

Editorial

# Tracking the Deep Biosphere through Time

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**Abstract:** The oceanic and continental lithosphere constitutes Earth's largest microbial habitat, yet it is scarcely investigated and not well understood. The physical and chemical properties here are distinctly different from the overlaying soils and the hydrosphere, which greatly impact the microbial communities and associated geobiological and geochemical processes. Fluid–rock interactions are key processes for microbial colonization and persistence in a nutrient-poor and extreme environment. Investigations during recent years have spotted microbial processes, stable isotope variations, and species that are unique to the subsurface crust. Recent advances in geochronology have enabled the direct dating of minerals formed in response to microbial activity, which in turn have led to an increased understanding of the evolution of the deep biosphere in (deep) time. Similarly, the preservation of isotopic signatures, as well as organic compounds within fossilized micro-colonies or related mineral assemblages in voids, cements, and fractures/veins in the upper crust, provides an archive that can be tapped for knowledge about ancient microbial activity, including both prokaryotic and eukaryotic life. This knowledge sheds light on how lifeforms have evolved in the energy-poor subsurface, but also contributes to the understanding of the boundaries of life on Earth, of early life when the surface was not habitable, and of the preservation of signatures of ancient life, which may have astrobiological implications. The Special Issue “Tracking the Deep Biosphere through Time” presents a collection of scientific contributions that provide a sample of forefront research in this field. The contributions involve a range of case studies of deep ancient life in continental and oceanic settings, of microbial diversity in sub-seafloor environments, of isolation of calcifying bacteria as well as reviews of clay mineralization of fungal biofilms and of the carbon isotope records of the deep biosphere.

**Keywords:** deep biosphere; geobiology; deep time; geochronology; microorganisms; evolution

## 1. Introduction

The deep biosphere is estimated to represent a significant portion of all live biomass on Earth [1,2]. Its realm ranges downwards into sediments, sedimentary and igneous rock [3–5]. The microbial communities here include mainly prokaryotes (bacteria and archaea) [6] but findings of eukaryotes such as active and fossilized fungi [7–15], as well as live and indigenous nematodes [16], have also been reported at great depth. The strict energy limitation, absence of sunlight and dominantly anoxic conditions of these systems make them different to other ecosystems on Earth in terms of metabolic flexibility and recycling [4,17–22]. The deep biosphere is the second largest reservoir of live biomass today, only surpassed by land plants. However, it has been put forward that most of Earth's live biomass (~80%) was to be found in the deep biosphere prior to plant colonization of land [23]. The deep

biosphere may thus have played a crucial role in the early evolution of both prokaryotes and eukaryotes, and dominated life on Earth for most of the planet's history. Yet, in addition to being an understudied environment in general when it comes to active communities, research on the deep time perspective of the deep biosphere has been particularly neglected. Microbiological and geobiological studies of the deep biosphere have, however, been the subject of increased interest in the last decade [10,18,24,25]. Similarly, the environmental conditions of these deep and more or less isolated settings have gained more attention lately [26]. By initiating the Special Issue, "Tracking the Deep Biosphere through Time", we aim to gather a collection of scientific contributions that provide a sample of forefront research in this emerging field of research. The contributions range from detection of ancient biosignatures in continental and oceanic settings, to microbial diversity in sub-seafloor environments and to isolation of calcifying bacteria. Two articles timely review clay mineral fossilization of fungal biofilms and the carbon isotope records of the deep biosphere.

## 2. An Overview of the Special Issue and the Contributions

A total of seven articles were published within this Special Issue; of which, five are research articles and two are reviews. A short summary for each contribution is given below.

Tillberg et al. [27] re-evaluate previously described fungal fossils [13] from the Lockne meteorite crater (age: 458 Ma), Sweden, by providing new in-situ Rb/Sr geochronological constraints of secondary calcite–albite–feldspar mineralization in veins and cavities of the impact structure, together with the fungal fossils. This new geochronological technique has shown to be feasible for dating complex discretely zoned mineral assemblages in fractured rock [28–32] and yielded a  $357 \pm 7$  Ma age for the Lockne mineral assemblage. The study concludes that fungal colonization took place at least 100 Myr after the impact event, thus long after the impact-induced hydrothermal activity ceased. Microscale stable isotope data of  $^{13}\text{C}$ -enriched calcite are also presented and revealed that microbial methanogenesis had occurred within the fracture system as well. Thus, the Lockne fungal fossils are not related to the impact event but represent later colonization of fractures formed by the impact.

