

## AEROBIC OXIDATION OF METHANE AND ETHANE BY UNCONVENTIONAL MICROBES AT A TERRESTRIAL METHANE SEEP IN SICILY, ITALY

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Methane and higher gaseous hydrocarbons (ethane, propane and butane) are important greenhouse gases that contribute to photochemical pollution and ozone production in the atmosphere. Worldwide, the aerobic oxidation of methane and higher gaseous hydrocarbons in soils is one of their largest sinks (Solomon et al., 2007). In natural terrestrial hydrocarbon seeps, microbes can act effectively as microbial filters to oxidize these gases into CO<sub>2</sub>. However, in contrast to aquatic systems, the microbes involved in these terrestrial systems are less well constrained, in particular with respect to diagnostic biomarker lipids.

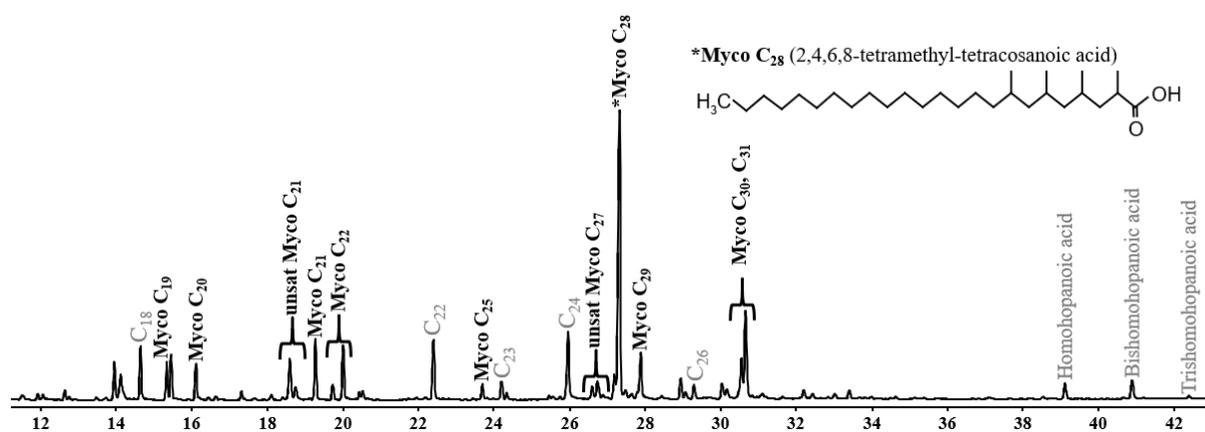
Here, we investigated the lipid inventory of soils from a natural terrestrial gas seep named Fuoco di Censo in southern Sicily, Italy. At this seep, thermogenic gas with a high methane concentration (85 %) and other gaseous hydrocarbons (in particular ethane and propane) are released (Grassa et al., 2004), likely stimulating aerobic microbial methane, ethane and propane oxidation in surrounding soils. We analyzed both the lipid composition as well as the microbial diversity by 16S rRNA gene amplicon sequencing of soils with increasing distance from the gas seep. Furthermore, stable isotope probing (SIP) with <sup>13</sup>C-labeled methane and ethane was used to investigate which microbes are involved in the gas oxidation processes at the Censo seep.

Lipid analyses showed an increasing abundance of <sup>13</sup>C-depleted unsaturated C<sub>16</sub> and C<sub>18</sub> fatty acids with decreasing distance to the gas seep. Close to the seep there was a high abundance of unusual methylated fatty acids (one to five additional methyl groups) characteristic for mycobacteria, mycocerosic acids (Fig. 1), which were also <sup>13</sup>C-depleted suggesting that these bacteria are involved in the oxidation of gases at this seep. The 16S rRNA gene amplicon sequencing confirmed a high relative abundance of mycobacteria together with conventional methanotrophs like *Methylococcales* and *Methylocystaceae* at the Censo site.

Incubations of the Censo soils with <sup>13</sup>C-labeled methane resulted in the incorporation of <sup>13</sup>C into various fatty acids in particular C<sub>16</sub>, C<sub>18</sub> and their unsaturated homologues which probably derive from members of conventional methanotrophic bacteria like alpha- and gammaproteobacteria (Bodelier et al., 2009). However, none of the methylated fatty acids potentially derived from mycobacteria showed any incorporation of <sup>13</sup>C suggesting they are unlikely to be involved in methane oxidation. Incubation with <sup>13</sup>C ethane indicated that the same fatty acids as with methane showed labelling suggesting that it was assimilated by methanotrophs also involved in methane oxidation. This fits with previous studies, especially on marine methanotrophs, showing that they can utilize methane as well as ethane (Redmond et al., 2010). Additionally, we could observe the incorporation of <sup>13</sup>C into mycocerosic acids which shows the involvement of mycobacteria in the oxidation of gaseous hydrocarbons at

this seep. This would agree with previous studies of hydrocarbon monooxygenase analysis which showed that mycobacteria can oxidize gaseous hydrocarbons but not methane (Coleman et al., 2012). Our results are also in agreement with studies from the 1950s which reported a high abundance of mycobacteria in areas of oil and gas production and in areas of petroliferous gas seeps as well as in other soils (e.g. Davis et al., 1959). Our study shows that mycocerosic acids may be potential biomarkers for gaseous hydrocarbon oxidation in terrestrial environments.

Further analysis on the lipid inventory, diversity estimations by 16S rRNA gene amplicon sequencing data, as well as metagenomic data of DNA extracted from the soil incubations are currently underway and will be used to better understand the role of mycobacteria and other methanotrophs as biofilters for greenhouse gases in these soils.



**Figure 1** GC-MS chromatogram of the apolar fraction of a Censo seep soil extract showing the abundance of mycocerosic acids. Also shown is the molecular structure of the most abundant mycocerosic acid C28 with 4 methyl groups.

## References

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