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Methanotrophic activity and diversity of methanotrophs in volcanic geothermal soils at Pantelleria (Italy)

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1. Gas sampling and characterization

Site	Depth	T	O_2	N_2	CH_4	CO_2
	cm	°C	mmol mol^{-1}			
FAV1	13	82.7	143.8	545.2	9.9	284.2
	25	103.7	41	97.3	38.7	732.7
	50	102.7	24.5	46.2	34.6	759.5
FAV2	13	75	159.2	606.8	8.5	216
	25	85.9	36.7	69.7	36	832.5
	50	111.6	32.5	62.7	38.9	808.4
FAV3	13	52.9	181.8	715.7	1.9	77.3
	25	68.5	162.3	633.2	6.2	187
	50	88.2	106.3	386.8	18.8	482.6

Table S1 - Chemical composition of soil gases in the Favara Grande area.

2. PCR-TTGE and soil diversity

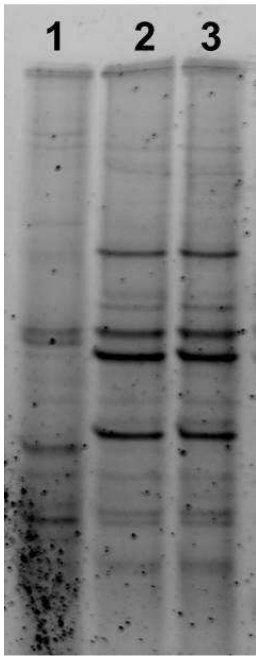

Figure S1. Bacterial diversity at the geothermal site Favara Grande. Temporal Thermal Gradient gel Electrophoresis (TTGE) profiles of PCR-amplified 16S gene fragments derived from soil DNA extracted from sites 1) FAV1, 2) FAV2, 3) FAV3. The hypervariable V3 region of the 16S rRNA gene was PCR amplified using the primer pair 341F-GC/534R (Muyzer et al., 1993) and soil DNA as template. The PCR reaction mixture (50 µl) contained about 100 ng of soil DNA, 1X PCR buffer, 0.20 mMdNTPs, 500 nM of each primer and 1 µl of Phire Hot Start II DNA Polymerase (Thermo Scientific, USA). PCR was carried out in a BiometraThermocycler using the following thermal cycling: initialdenaturation at 98°C for 30 sec, followed by 35 cyc les of 10 sec at 98°C, 10 sec at 66°C, 10 sec at 72°C and final extension at 72°C for 1 min. For TTGE analysis, 10 µl of each PCR mix were loaded in a 8% (w/v) acrylamide gel (acrylamide:bisacrylamide 29:1) containing 7 M urea and 10% formamide in 1.5X TAE buffer (60 mMTris-Acetate, 1.5 mM Na2 EDTA; pH 8). The gel was run in a DCode (Bio-Rad, Richmond, CA, USA) apparatus, at 70 V for 17 h, with a temperature ramping rate of 0.4°C/h with a starting temperature of 57°C. The gel was stained with SYBR Gold (Invitrog en, USA) in 1X TAE for 45 min and visualized under a UV light using the ChemiDoc apparatus (BioRad).

References

Muyzer, G., De Waal, E.C, and Uitierlinden A.G.: Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA, Appl. Environ. Microb., 59, 695-700, 1993.