In a study from the deep borehole, COSC-1, drilled into the Silurian–Devonian Scandinavian Caledonide mountain range in Central Sweden, Drake et al. [33] present isotopic and geochronological constraints for ancient microbial life in deep fractures and micro-karst formations in the nappe system. Micro-karsts at 122–178 m depth featured several generations of secondary calcite and pyrite growth. The younger of these precipitation phases showed  $^{34}\text{S}$ -depleted  $\delta^{34}\text{S}_{\text{pyrite}}$  values consistent with microbial sulfate reduction in situ. Laser ablation inductively coupled mass spectrometry carbonate U–Pb geochronology [34] was applied to the late stage calcite of this assemblage and gave two separate ages ( $9.6 \pm 1.3$  Ma and  $2.5 \pm 0.2$  Ma). These ages mark fluid circulation, related bacterial activity and mineral precipitation, following karst formation. The results show that the combined high spatial-resolution stable isotope and geochronology approach is suitable for characterizing paleo-fluid flow in micro-karst in nappe units.

Carlsson et al. [35] describe filamentous fossils interpreted as fungal remains from the Troodos ophiolite (91 Ma), Cyprus. Mineralogy and fluid inclusion studies show that the microorganisms lived in the system while the volcanism-related hydrothermal activity still was active in the seafloor, thus before the crust was emplaced on land. The paper highlights the importance of detailed geochemical investigations associated with descriptions of potential microfossils in the deep subsurface. Reconstructing the past habitats, fluid regimes and geochemical conditions are key in understanding fossilization and addressing biogenicity criteria.

Martino et al. [36] explore the microbial community of the sub-seafloor sediments obtained during IODP (International Ocean Discovery Program) Expedition 334 at Costa Rica Margin. The correlation assessment between microbial taxa (Bacteria, Archaea and Eukaryotes) and environmental chemistry surprisingly diverge despite their geographical proximity, similar lithology and similar pore water chemistry. The major taxonomic lineages identified have also been found in sub-seafloor environments worldwide. Up to now, knowledge about habitat preferences and metabolic specializations is rather

low regarding this environment and therefore variations in their relative abundance are just partly explained. However, this study also shows that contamination issues for such subsurface drilling projects seem well manageable and therefore should not hinder further necessary research.

Cacchio and Gallo [37] provide a methodical study describing calcium-carbonate precipitation processes in bacterial strains isolated from the rhizosphere. In contrast to bacterial carbonate precipitation in speleothems, the biomineralization potential in rhizosphere soils is understudied. Assessing the carbonate precipitation in the original environment and in enrichment cultures, this is the first study to show the biomineralization ability of the bacterial strains *Vibrio tubiashii*, *Clavibacter agropyri* (*Corynebacterium*), *Corynebacterium urealyticum*, *Sanguibacter suarezii*, *Sphingomonas sanguinis*, and *Pseudomonas syringae*, which are commonly not related to mineral precipitation. Initial mineral precipitation was induced in direct vicinity of the bacterial cells and thus attributable to the metabolic activity of the cells. Besides presenting well adapted and versatile subsurface organisms, the authors highlight methodical approaches for efficient and cost-optimized microbial community analysis and rapid screenings.

Sallstedt et al. [38] review the current data on clay mineralization by fossil fungal biofilms from oceanic and continental subsurface igneous rock. The aim of the review is to compare the nature of subsurface fungal clays from different igneous settings to evaluate the importance of host rock and ambient redox conditions for clay speciation related to fossilization of microorganisms. Montmorillonite-like smectite is the dominant clay involved in subsurface microbial fossilization, independent of host rock type and geochemical regime. The paper highlights the role of fungal clay authigenesis in the cycling of elements between host rock, ocean and precipitation of secondary minerals.

Meister and Reyes [39] review the carbon isotope record of the sub-seafloor biosphere, which exhibits large carbon-isotope fractionation effects as a result of microbial enzymatic reactions. The review presents a brief and timely overview on carbon isotopes, including microbial fractionation mechanisms, transport effects, preservation in diagenetic carbonate archives, and their implications for the past sub-seafloor biosphere. Particularly large carbon-isotope fractionation linked to methanogenesis and fractionations occurring during this process is a focus of the review. Methane metabolisms in Archaea and Bacteria essentially mediate through the enzymatic Wood–Ljungdahl pathway, but it remains unclear where in the pathway carbon-isotope fractionation occurs. In marine porewaters (and diagenetic carbonates), the highly varying C isotopic signature of dissolved inorganic carbon and methane reflects different microbial metabolisms [40]. Carbon isotope compositions of diagenetic carbonates may therefore act as biosignatures for ancient deep biosphere conditions.

### 3. Summary and Outlook

As stated in the introduction, the deep biosphere is understudied, particularly in a deep time perspective. With this Special Issue, we have gathered a collection of well-needed additions to take the state of knowledge further, and that we hope can inspire new research on both fossil and active communities in this vast realm.

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