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The importance of methanotrophic activity in geothermal soils of Pantelleria island (Italy)

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Methane is a major contributor to the greenhouse effect, its atmospheric concentration being more than doubled since the XIX century. Every year 22 Tg of methane are released to the atmosphere from several natural and anthropogenic sources. Natural sources include geothermal/volcanic areas but the estimation of the total methane emission from these areas is currently not well defined since the balance between emission through degassing and microbial oxidation within the soils is not well known.

Microbial oxidation in soils contributes globally for about 3-9% to the removal of methane from the atmosphere and recent studies evidenced methanotrophic activity also in soils of volcanic/geothermal areas despite their harsh environmental conditions (high temperatures, low pH and high concentrations of H_2S and NH_3). Methanotrophs are a diverse group of bacteria that are able to metabolize methane as their only source of carbon and energy and are found within the Alpha and Gamma classes of Proteobacteria and within the phylum Verrucomicrobia.

Our purpose was to study the interaction between methanotrophic communities and the methane emitted from the geothermally most active site of Pantelleria island (Italy), Favara Grande, whose total methane emission has been previously estimated in about 2.5 t/a.

Laboratory incubation experiments with soil samples from Favara Grande showed methane consumption values of up to 9500 ng g^{-1} dry soil per hour while soils collected outside the geothermal area consume less than 6 ng g^{-1} h^{-1}. The maximum consumption was measured in the shallowest part of the soil profile (1-3 cm) and high values (>100 ng g^{-1} h^{-1}) were maintained up to a depht of 15 cm. Furthermore, the highest consumption was measured at 37°C, and a still recognizable consumption (>20 ng g^{-1} h^{-1}) at 80°C, with positive correlation with the methane concentration in the incubation atmosphere. These results can be considered a clear evidence of the presence of methanotrophs that were investigated by culturing and culture-independent techniques.

The diversity of proteobacterial methanotrophs was investigated by creating a clone library of the amplified methane mono-oxygenase encoding gene, *pmo*A. Clone sequencing indicates the presence of Gammaproteobacteria in the soils of Favara Grande. Enrichment cultures, on a mineral medium in a CH_4-enriched atmosphere, led to the isolation of different strains that were identified as *Methylocistis* spp., which belong to the Alphaproteobacteria. The presence of Verrucomicrobia was detected by amplification of *pmo*A gene using newly designed primers.

Soils from Favara Grande show therefore the largest spectrum of methanotrophic microorganisms until now detected in a geothermal environment.

While the presence of Verrucomicrobia in geothermal soils was predictable due to their thermophilic and acidophilic character, the presence of both Alpha and Gamma proteobacteria was unexpected. Their presence is limited to the shallowest part of the soil were temperatures are lower and is probably favored by a soil pH that is not too low (pH ~5) and their contribution to biological methane oxidation at Pantelleria is significant.

Understanding the ecology of methanotrophy in geothermal sites will increase our knowledge of the role of soils in methane emissions in such environments.