (Fig. 2g) but no C isotope signature specific for methanogenesis or methane oxidation, in agreement with previous findings of blocky bitumen-related calcite at Forsmark, Sweden (Fig. 1)23. At Forsmark and also at Laxemar, the calcite of this type has fluid inclusion compositions that suggest formation from a 80–95 °C brine fluid and Rb-Sr geochronology of coeval calcite-K-feldspar indicates Devonian–Carboniferous precipitation22,23,34. This suggests that bitumen infiltrated the deep igneous-rock fracture

(C18:1, Fig. 5). Only a few other compounds were detected, but could not be identified due to low abundance and/or coelution (i.e., stayed in the fatty acid methyl ester fraction after column chromatography; see “Methods” section). C16:0 and C18:0 were most abundant in all calcite samples, with highest amounts in the Kallmora samples (ca. 4 p.p.m.; Supplementary Data 7, Supplementary Fig. 6). Kallmora #19334861 shows the highest concentrations of methyl-branched fatty acids. GC–combustion–isotope ratio MS (GC–C-IRMS) analysis of its purified fatty acid fraction revealed δ13C values of −29.3 ± 0.2‰ for C16:0, −41.0 ± 3.5‰ for C17:0, −38.6 ± 3.8‰ for C18:0, and −29.7 ± 0.3‰ for C19:0. The δ15N values of C16:0, C18:0, and C24:0 could not be determined due to coelution or low abundance.

Solid bitumen from Kallmora was analyzed with GC-MS (maltene fraction) and elemental analysis. The maltene fraction only contained an unresolved complex mixture (UCM, Supplementary Fig. 5), which could not be characterized further. The solid bitumen has a bulk δ13C of −30‰ and a carbon content of 79 wt%.
networks at tectonic events when temperatures were elevated in the sedimentary rock pile due to thickening of a Caledonian foreland basin. These elevated temperatures caused bitumen expulsion from organic-rich black shales of various types (e.g., lower Cambrian alum shale and Silurian Fjäcka shale). The bitumen at Kallmora and at other study sites suggest that bitumen expulsion from overlying (but presently largely eroded) sedimentary rocks was ubiquitous in the deep igneous-rock fracture network of the Fennoscandian shield during post Caledonian foreland basin development. We focus the following discussion on biosignatures in mineral assemblages that succeed the bitumen emplacement, in particular to calcite overgrowths that show highly variable δ^{13}C values between different growth zones, pointing toward shifts in microbial metabolisms.

Isotopic evidence for microbial methanogenesis. The first stage of euhedral calcite overgrowths has δ^{13}C_{calcite} of up to +10.2‰ at Kallmora and +27‰ elsewhere. δ^{13}C-rich residual CO2 is a commonly used diagnostic marker for the formation of secondary methane from biodegradation of seep oils and petroleum, owing to the discrimination that occurs against δ^{13}C during methanogenesis. The significant δ^{13}C-enrichment observed in OG1–2 calcite at Kallmora is therefore proposed to reflect calcite formation following secondary microbial methane formation in situ. Microbial methanogenesis is commonly linked to sulfate-poor biodegraded petroleum reservoirs. It may involve initial biodegradation steps of fermentation of the expelled hydrocarbons (bitumen/seep oil) leading to the formation of, e.g., acetic acid, H2 and CO2, which can fuel...
methanogenesis, but as bitumen hydrocarbons are fully saturated, an oxidant may be required, potentially in the form of sulfate reducers oxidizing the organic carbon. These bitumen biodegradation pathways were proposed for formation of microbial methane and $^{13}$C-rich CO$_2$, and authigenic calcite in deep basement fractures of the Siljan impact structure in Sweden. Studies of deep granite fracture networks in the UK have also suggested that the presence of solid bitumen/seep oils may have provided an energy source for in situ microbial activity. Large C isotope fractionation and, consequently, production of a $^{13}$C-rich residual CO$_2$ is common for the CO$_2$ reduction methanogenesis pathway, in contrast to the acetate fermentation pathway, which suggests that the methanogens in the studied mines utilized the carbonate reduction pathway.

