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Opinion Article

The role of anaerobic fungi in fundamental biogeochemical cycles in the deep biosphere

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ABSTRACT

A major part of the biologic activity on Earth is hidden underneath our feet in an environment coined the *deep biosphere* which stretches several kilometers down into the bedrock. The knowledge about life in this vast energy-poor deep system is, however, extremely scarce, particularly for micro-eukaryotes such as fungi, as most studies have focused on prokaryotes. Recent findings suggest that anaerobic fungi indeed thrive at great depth in fractures and cavities of igneous rocks in both the oceanic and the continental crust. Here we discuss the potential importance of fungi in the deep biosphere, in particular their involvement in fundamental biogeochemical processes such as symbiotic relationships with prokaryotes that may have significant importance for the overall energy cycling within this vast subsurface realm. Due to severe oligotrophy, the prokaryotic metabolism at great depth in the crust is very slow and dominantly autotrophic and thus dependent on e.g. hydrogen gas, but the abiotic production of this gas is thought to be insufficient to fuel the deep autotrophic biosphere. Anaerobic fungi are heterotrophs that produce hydrogen gas in their metabolism and have therefore been put forward as a hypothetical provider of this substrate to the prokaryotes. Recent *in situ* findings of fungi and isotopic signatures within co-genetic sulfide minerals formed from bacterial sulfate reduction in the deep continental biosphere indeed seem to confirm the fungi-prokaryote hypothesis. This suggests that fungi play a fundamental biogeochemical role in the deep biosphere.

1. Introduction

The deep biosphere comprises microorganisms several kilometers below the ground surface and ocean floor (Lin *et al.*, 2006). Active deep ecosystems have been reported from such diverse settings as marine sediments (Parkes *et al.*, 2005), deep-sea hydrothermal vents (Jorgensen *et al.*, 1992), subseafloor igneous rocks (Schrenk *et al.*, 2009), and terrestrial sedimentary (Fredrickson *et al.*, 1995) and igneous rocks (Pedersen *et al.*, 2008). The deep biosphere in igneous rocks is among the least understood ecosystems on Earth. Although the microbial processes are relatively slow because of the low energy supply (Wu *et al.*, 2016) the deep ecosystems are proposed to play an important role in the energy cycling of the

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Earth and comprise a substantial part of the Earth's biomass (McMahon and Parnell, 2014). Until recently, the majority of the microbiological investigations in the deep biosphere have been focused on prokaryotes, and the potential presence of eukaryotes such as fungi has been largely neglected. In this respect, recent identifications of fungi in a wide variety of deep environments, including various marine settings (Connell *et al.*, 2009; Le Calvez *et al.*, 2009; Nagano and Nagahama, 2012; Orsi *et al.*, 2013), subseafloor basalt (Ivarsson *et al.*, 2012, 2016a), and in the deep continental crust (Sohlberg *et al.*, 2015; Drake *et al.*, 2017a) definitely shed new light on the presence of fungi in the deep biosphere and suggest fungi to play a major role in the energy cycling. Identification of fungi from anoxic deep sea environments (Jebaraj *et al.*, 2010; Raghukumar *et al.*, 2010) and at depths in the crust where strictly anoxic conditions prevail indicates that anoxic conditions are not a limiting factor for fungal growth, and that the fungi have adapted to anaerobic metabolism.

Anaerobic fungi are so far poorly understood in an environmental context, and the most thorough description is from rumina of ruminating herbivores (Khejornsart and Wanapat, 2010; Liggenstoffer *et al.*, 2010), where the anaerobic fungi produce H_2 during their respiration and consort with H_2-dependent methanogenic and acetogenic archaea, which enhances growth of both organisms. The fungi that form symbiotic relationships with acetogens and methanogens in the rumen have recently been described from marine sediments (Picard, 2017). Potentially any H_2-dependent chemoautotrophic microorganism could be fueled by H_2 produced by anaerobic fungi in an anoxic environment (Ivarsson *et al.*, 2016b), which is supported by recent findings of fungi-prokaryote consortia in the igneous crust (Drake *et al.*, 2017a). Consequently, anaerobic fungi have been proposed to be a neglected geobiological force in subsurface ecosystems (Ivarsson *et al.*, 2016b; Drake *et al.*, 2017a). Here we discuss this previously unknown biogeochemical agent and its potential implications.

