Methane efflux from the soil and methanotrophic activity in volcanic-geothermal areas: Examples from Italy and Greece

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Methane plays an important role in the Earth's atmospheric chemistry and radiative balance being the most important greenhouse gas after carbon dioxide. It is released to the atmosphere by a wide number of sources, both natural and anthropogenic, with the latter being twice as large as the former. It has recently been established that significant amounts of geological methane, produced within the Earth's crust, are currently released naturally into the atmosphere. Active or recent volcanic-geothermal areas represent one of these sources of geological methane. Due to the fact that methane flux measurements are laboratory intensive, very few data have been collected until now and the contribution of this source has been often indirectly estimated. Both the Italian and the Hellenic territories are geodynamically very active with many volcanic and geothermal areas. Here we report on methane flux measurements made at Pantelleria (Italy), Sousaki and Nisyros (Greece). The total outputs of these three systems are about 10, 19 and 2 t a^{-1} respectively. These figures are up to one order of magnitude lower than those obtained through indirect estimations.

At the global scale, microbial oxidation in soils contributes to the total removal of methane from the atmosphere. Environmental conditions in the soils of volcanic/geothermal areas (i.e. low pH, high temperature, etc.) have been considered inadequate for methanotrophic microrganisms. But recently, it has been demonstrated that methanotrophic consumption in soils occurs also under such harsh conditions due to the presence of thermo-acidophilic Verrucomicrobia.

Here we present the results of laboratory incubation experiments on soil samples collected at the main exhalative areas that highlighted methanotrophic activity also at Pantelleria and Sousaki.

Soil metagenomic DNA was extracted from some of the Pantelleria samples and analysed using Temporal Temperature Gradient Electrophoresis (TTGE) of the amplified Bacterial 16S rRNA gene in order to evaluate the total bacterial diversity. Soil DNA amplification with primers targeting Proteobacterial and Verrucomicrobial methane monooxygenase genes (*pmmo*) revealed the presence of methanotrophs affiliated to both phyla up to a depth of 11 cm and a temperature of 80°C. The diversity of proteobacterial methanotrophs was investigated by creating a clone library of the amplified methane mono-oxygenase encoding gene, *pmmo*A. The clone sequences are close to those of uncultured type I methanotrophic proteobacteria.

An attempt to isolate methanotrophs was carried out on soils from Pantelleria, sampled at different depths, by enrichment cultures on a mineral medium in a methane-enriched atmosphere. No isolates were obtained from enrichments carried out at 65°C while incubation at 37°C allowed to isolate a few methanothropic strains that were identified as *Methylocystis* spp.