The timing of methanogenesis is assigned directly by the authigenic $^{13}$C-rich calcite formation ages derived from the in situ U-Pb dating and gives a 51 ± 2 Ma age for the first $^{18}$O-rich calcite stage (OGI) and a 35–30 Ma age range for the following stage (OGII) (Fig. 2). The shift in $^{18}$O of the calcite indicates that methanogens were active in fluids of two distinctly different origins, or at various temperatures, as O isotope fractionation between calcite and water is highly temperature sensitive. These ages overlap with ages of methanogenesis-related calcite from Siljan and to discrete, supposedly Alpine-related reactivation events of platform sediment fractures in Sweden, derived by slickenfibre U-Pb geochronology.

Isotopic evidence for microbial sulfate reduction. Later calcite overgrowths at Kallmora, in particular OG4–5, which feature $^{13}$C-depleted signatures (−15.8‰), indicate that methanogenesis ceased to dominate in the fracture system. Instead, the $^{13}$C-depleted signatures suggest organotrophy. At this stage, pyrite started to precipitate. Pyrite forming due to microbial
sulfate reduction (MSR) inherits the S isotopic composition of the hydrogen sulfide\(^{48}\). As the MSR metabolism produces hydrogen sulfide strongly depleted in \(\delta^{34}S\), \(\delta^{34}S_{\text{pyrite}}\) serves as a marker for MSR, whereas heavy values commonly develop in systems undergoing Rayleigh isotope fractionation\(^{49}\). In several of the mines, the \(\delta^{34}S_{\text{pyrite}}\) values are significantly depleted, down to \(-39\%\). The initial sulfate \(\delta^{34}S\) values are unknown but similar settings (Laxemar and Forsmark, Sweden) have been shown to hold \(\delta^{34}S\) values in the +16–27\% range for sulfate minerals and dissolved sulfate in the fracture systems\(^{50}\). This implies a maximum \(34\varepsilon\) value (\(34\varepsilon = \delta^{34}S_{\text{SO}_4} - \delta^{34}S_{\text{pyrite}}\)) of 55–66\%, in line with MSR\(^{51,52}\), and rules out thermochemical sulfate reduction (TSR), which produces \(34\varepsilon\) of up to 22\%, but usually much less\(^{53}\). Low minimum \(\delta^{34}S_{\text{pyrite}}\) values implying apparent MSR-related \(34\varepsilon\) values occur in 10 of 19 mines analyzed for \(\delta^{34}S_{\text{pyrite}}\). The S isotope composition of the bitumen is not known, but sulfur isotope determinations of solid bitumen from other areas point to similar \(\delta^{34}S\) values of this material as the anticipated initial sulfate described above, c.f. the Big Piney–La Barge oil and gas field in Wyoming (+18.9 ± 3.9\%)\(^{54}\), and the Puguang and Yuanba gas fields (+12.0 to +34.2\%) of the Sichuan Basin, China\(^{55}\).

For the Kallmora samples, the minimum \(\delta^{34}S_{\text{pyrite}}\) values are relatively heavy, +18.4\%, and a complex evolution towards heavier values is evident (Fig. 4 and Supplementary Fig. 4). After the peak in values at +147\%, the values drop towards the rim. To achieve superheavy \(\delta^{34}S_{\text{pyrite}}\) values, Rayleigh reservoir effects in semi-closed systems are generally needed\(^{56,57}\). These effects arise when MSR occurs at rates higher than the supply by advection and diffusion such that the dissolved sulfate pool shrinks, whereby the \(\delta^{34}S\) increases. However, if there are large relative quantities of superheavy pyrite formed, there is an obvious mass balance problem that cannot be explained by a simple Rayleigh

