

## Aerobic methane oxidation in soils as revealed by lipid biomarkers at terrestrial methane seeps in southern Sicily, Italy

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The greenhouse gas methane ( $\text{CH}_4$ ) is an important contributor to natural and anthropogenic global climate changes in present and past environments. Worldwide, microbial methane oxidation in soils is one of the largest sinks for atmospheric methane and accounts for up to 10% of total oxidized methane (Bull et al., 2000; Maxfield et al., 2006). However, applicable tools for determining past methane concentrations in the atmosphere and the intensity of methane cycling in soils are currently lacking.

Here, we have investigated soils from terrestrial methane seeps in southern Sicily, Italy where gases with methane concentrations of up to 95% are naturally released, likely stimulating microbial aerobic methane oxidation (AMO) in surrounding soils. Gas flux and isotope data from previous studies show a diffuse soil degassing at the investigated methane seeps (mud volcanoes and dry seeps) even at large distances (Etiope et al., 2002). At the dry methane seep named Fuoco di Censo ('Everlasting Fire') a high flux of  $10^6 \text{ mg m}^{-2} \text{ d}^{-1}$  was recorded. Furthermore,  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta\text{D}_{\text{CH}_4}$  values indicate a thermal origin of the gas (Grassa et al., 2004). However, these studies did not investigate microbial communities in the surrounding environment. Studies from other environments show that certain lipids like bacteriohopanepolyols (amino-BHPs or 3-methylated BHPs), hopanoids (diplopteroles, hopanoic acids) or isotopically depleted fatty acids can be linked to microbial AMO (Birgel et al., 2006; Bull et al., 2000; Talbot and Farrimond, 2007; Talbot et al., 2014).

Soils from Fuoco di Censo were sampled with increasing distance from the methane seep. GC-MS analysis of the soils indicate an increasing abundance of odd over even fatty acids and hopanoids (diplopteroles, hopanoic acids) with decreasing distance to the methane seep. The occurrence of unusual glycosidic alkyl lipids, proposed to be of bacterial origin, and their spatial variations in concentration at the sampling site, suggest they may be novel lipid biomarkers for methane oxidizing bacteria. UHPLC-high resolution mass spectrometry (HRMS) measurements of BHPs revealed an abundance of adenosylhopane, aminotriol and BHP-tetrol (BHT) with methylated counterparts, novel isomers of methylated BHT and adenosylhopane. The abundance of these methylated BHPs show a positive correlation with methane concentration. Previous studies on AMO bacteria suggest that 3-methylated BHPs and amino-BHPs can trace active methane oxidation and can therefore be used to describe the community as well as changes in methane concentrations (Talbot and Farrimond, 2007; Talbot et al., 2014). Further investigations on the lipid inventory and lipid isotopes of the sample set will potentially lead to new AMO biomarkers for present and past environments.