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Mineral substrate and fluid redox conditions control cell density in attached biofilms: *in-situ* incubations in deep groundwater

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The continental deep subsurface sustains most of Earth's microbial life. Recent studies have shown that about 80 % of Earth's microbial biomass lives in the deep continental subsurface, mostly in the form of biofilms, with important consequences for biogeochemical processes and element fluxes in the critical zone. However, one of the major uncertainties in those studies comes from cell density estimations that have been obtained from microbial communities filtered from fluids. Little is known about cell densities in microbial biofilms attached to minerals in the subsurface, mainly due to difficulties to access and sample those environments. In particular, a comprehensive and quantitative view of how the mineral substrate or the lithology affect cell density in biofilms is still missing.

In this study, we engineered a set of probes to incubate well-characterized mineral samples at key locations of the fractured-bedrock aquifer of the Ploemeur-Guidel critical zone observatory (Brittany, France). We incubated a set of 8 environmentally relevant mineral types with different geochemical compositions. The samples were incubated *in situ* during 10 months in two 100-meter deep boreholes with redox contrasted groundwater typical of recharge (oxic) and discharge (anoxic) zones of the aquifer. After incubation, cell density was quantified on the colonized surfaces by using epifluorescence microscopy. For samples with thick biofilms, an approach combining microtopography analysis and confocal microscopy was used to estimate both the volume of the biofilm and the associated volumetric cell density. The mineralogy and chemistry of the biofilms were studied using spectroscopy methods. The microbial communities associated with different redox conditions and mineral types were characterized using 16S rRNA gene surveys.

Our preliminary findings show that the redox conditions play a first order control on cell abundance, as well as on the structure and mineralogy of the biofilm. Likewise, the mineral substrate acts as a secondary control in which the highest cell densities are associated with the most reactive minerals. This study provides quantitative and field-relevant information on biofilms attached to minerals which should allow to better constrain biomass estimations as well as reactive transport models looking to account for microbially mediated mineral dissolution.