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**Fig. 6 Filaments and degraded bitumen.** a Photograph and c–e BSE-SEM images of mineralized filaments in a mycelium-like network on bitumen, adjacent to calcite (and younger calcite exist on the filaments as well, d). Branching is frequent and anastomoses between branches occur occasionally (e). The morphology, size, and occurrence indicate a fossilized fungal mycelium. The complete mineralization and presence of pyrite (d) on the filaments further indicate the mycelium to be an indigenous feature and not a modern contaminant. b Photograph and f BSE-SEM image of corrosion textures in bitumen on calcite from Riddarhyttan (#201803013). g Photograph and h BSE-SEM image of a curvi-linear filament growing from a biofilm associated with pyrite and solid bitumen, on a fracture surface with an older quartz coating and calcite, from Grängesberg (#19670390). Fine-grained pyrite (BSE-bright spots at arrow) occurs on the filaments. Photographs include mm-marked ruler for scale.
fractionation cycle, as isotopically heavy pyrite would only form in the very last portion of closed system MSR\(^{35}\). In addition, if all sulfate is consumed, the mean \(\delta^{34}S\) of all pyrite formed should be equal to the \(\delta^{34}S\) of the initial sulfate. At Kallmora, the bulk values of aliquots of several ground pyrite crystals are at \(+81 \pm 1\%o\). This means that the \(\delta^{34}S\) of the sulfate was already significantly affected by MSR before reaching the investigated cavities. The relatively heavy inner part of the crystals (+18\%o) is also indicative of an onset of pyrite precipitation from a \(\delta^{34}S\)-enriched initial sulfate, which, if a \(34\%\) of 55–66\%o is assigned, gives an initial \(\delta^{32}S\) of 73–84\%o, which overlaps with the bulk pyrite at +81\%o. The evolution towards the heaviest value in the crystal is not straightforward, but features dips that may be due to fluid inflow and mixing events. Similarly, the decreasing \(\delta^{34}S\) pyrite values in the outermost overgrowths can be due to dilution of the \(\delta^{34}S\)-enriched sulfate pool by infiltrating fluids. Superheavy SIMS values for samples from Storstreck and Grängesberg are also associated with significantly \(\delta^{34}S\)-enriched bulk values (Supplementary Data 1).

Further environmental support for microbial origin for the superheavy \(\delta^{34}S\) values is provided by the documented low-temperature conditions at the time. The U-Pb geochronology shows the pyrite-calcite assemblage at Kallmora formed at 19–13 Ma (Fig. 2). Thermochronological investigations of the south and central Fennoscandian shield show that the temperatures of the investigated crustal level experienced a maximum of 50–70 °C during the time of calcite–pyrite precipitation\(^{35,38}\). These temperatures are much lower than those needed for TSR (>100 °C)\(^{59}\), which can thus be ruled out as a pathway for pyrite formation. Taken together, the isotopic signatures detected, together with previously documented MSR-related pyrite \(\delta^{34}S\) values from Olkiluoto, Finland\(^{60}\), suggest widespread ancient MSR in the deep fracture networks of the Fennoscandian shield.

Molecular evidence for deep ancient life. The UCM detected in the solid bitumen extract of Kallmora #19334861 together with the absence of \(n\)-alkanes in that material (Supplementary Fig. 5) is a typical sign of biodegradation\(^{41,61}\) and therefore provides further evidence for microbial utilization in situ of the primary expelled hydrocarbons of proposed shale origin. In addition, methyl-branched (\(i-C_{17:0}\), \(i-C_{17:0}\), and \(i-C_{18:0}\)) and straight-chained (especially \(C_{16:0}\), \(C_{17:0}\), and \(C_{18:0}\)) fatty acids were preserved within the calcite coating in paragenesis with pyrite (Fig. 5). \(C_{16:0}\) and \(C_{18:0}\) are common in many organisms, whereas \(C_{17:0}\) and the methyl-branched fatty acids can be used as biomarkers for sulfate reducing bacteria (SRB)\(^{62–64}\), especially in combination with their \(\delta^{13}C\) signature. \(a\)-\(C_{17:0}\) and \(C_{17:0}\) are depleted in \(\delta^{13}C\) compared to co-occurring solid bitumen by 9–11\% (−41 and −39\% in the host calcite (Fig. 2). SRB biomarkers are less abundant in Kallmora #18921093 (Supplementary Fig. 6), which only includes the methanogenesis-related \(\delta^{34}S\)–calcite values. It is therefore underlined that MSR caused the superheavy \(\delta^{34}S\) values in pyrites of Kallmora #19334861. This secondary biogenicity evidence is crucial, as there have been reports from the sedimentary record that superheavy pyrite may form also from TSR\(^{65}\).

