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Unraveling the role of oxygen fluctuations on microbial iron oxidation and biomass production in the fractured continental subsurface.

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Microbial communities in the subsurface gain energy from redox reactions sustained by the disequilibrium between fluids and rocks. Meteoric flows maintain this disequilibrium by transporting reactive elements and gases to the subsurface. Through preferential flowpaths, oxygenated water can be transported to great depths where it can mix with reduced waters, creating localized reactive zones. The mixing of oxygenated and ferrous iron-enriched waters can in particular promote the development of chemolithoautotrophic iron-oxidizing bacteria (FeOB). To assess the extent to which FeOBs are major players in these biogeochemical cycles, two important points remain to be understood: the microaerobic range over which subsurface FeOB can grow and the consequences of fluctuations in oxygen concentrations on the amount of biomass produced. To answer, we explored the sensitivity of FeOB communities to oxygen concentrations with enrichment experiments.

We sampled iron-rich groundwater from the fractured-rock observatory of Ploemeur (SNO H^+, France) where FeOB are abundant. Groundwater samples were enriched with four oxygen concentrations: 0.3, 3, 13, and 60 µM, which were precisely maintained during the incubations. We observed growth of rusted FeOB mats after about 24-hours in microaerobic conditions (≤ 13 µM O_2), with abundant FeOB extracellular structures and Fe-oxyhydroxides in the mats. Biomass measurements and Fe kinetics show the FeOB activity. Unexpected non-rusted white colored FeOB mats with low content in iron oxides were observed under near anoxic conditions (0.3 µM O_2)(Fig.1). The incubations were analyzed by metagenomics in order to determine the impact of oxygen concentrations on community diversity and metabolism. These latter results, FeOBs coexisted with Fe and S-reducing bacteria at 0.3 µM O_2, indicating that a cryptic Fe cycle is occurring. This would explain the low level of mineral incrustation observed, as Fe-oxyhydroxides would be reduced as soon as they are formed. Our study shows that in the almost absence of oxygen, FeOBs are able to grow and that a microbial biomass not encrusted with Fe-oxyhydroxides can be produced at depth and recycled by heterotrophic bacteria. Gaining knowledge on subsurface primary producers and environmental conditions that promote their growth would be a stepping-stone to understand the functioning of biogeochemical cycles.