2. Fungi in oceanic crust

Deep sea environments host diverse fungal communities, mainly represented by Ascomycota, but also Basidiomycota and Chytridiomycota (Nagano and Nagahama, 2012). A majority of Chytridiomycota in deep sea environments represent novel deep-branching lineages, including a new branch forming an ancient evolutionary lineage (Le Calvez *et al.*, 2009). Ascomycetes and Basidiomycetes have been isolated from seafloor-exposed basalt (Connell *et al.*, 2009), but from the underlying igneous crust, only one fungal isolate (genus *Exophiala* of the order Chaetothyriales) has been reported so far (Hirayama *et al.*, 2015). Apart from this single observation our understanding of the fungal presence in the oceanic crust is based on a fossil record, such as the findings by Ivarsson *et al.* (2012) describing mycelium-like networks of hyphae with preserved chitin in their cell walls, in vesicular basalts from the Emperor Seamounts in the Pacific Ocean. Bengtson *et al.* (2014) revealed a close symbiotic-like relationship between fungi and two types of chemoautotrophic prokaryotes in subseafloor basalts, and that the prokaryotes used the structural framework of the mycelia for their growth (Fig. 1). The close relationship with chemoautotrophic prokaryotes was also described by Ivarsson *et al.* (2015a) and probably was essential for fungal colonization in the oligotrophic environment. The most likely source of carbohydrates that are essential for the fungi in these oligotrophic environments has been proposed to be living or dead bacterial biofilms (Gadd, 2006). Fungi from the Emperor Seamounts were interpreted as Ascomycetes or stem-group Dikarya and fossilized fungi from the Vesteris Seamount in the Greenland Basin (Ivarsson *et al.*, 2015b) were interpreted as Zygomycetes. Thus, the fungal diversity seems high in both marine sediments and in subseafloor basalts. Fossilized fungi have been found in ophiolites as old as 2.4 Ga (Bengtson *et al.*, 2017). The igneous oceanic crust is a fungal niche that likely is of great importance considering the spatial and temporal distribution of fungi.

3. Fungi in continental crust

The deep subsurface of continental igneous rocks has not been as extensively studied as deep sea sediments and subseafloor basalts, and the studies here have focused on prokaryotes. Therefore, observations of fungi are very few. Fossilized fungal hyphae have been reported from the bedrock in Sweden and Germany (Reitner *et al.*, 2006; Ivarsson *et al.*, 2013). Ekendahl *et al.* (2003) isolated a limited number of yeast fungi strains from waters at Äspö, Sweden and Sohlberg *et al.* (2015) found a high fungal diversity in bedrock aquifers at 300–800 m depth at Olkiluoto, Finland. Most of the observed fungal sequences in the latter study belonged to the phylum Ascomycota, with minor contribution of the Basidiomycota and Chytridiomycota phyla. Anoxic conditions prevail at these depths in the crust and the fungi are thus considered to be anaerobic, but still, the possible role of the detected fungi in this oligotrophic environment is unknown. Recently, we presented *in situ* evidence of fungi from great depth within the continental crust, from a fracture cavity in Proterozoic igneous rocks at Laxemar, Sweden (Fig. 2), giving a new piece in the deep biosphere puzzle (Drake *et al.*, 2017a) and suggesting that anaerobic fungi are widespread in the continental crust, down to almost 1 km depth.

4. The biogeochemical role of fungi in the deep biosphere

Anaerobic fungi

Facultative anaerobic fungi are known from all major fungal divisions and frequently found in extreme environments including deep subsurface environments (Sohlberg *et al.*, 2015). A correlation between facultative anaerobic fungi and nitrogen cycling seems plausible as fungi use nitrate or nitrite as alternative terminal electron acceptors in the absence of oxygen (Kurakov *et al.*, 2008). Obligate anaerobic fungi, however, are only known from the phylum Neocallimastigomycota, which is best known from rumen of herbivores but have been reported from the gut and coelomic fluid of the coastal sediment-dwelling sea urchin *Echinocardium cordatum* (Thorsen, 1999) and in the guts of the algae-grazing marine

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