Morphological evidence for deep ancient life. As most of the samples are not newly retrieved from the mines, focus is on completely mineralized morphological remains of microbial communities, interpreted as indigenous. The remnants of completely mineralized interconnected networks of filamentous structures with anastomosing and branching filaments occurring in spatial relation to bitumen in a sample from Lund (Fig. 6c–e) resemble in morphology, size, mineralization, and occurrence of previously described endolithic fungal fossils reported from both the Fennoscandian shield\(^{31,66}\) and from the ocean floor\(^{67}\). Desiccation of biofilms can sometimes result in patterns of inter-connected ridges with filamentous-like appearances. However, these desiccation features are the result of biofilm contraction with non-circular ridges of highly variable diameters and with a topped morphology. In contrast, the described microstructures are distinct filaments with circular and even diameters throughout their lengths (Fig. 6c), and hence in favor of a microbial interpretation over an abiotic explanation. Without supporting organic data or biomarkers such as chitin, it is difficult to assign a certain biological affinity to the purported biofilms. However, the frequent anastomoses between branches (Fig. 6c, e) exclude a prokaryotic interpretation of the fossils and favors of a fungal interpretation\(^{66,67}\). The partly carbonaceous filamentous structures from Grängesberg (Fig. 6h) also correspond in size and occurrence to previously described chitin-bearing fungal hyphae from the Fennoscandian shield\(^{31}\). Similar, partly carbonaceous preservation has been seen in several fungal fossils previously\(^{66}\). Lack of further diagnostic morphology makes a final fungal conclusion frail, although the behavior of potential hyphae reaching between the bitumen spheres suggests a trophic exploration.

The longitudinal shape with coherent diameter and rounded tip of the corrosion structures suggest that they could be produced by microorganisms (Fig. 6f), in particular an organism with grazing behavior as the shape with a rounded tip could correspond to known morphology of fungal hyphae. However, a biological explanation cannot satisfactorily be supported based solely on the morphology.

A temporal shift in metabolisms. Spatially coupled analytical spots for stable isotopes and geochronology within zoned calcite–pyrite assemblages provide distinction of the shift from methanogenesis at ca. 50–30 Ma to sulfate reduction at 19–13 Ma. The onset of the methanogenesis stage may be related to far-field fracture reactivation during the Pyrenean–Alpine orogeny that led to infiltration of microbial communities with descending surficial waters, at temperatures and salinities that were more suitable for microbial colonization than the previous bitumen infiltration stages\(^{21}\). Similar Sr isotope composition of the \(13C\)-rich calcite samples in Bergslagen points to a regional infiltration event during the Cenozoic methanogenesis event. The Sr isotope values are, however, much higher than contemporaneous seawater values (0.7075–0.7090\(^{68}\)), which means that water–rock interaction along the fracture flow paths has a large control on the Sr isotope values of the deep fluids. This is in agreement with findings at the tunnel system Äspö, Sweden, where Baltic seawater infiltrated to several hundred meters deep rapidly inherits more radiogenic Sr compositions due to ion-exchange along the flow path\(^{69}\). Although methanogens and SRB can coexist, SRB usually successively outcompete methanogens for substrates as sulfate concentrations elevate\(^{70}\). The BSR stage is thus proposed to have occurred when a sulfate-rich water infiltrated the fracture system. At this stage, BSR exhausted the sulfate pool to a degree that led to precipitation of substantially \(34S\)-rich pyrite (Fig. 4). The BSR stage is temporally related to subsidence due to burial beneath Upper Cretaceous to Oligocene sediments, at least in the southern parts of Fennoscandia\(^{38}\). Fracture reactivation during an early Miocene uplift that affected the craton\(^{38}\) may have introduced sulfate-rich basal fluids to the deeper igneous fracture system and shifted the metabolisms to dominantly BSR. At Grängesberg, the shift from methanogenesis to BSR is associated
with a shift in $\delta^{18}O_{\text{calcite}}$ to higher values, which indicates a change in fluid origin and/or temperature (Supplementary Fig. 2), and at Kallmora, the outer calcite overgrowths have less radiogenic Sr isotopic composition, also in agreement with a new fluid infiltration event. Overall, the signs of microbial activity that are preserved in the mineral record seem to reflect separate episodic events. The U-Pb dating shows that the events that led to calcite precipitation can last for up to ~6–8 Myr (Figs. 2h and 3g, and Supplementary Fig. 3). The determined ages of microbial processes are >1.5 Gyr younger than the host rock, which suggests that the environmental conditions have only been favorable for subsurface life during late Phanerzoic in the Fennoscandian shield, in agreement with thermochronological data of the host rocks and previous timing constraints of ancient microbial life in this shield, e.g., in the Siljan impact crater. The determined ages are, however, from a time when land plants already had colonized the continents and can therefore not be used to test the hypothesis of whether life developed in the deep biosphere.

