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Moonmilk deposits: a case of biomineralization and greenhouse gases regulation in subterranean environments

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Moonmilk deposits is a well-known and widespread example of calcium carbonate biomineralization in subterranean environments. Moonmilk structure is spongy and wet during an emerging and growing active phase, while becoming crumbly, powdery and, finally, crusting during an evolved phase. The microbiota of moonmilk deposits varies throughout these development stages [1]. The RNA/DNA ratios also indicate an inactivation of microorganisms from incipient moonmilk toward consolidated deposits of calcium carbonate [2]. Therefore, a variety of biochemical mechanisms would be involved at each stage of moonmilk formation that could play a significant role in the carbon geochemical cycle.

We quantify the CO_2 and CH_4 fluxes from microbial communities associated to moonmilk from Pindal cave (northern Spain), using a field-deployed chamber-based gas exchange system coupled with a cavity ring-down spectrometer for a simultaneous and real-time $\delta^{13}C$ geochemical tracing. Moonmilk deposits were characterized by metagenomics analysis of 16S rRNA gene amplicons in terms of composition and ecological functionality, particularly those related with the cycle of both gases.

Gas flux measurements show a continuous one-way CO_2 efflux from moonmilk as well as a continuous one-way CH_4 consumption from the cave air. These fluxes are intensified in the emerging and growing active phase of moonmilk. We found the key functional microbial groups in the moonmilk formation (*Crossiella* and the lineage wb1-P19) concluding that syntrophic relationships are established between bacteria, including ammonification (production of NH_3 and CO_2) → nitrification → CO_2 capture and NH_3 consumption triggering the fixation of the excess CO_2 as calcite. *Methylomonaceae* is the main methanotrophic family responsible for the intense CH_4 oxidation, up to 90% the atmospheric CH_4 entering the cave environment. This research reveals moonmilk as a remarkable effective deposit in carbon sequestration.

[1] Park et al (2020), *Frontiers in Microbiology* 11: 613.

[2] Sanchez-Moral et al (2012), *Geomorphology* 139-140, 285-292.

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