Transition of microbial communities and laminated structures in travertines: a case study in northern Sumatra, Indonesia

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Travertines, carbonate developed in calcareous and high  $pCO_2$  hot spring. Because these often exhibit laminated structures similar with stromatolites that had been extensively developed in ocean in Archean and Proterozoic. Previous researches of the modern analogs of the ancient stromatolites have indicated that cyanobacteria played a central role to form the laminated structure in previous studies. However, this may contradict to very low oxygen concentration in Archean. To solve this paradox, we researched a travertine-bearing hot spring at Dolok Tinggi Raja in northern Sumatra, Indonesia. Here, the water of 62 degrees, high in  $H_2S$  and  $CO_2$  discharged from the vent formed a travertine mound of ~50 m wide as a consequence of geochemical and microbial processed. The water rapidly degassed H<sub>2</sub>S and CO<sub>2</sub> immediately after discharge, increased dissolved oxygen concentration and pH and  $CaCO_3$  saturation index, and precipitated carbonate mineral (aragonite and calcite) along the flow passages. Responding to the change in the watery chemistry from upstream to downstream, the dominant microbes changed in order: chemical synthesis bacteria, purple sulfur bacteria, green non-sulfur bacteria, green sulfur bacteria. Although cyanobacteria were recognized in the downstream sites, they were subordinate. In the environment of high  $H_2S$ , anoxygenic photosynthesis (sulfur bacteria) can be more profitable than oxygenic photosynthesis (cyanobacteria), and takes first priority. In the places where microbial mat is developed, the travertines often form laminated structure. The travertines with sulfur bacterial mat appear the structures that resemble the daily lamination reported from Nagayu hot spring in Oita Prefecture and Pancuran Pitu in Java. Anoxygenic sulfur bacteria have a potential to form the stromatolitic lamination, and therefore, ancient stromatolites were not necessarily made by cyanobacteria.

Keywords: travertine, sulfur bacteria, stromatolite

Biogeochemical cycle of methanol in anoxic deep-sea sediments of the eastern Japan Sea

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Methanol is one of the most important carbon and energy sources in anoxic environments. However, the biological flux and lifetime of methanol in anoxic marine sediments are largely unknown. In this study, we report quantitative methanol removal rates in subsurface sediments for the first time. Methanol concentrations in pore water from Japan Sea sediments gradually increased with depth below the sulfate-methane transition zone. Based on anaerobic incubation experiments with radiotracers, high rates of microbial methanol consumption were detected in the sediments. Our experiments also showed that the methanol oxidation to  $CO_2$  surpassed methanol assimilation and methanogenesis from  $CO_2/H_2$  and methanol. Nonetheless, a significant decrease in methanol was not observed after incubation, likely because of the microbial production of methanol in parallel with its consumption. This study suggests that microbial reactions play an important role in the sources and sinks of methanol in subseafloor sediments.

Keywords: methanol, deep-sea sediment, subseafloor biosphere

Co-occurrence and Metabolic Consequences of Candidate Bacterial Phyla and Anaerobic Methane Oxidizing Archaea in the Deep Crustal Biosphere

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The terrestrial crust is known to harbor deep microbial life energetically dependent on organic matter and/or H<sub>2</sub>. Recent studies have provided fragmented pieces of evidence suggesting that anaerobic oxidation of methane (AOM) is microbiologically mediated in the terrestrial subsurface as well as the deep oceanic crust. As the abundance of methane is extremely common in the deep aquifers, the existence of subsurface microbial ecosystems capable of harvesting the energy from AOM can dramatically change our view of the Earth's biosphere. Here we show the integrated evidence of the anaerobic methanotrophy based on geochemical, stable isotopic, molecular phylogenetic and metagenomic data from the deep granitic aquifer. High-quality groundwater was collected from two adjacent boreholes drilled into highly and sparsely fractured domains at a 300-m deep stage of the Mizunami underground research laboratory (URL), central Japan. The highly fractured domain was associated with groundwater dominantly colonized by AAA (AOM associated Archaea) and the candidate phyla OD1 and OP3, neither of which were detected from the sparsely fracture domain with groundwater enriched with  $H_2$  (~10-100 nM) and depleted in sulfate (<5  $\mu$ M). Consistent with 16S rRNA gene sequences, methyl-coenzyme M reductase gene sequence analysis revealed the habitat segregation of AAA and methanogens corresponding to the fracture domains. As the strong correlation of AAA and sulfate was statistically indicated by canonical correspondence analysis (CCA), anaerobic methane oxidation coupled to sulfate reduction was experimentally demonstrated by the amendment of  $^{13}CH_{4}$  of, and the subsequent detection of <sup>13</sup>C-enriched dissolved inorganic carbon from, microbial cells incubated in groundwater with and without the molybdate inhibition of dissimilatory sulfate reduction. Heatmap of 16S rRNA gene abundance in the boreholes within the two domains over two years showed the highly correlated distributions of AAA and the candidate phylum OP3, the nearly completed genome of which has functional genes involved in sulfur metabolisms such as a potential reductase gene of nitrite and sulfite. Although the syntrophic partnership among AAA and the candidate phyla needs to be further investigated, our results clearly demonstrate that the deep methanotrophy coupled to sulfate reduction is microbiologically mediated, which leads to the reconsideration of the biomass production and the cycling of hydrogen, carbon and sulfur in one of the largest microbial habitats on Earth.