Omnipresence of biosignatures and their implications. The findings of pervasive $^{13}$C-rich calcite confirm previous hypotheses of widespread long-term microbial methane formation in the vast environment that the igneous upper continental crust represents; however, these hypotheses were hitherto only based on biosignatures from two sites in Sweden and on live communities of methanogens/methanotrophs from South Africa, Finland, Sweden, and Japan. In particular, but not exclusively, the methanogenesis signatures are spatially related to seep oils and bitumen expelled from overlying sedimentary successions into the igneous basement fracture systems, to depths of almost 1 km. The widespread methanogenesis signatures are of particular interest, owing to that methane is a very potent greenhouse gas if released to the atmosphere. Furthermore, $^{13}$C-depleted calcite that is indicative for incorporation of C originating from microbial decomposition of organic matter, and potentially methane, occurs in nine additional mines. For $\delta^{34}S_{\text{pyrite}}$, there is substantial variability and superheavy values at several of the investigated sites. A majority of the mines show $\delta^{34}S_{\text{pyrite}}$ values indicative for BSR, of which most feature superheavy $\delta^{34}S_{\text{pyrite}}$ values (i.e., $>$anticipated initial $\delta^{34}S_{\text{SO}_4}$).

From the marine sedimentary record, superheavy pyrite has gained interest, as it may be a proxy for oxygen levels in the oceans and atmosphere, and more locally for the sulfate–methane transition zone. Our results show, in similarity to findings from Laxemar and Forsmark, no correlation between superheavy $\delta^{34}S_{\text{pyrite}}$ and AOM-related $\delta^{13}C_{\text{calcite}}$ values. Our nine new sites with superheavy pyrite determinations are in agreement with previously documented Swedish sites Laxemar ($\delta^{34}S_{\text{pyrite}}$: −54 to +132‰), Forsmark ($–42$ to $+67$‰) and Siljan ($–42$ to $+78$‰), as well as Olkiluoto in Finland ($–50$ to $+82$‰). Large $\delta^{34}S_{\text{pyrite}}$ variability, including superheavy values, thus appears to be a widespread biosignature for deep, ancient rock-hosted BSR in the Fennoscandian shield, a metabolic pathway also indicated by calcite-trapped fatty acid biomarkers and their compound-specific C isotope signatures (Fig. 5), relative to the potential substrate (bitumen).

It has been hypothesized that microbial activity in the continental igneous crust has been widespread during Earth’s history, but direct evidence for this omnipresence is scarce. Our approach using a comprehensive set of subsurface mineral samples collected from 33 mines across the Fennoscandian shield reveals omnipresent biosignatures of deep ancient life across this craton, particularly in the central part where most the samples in this study are from. There are also isotopic signatures for MSR in pyrite from the eastern part of the Fennoscandian shield (Finland). Although no timing constraints have been presented for the Finnish biosignatures, they indicate that the eastern part of the shield has been colonized in similar manner as the investigated area of the current study. Taken together, the findings are complementary to previous scattered investigations of deep ancient and active microbial life from scientific boreholes and underground facilities on land and in caves and suggest that life in the continental igneous crust is not an anomaly in time and space. This supports the idea of large-scale long-term microbial activity in the continental igneous crust.

It is, however, important to note that heating of organic-rich source rocks and migration of the expressed oils (bitumen) have been required to create many of the features documented in this study.

Furthermore, the findings show that diverse biosignatures of deep ancient microbial activity are preserved within mineral coatings over millions of years. Visualization of the time evolution in $\delta^{13}C$ and $\delta^{34}S$ enables to trace temporal shifts in dominating metabolisms. Preserved organic molecules and their compound-specific stable C isotope compositions in mineral coatings provide further line of evidence for biogenicity. Strategies for Mars exploration tackling life (ancient and recent) at depth have been suggested as favorable over search in surficial environments, mainly owing to high radiation that destroys organic remains of life rapidly. The omnipresence of terrestrial biosignatures from deep ancient life in calcite and pyrite veins described in this study further emphasizes the importance of subsurface investigations in the search for extraterrestrial life.

