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Light-independent coupled C, H, N, S and Fe biogeochemical cycles operating in the deep subsurface of the Iberian Pyrite Belt

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Microbial activities are major contributors to the biogeochemical cycles that constitute the life support system of planet Earth. Recent estimates suggest that an important part of prokaryotic biomass and diversity reside in the deep subsurface. Despite their importance, we know little about such microbes and their activities, in particular those dwelling in the rock matrix of the continental subsurface, because of difficulties of sample accessibility and handling. By means of a devoted 613 m deep geomicrobiological drilling and systematic multi-analytical characterization an unexpected diversity associated to the rock matrix microbiome operating in the subsurface of the Iberian Pyrite Belt was unravel. Members of one class and sixteen genera identified by at least two independent methodologies (hybridization, sequencing, cloning, immunological-detection, enrichment cultures or isolation) at five depth intervals are considered the most representative microorganisms of the deep subsurface of the IPB and were selected for a deeper analysis. The use of Fluorescence in situ Hybridization allowed not only to identify, but to detect the co-occurrence of microorganisms able to maintain complementary metabolic activities, like ferric and sulfur oxidation and reduction; the anaerobic ammonium and methane oxidation and the existence of biofilms interconnecting microniches in the oligotrophic conditions existing in the deep subsurface of the IPB. Enrichment cultures allowed the detection of different complementary anaerobic metabolic activities (methanogenesis, methanotrophy, acetogenesis, autotrophic nitrate reduction, sulphate reducing, iron oxidation and reduction). The genomic analysis of nine isolates allowed identifying the presence of genes involved in the operation of the complete and coupled C, H, N, S and Fe biogeochemical cycles. This study revealed the key role of the nitrogen cycle and the importance of nitrate reduction microorganisms in the oxidation of iron in the anoxic conditions existing in the subsurface of the Iberian Pyrite Belt.