Keywords: Deep biosphere, Mizunami underground research labolatory, Anaerobic oxidation of methane

Effects of sedimentary redox conditions on Eukaryotic DNA recorded in deep-sea sediments

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Eukaryotic DNA in marine sediments can be a useful indicator of both ancient marine ecosystem in water column and living microbial eukaryotes in sediments. However, the environmental factors that influence the composition and preservation of eukaryotic DNA in marine sediments are poorly characterized. In this study, we examined effects of sedimentary redox conditions on the diversity of eukaryotic communities recorded in deep-sea surface sediment samples from 8 sites of the Japan Sea (from oxygenated abyssal sediments to sulfidic sediments in methane seeps) using a combination of various geochemical and molecular-biological tools. Sedimentary redox conditions were characterized by depth profiles of pore water (oxygen, nitrate, iron, sulfide, etc.) and bulk sedimentary organic matter (TOC, TN,  $\delta^{13}$ C,  $\delta^{15}$ N). The concentration and diversity of 18S rDNA in the sediment samples were investigated by qPCR and pyrosequencing. Decreases in 18S rDNA sequences also varied with the sedimentary redox conditions. These results suggest that redox conditions of surface sediments can be important factors controlling the composition and preservation of eukaryotic DNA in deep-sea sediments.

Keywords: Redox condition, Marine sediments, Biodiversity, DNA, Eukaryote

Biogeochemistry and subglacial meltwater limnology in East Antarctica: insight from microbial response with subglacial silica input in a perennially ice-covered lake at Rundvagshetta

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Since the discovery of subglacial Antarctic lakes by radio-echo sounding in the late 1960s, numerous subglacial lakes including extensive networks of subglacial meltwater channels (e.g., Anderson et al., 2002; Wingham et al., 2006) have been identified during the past two decades (e.g., Priscu et al., 1999; Christner et al., 2006). To date, Siegert and co-workers compiled an inventory of 145 subglacial lakes beneath the East and West Antarctic Ice Sheet (Siegert et al., 2005). The subglacial water, which is initially derived from melting due to geothermal heat (heat flow rates, ~50 mW m<sup>-2</sup>; Siegert et al., 2012), is involved in various water-rock interaction processes beneath the ice sheet, and these interactions play important roles in the supply of nutrients, including trace metals, to organisms in Antarctic environments.

Firstly, silicon (Si) is one of the critical elements limiting the growth of Antarctic diatoms (e.g., Nelson and Treguer, 1992), likewise nitrogen and other elements (e.g., Hutchins and Bruland, 1998). Moreover, glacial Fe inputs including particulate and dissolved Fe are significant as a biologically essential element to the Southern Ocean (Raiswell and Canfield, 2012). In addition to nutrient input by seasonal snowmelt, subglacial meltwater flowing through channels may influence the productivity and diversity of microbial communities by controlling the concentrations of nutrients and the physico-chemical conditions of glacial environments. Secondly, glacial lakes affected by subglacial water input can be observed at the retreating margins of the Antarctic ice sheet. In the Rundvågshetta area on the Soya Coast of Lützow-Holm Bay, East Antarctica, fresh water flows from subglacial drainage channels of the EAIS.

The objective of this study is to examine the interaction between the limnology of subglacial water input and microbiological responses in the perennially-ice covered glacial lake in Rundvagshetta (i.e., Lake Maruwan) over the last 6000 years. Greenish-grayish organic-rich laminations in sediment cores from the lakes indicate continuous primary production affected by inflow of subglacial meltwater containing relic carbon, nitrogen, sulfur and other essential nutrients. Biogenic silica, amorphous hydrated silica, and DNA-based molecular signatures of sedimentary facies indicate that diatom assemblages are the dominant primary producers supported by the input of inorganic silicon (Si) from the subglacial inflow.This study highlights the significance of subglacial water-rock interactions during physical and chemical weathering processes for an important source of bioavailable nutrients.

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Keywords: Biogeochemistry within subglacial weathering, Subglacial meltwater limnology and microbial ecology



(a) Lake Maruwan, a perennially ice-covered glacial lake at the Rundvågshetta on the Soya Coast of Lützow-Holm Bay (LHB), East Antarctica. (b) a drainage map of the Antarctic ice sheet. Modified after Anderson *et al., Quaternary Sci. Rev.*, 2002 and Takano *et al.*, this study.