Methods

Materials. A total of 49 mineral samples were examined from 33 open pit and underground mines from across the Fennoscandian shield (Fig. 1, including a few samples from quarries and excavations). The samples are dominantly from Paleoproterozoic host rocks, spanning from the Kiruna region in the north to southern Sweden, mainly from the Bergslagen mining area in central Sweden (Supplementary Fig. 1). This area hosts numerous base metal sulfide deposits occurring in volcanic ash interbedded with, e.g., marbles, and banded iron formations. The succession of supracrustal rocks form inliers bound by plutonic and granitic rocks intruded at 1.9–1.8 Ga and shear zones. Low-temperature euhedral druse mineral assemblages of clay minerals, pyrite, calcite, zeolites, and solid bitumen occur in late-stage cavities and veins of the reactivated ore and shear zones, and are the focus of the current study. The host rock of each mine is listed in Supplementary Data 1. Most of the mineral samples were originally collected during mining operations in deep mines that are no longer accessible. Therefore, they represent a unique widespread sample record of the subsurface fracture systems. Many of the sample specimens belong to the collection of the Fennoscandian Institute of Natural History, Stockholm, Sweden, except for the samples from accessible underground mines at Garpenberg and Dannemora, which were curated and provided by mining geologists.

The mineralogy and appearance of the fracture coating specimens were examined using a Hitachi S-3400N scanning electron microscope (SEM) equipped with an integrated energy dispersive spectrometer (EDS) system and a environmental SEM with a FEI QUANTA FEG 650 (ThermoFisher Scientific, USA) fitted with an Oxford X-Max 80 mm$^2$ EDS detector (Oxford Instruments, UK). The analyses were performed in low vacuum to minimize surficial charging effects. This enables the use of uncoated samples and, thus, EDS analyses of the C content. The acceleration voltage was 20 or 15 kV depending on the nature of the sample and the instrument was calibrated with a cobalt standard. Peak and element analyses were done using INCA Suite 4.11 software and normalized to 100 wt%.

Element mapping was done using Aztec software. The calcite and pyrite crystals were hand-picked under the microscope for stable isotope analysis and geochronology, and solid bitumen aggregates were hand-picked for biomarker analysis.

Secondary ion mass spectrometry. Calcite and pyrite crystals were mounted in epoxy, polished to expose cross-sections, and examined with SEM to trace zonation and impurities prior to SIMS analysis. SIMS analysis (10 μm lateral beam dimension, 1–2 μm depth dimension) of carbon and oxygen isotopes in calcite and sulfur isotopes in pyrite was performed on a CAMECA IMS1280 ion microprobe following the analytical settings and tuning reported previously with some differences; O was measured on two Faraday cups (FCs) at mass resolution 2500, whereas C used an FC/electron multiplier (EM) combination, with mass resolution 1500. High-13C peak and 4000 ppm on the 13C peak to resolve it from 12CH4. The magnetic field was locked at the beginning of the session using an nuclear magnetic resonance field sensor. Secondary ion signals for $^{34}S$ and $^{32}S$ were detected.
Simultaneously using two Faraday detectors with a common mass resolution of 4860 (M/ΔM). Data were normalized for instrumental mass fractionation (IMF) using the deuterium, a standard material, and measured after the sample mounts and analyzed after every sixth sample analysis. Analyses were performed in automated sequences, with each analysis comprising a 70 s pre-sputter to remove the gold coating, centering of the secondary beam in the field aperture to correct for small variations in surface relief and deadtime of 44 ns. For each image, 200 cycles were measured, each analysis taking ca. 44 min that included a 10 min pre-sputter over an area of 150 × 150 μm. Results are reported as δ13C and δ18O based on the Pee Dee Belemnite (V-PDB) reference value, and pyrite results are reported as δ34S based on the Canon Diablo Troilite (V-CDT) reference value. Data were normalized for IMF using matrix-matched reference material mounts together with the sample mounts and analyzed after every sixth sample analysis. The calcite reference material S0161, from a granulite facies marble in the Adirondack Mountains, was kindly provided by R.A. Stern (University of Alberta). The values used for IMF correction were determined by conventional stable isotope MS at Stockholm University on ten separate pieces, in duplicate, with seven runs of each in a 4 s integration cycle. Results are reported as δ13C and δ18O.

