

# Metagenomic insights into the importance of organic sulfur in the deep biosphere

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Inorganic sulfur has been a primary focus of investigations into the sulfur cycle, as microorganisms can generate energy through reduction or oxidation of these compounds. However, all life requires organic sulfur for building biomass and general metabolism. While inorganic sulfur may dominate the sulfur cycle in high-sulfate systems such as the modern ocean, organosulfur mineralization is an important source of oxidized and reduced sulfur compounds in low-sulfate environments like modern freshwater systems or the Archean ocean. Thus, we hypothesize that organic sulfur will play a significant role in the biogeochemical cycling of sulfur in the nutrient-deprived deep biosphere. To investigate this question we mined shotgun metagenomic datasets from a deep, terrestrial subsurface environment at Soudan Underground Iron Mine, MN. Legacy boreholes in Soudan Mine reach over 800m below the surface and transect a Neoproterozoic (~2.7 Ga) massive hematite iron formation, providing access to the fractured rock aquifer below the mine. The conditions in this aquifer are thought to resemble Archean oceans, with reducing, anoxic brines that have sulfate levels much lower than present day oceans; thus, this setting provides a useful analog to the oceans on early Earth, and may provide insight into the contribution of organic sulfur to the subsurface sulfur cycle in other systems like Mars.

Our Metagenome Assembled Genomes (MAGs) show evidence of both assimilatory and dissimilatory sulfur metabolisms. Over 77% of MAGs have one or more genes involved in the degradation of cysteine to sulfide, and over 65% have the sulfur dioxygenase (*sdo*) gene that produces sulfite from S-sulfanylglutathione. The cryptic cycling of organosulfur may be a previously overlooked source that is key to refueling dissimilatory sulfur metabolisms in this highly oligotrophic setting. Additionally, 93% of MAGs contain an incomplete sulfur assimilation pathway. Since sulfur assimilation is necessary for life as we know it, this could indicate the importance of inter-organism interactions and metabolic handoffs in deep subsurface communities. Further examination of organosulfur assimilation, production, and utilization pathways will improve our understanding of biogeochemical sulfur cycling in the deep terrestrial biosphere and may have implications for our understanding of the evolution of life in Archean oceans.