On the role of cave-soil in the carbon cycle. A first approach.

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Methods:

(1) In situ real-time automatic monitoring of CO₂ and CH₄ cave-soil fluxes: Seasonal campaigns with daily continuous monitoring by a closed chamber-based gas exchange system (LICOR Automated Soil Gas Flux System LI-8100A), equipped with a Long-Term Chamber 8100-104, in conjunction with a compatible Gasmet FTIR gas analyser (Gasmet DX4015).

(2) Microbial Analyses: Structural and functional characterization by meta-barcoding analyses of bacteria 16S rRNA genes and Shotgun Metagenomics.

Objectives: The aim of this preliminary study is monitoring the carbon fluxes, CO₂ and CH₄, from cave soil directly exchanged with the cave atmosphere.

Study site: Pindal Cave (Asturias, north of Spain), is a touristic shallow cave with Paleolithic (Magdalenian) paintings.

Particular environment: absence of light, small thermohygrometric variation and large CO₂ oscillation.

<table>
<thead>
<tr>
<th>Annual:</th>
<th>Average</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Cave air temperature, °C</td>
<td>11.7</td>
<td>2</td>
</tr>
<tr>
<td>Cave air CO₂, ppm</td>
<td>700</td>
<td>1000</td>
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• Predominance of CO₂ emission flux, with daily variations in magnitude, and occasionally alternating emission or uptake.
• The daily-averaged CO₂ flux ranges from +0.1 to +0.25 μmol m⁻² s⁻¹, but less than 0.1 μmol m⁻² s⁻¹ in presence of moonmilk deposits on the soil surface.

• CH₄ uptake flux from cave soil.
• The daily-averaged CH₄ flux ranges from -1 to -3 nmol m⁻² s⁻¹.
Proteobacteria, Acidobacteria, Planctomycetes and Chloroflexi were the major phyla in the soil samples, with an increase of Actinobacteria in the most superficial ones.

- The CO₂ emission flux observed are the result of respiration by chemotrophic microorganisms.
- Crossiella found in moonmilk have the ability to capture CO₂ from the underground atmosphere, resulting in precipitation of calcium carbonate as a by-product of the action of carbonic anhydrase.

- Methanotrophic bacteria were represented by Rokubacteria (NC10) that mediates the anaerobic oxidation of methane (AOM) coupled to nitrite reduction.
- CH₄ is consumed by the action of methanotrophic bacteria of NC10 phylum.
Final remarks

The results reveal that the cave soil in Pindal cave is acting as net uptaker of CH$_4$ and as an emitter / sink of CO$_2$ alternately. Their uptake and turnover rates appear to be meaningfully high.

These preliminary results confirm that cave-soil is playing an outstanding role in the processes of production and consumption of CO$_2$ and CH$_4$, that may be partially determining the strong variations of these major GHGs in natural subterranean ecosystems.

This research line is crucial to achieve a more accurate assessment of the effective contribution of karst ecosystems to the global carbon cycle.