Sulfur isotopes in pyrite samples (n = 7) were determined by MC-ICP-MS at the Vaggecenter, Swedish Museum of Natural History, Stockholm University. Sample analysis, using solutions from 2.1996 and #19334861 was performed with the same CAMECA IMS1280 instrument, utilizing a 20 kV incident energy, critically focused Ca+ primary beam rastered over an area of 140 × 140 μm, following ref. 2. The primary beam current of 20 pA correspond to a spatial resolution of <1 μm. Secondary ions were processed through a dynamic transfer optical system, a secondary beam rastered before the entrance slit to the mass spectrometer that steers the secondary beam back onto the ion optical axis of the instrument, to preserve its ability to generate flat-topped peaks at high mass resolution. The ion image acquisition software then reconstructs a given count to the correct emission point within the primary rastered beam. The secondary beam raster was operated in peak-hopping mode at a mass resolution of 4500, sufficient to resolve 33S from 34SH, and the two species 34S and 35S were measured in an ion-counting EM with an electronically gated detection system. Processing assuming only mass-dependent fractionation. Raw ion images were processed using the Winimagne2 software, using previously determined δ34S values from spot analyses to normalize the images to true values. Line scans were generated in the Winimagne2 software using a 5 μm square box. The typical uncertainty on δ34S in the line scans was ±3‰ (1σ, compared to ±0.1‰ from the spot analysis).

**Bulk sample S isotope analysis.** Sulfur isotopes in pyrite samples (n = 7) were determined by MC-ICP-MS analysis at the Vaggecenter, Swedish Museum of Natural History, Stockholm University. Sample analysis, using solutions from 2.1996 and #19334861 was performed with the same CAMECA IMS1280 instrument, utilizing a 20 kV incident energy, critically focused Ca+ primary beam rastered over an area of 140 × 140 μm, following ref. 2. The primary beam current of 20 pA correspond to a spatial resolution of <1 μm. Secondary ions were processed through a dynamic transfer optical system, a secondary beam rastered before the entrance slit to the mass spectrometer that steers the secondary beam back onto the ion optical axis of the instrument, to preserve its ability to generate flat-topped peaks at high mass resolution. The ion image acquisition software then reconstructs a given count to the correct emission point within the primary rastered beam. The secondary beam raster was operated in peak-hopping mode at a mass resolution of 4500, sufficient to resolve 33S from 34SH, and the two species 34S and 35S were measured in an ion-counting EM with an electronically gated detection system. Processing assuming only mass-dependent fractionation. Raw ion images were processed using the Winimagne2 software, using previously determined δ34S values from spot analyses to normalize the images to true values. Line scans were generated in the Winimagne2 software using a 5 μm square box. The typical uncertainty on δ34S in the line scans was ±3‰ (1σ, compared to ±0.1‰ from the spot analysis).

**Sulfur isotope ratios of the solutions were determined using a Nu Plasma II MC-ICP-MS and an Aridus II for sample introduction. A resolving power of ~10 000 was achieved with a 2 s dwell time. The analysis of the isotopic ratio isobaric interferences and presents an interference-free plateau for precise measurements. We confirmed previous observations27 that the addition of Na to the samples increases the measured sensitivity with a preferred rate of 1.5 to 3 for Na2SO4. The setup resulted in a sensitivity of ~5.5 V/pFm for 26S, for solutions measured at 1.3 p.p.m. sulfur and 2 p.p.m. sodium. The sample uptake rate was ~100 μl/min, which resulted in ~600 μl sample consumption per analysis. Fractionation correction was performed by standard-sample bracketing with the IAEA-S-1 standard (δ34S = −0.35%o). An on-mass correction was applied at the beginning of the acquisition. The setup consisted of a pure 0.3 M HNO3 to account for instrumental sulfur background. The measured background was in the range of 80–100 mV for 26S, which accounts for ~1% of the sample intensity. Each sample was measured three times in a row, in two separate analytical sessions. Results are presented as δ34S normalized to the V-CDT scale. For quality control, reference solutions IAEA-S-4 (Soufre de Laca) and CTG39 were run repeatedly and treated as unknowns. The resulting δ34S, CDI of 16.37 ± 0.67‰ (n = 19) and 20.92 ± 0.94‰ (n = 9), respectively, overlap with their corresponding literature values (16.90 ± 0.24‰ and 20.99‰). All samples and standards plot on a mass-dependent fractionation line between δ34S and δ18O with a slope of 0.515 (Supplementary Data 4), which indicates that isotopic interferences are properly corrected for.
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Author contributions
H.D. conceived the study. H.D. carried out SEM investigations, sample preparation, and microscopy of the mineral assemblages. H.D. and M.I. did microscopy and staining of filaments. N.M.W.R. carried out U-Pb carbonate geochronology. M.W. and H.D. carried out SIMS analysis. M.R. carried out GC-MS investigations and extraction. A.K. carried out XRD. E.K. and M.K.-S. handled (LA)-MC-ICP-MS. H.D. wrote the initial draft and N.M.W.R., M.R., and M.I. provided constructive edits to the text.

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