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HYDROTHERMAL METHANE FLUXES FROM THE SOIL AT SOUSAKI (GREECE)

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Abstract: Methane soil flux measurements have been made in 38 sites at the geothermal system of Sousaki (Greece) with the closed chamber method. Fluxes range from -47.6 to 29,150 mg m⁻² d⁻¹ and the diffuse CH₄ output of the system has been estimated in 19 t/a. Contemporaneous CO₂ flux measurements showed a fair positive correlation between CO₂ and CH₄ fluxes but the flux ratio evidenced methanotrophic activity within the soil. Laboratory CH₄ consumption experiments confirmed the presence of methanotrophic microorganisms in soil samples collected at Sousaki. These results further confirm recent studies on other geothermal systems that revealed the existence of thermophilic and acidophilic bacteria exerting methanotrophic activity also in hot and acid soils thereby reducing methane emissions to the atmosphere.

Keywords: Sousaki, accumulation chamber, soil degassing, hydrothermal systems, methane output, methanotrophic activity

1. Introduction

Methane, the most abundant hydrocarbon in the atmosphere, plays an important role in the Earth's atmospheric chemistry and radiative balance being the second most important greenhouse gas after CO_2 . It has a relatively short lifetime in the troposphere (8–12 years), but its strong IR absorption band at 7.66 µm, where water and CO_2 absorb weakly, makes CH_4 an effective contributor to the radiative forcing with a global warming potential about 21 times that of CO_2 (IPCC, 2001).

Methane is released to the atmosphere by a large number of sources, both natural and anthropogenic, with the latter being twice as large as the former (IPCC, 2001). It has recently been established that significant amounts of geological CH₄, produced within the Earth's crust, are currently released naturally into the atmosphere. The preliminary global estimate of these CH₄ emissions indicates that there are probably more than enough sources to provide the amount required accounting for the suspected missing source of global CH₄ (Etiope et al., 2008). Despite of the large number of flux measurements conducted in Europe, Asia and the USA, considerable uncertainties still affect both the total emission of geological methane and the apportioning of its sources. Among these the volcanic/geothermal source is probably one of the least constrained. Recently Etiope et al. (2007) made an effort to estimate the total methane emissions from geothermal and volcanic systems in Europe, but their provisional range (4-16 kt a^{-1}) is rather large and claims for more field measurements in order to widen the current database and decrease the present uncertainties. The same authors recognised Greece as one of the three European countries that most contribute to the release of geothermal CH₄.

Microbial oxidation in aerobic soils contributes 3– 9% of the total annual removal of CH_4 from the atmosphere (IPCC, 2001; Dutaur and Verchot, 2007). Methanotrophic bacteria in soils are unique in their ability to utilize CH_4 as a sole source of carbon and energy and Hütsch (2001) suggested that if this sink were absent, the rate of atmospheric increase would be 1.5 times greater.

In volcanic/geothermal areas diffuse degassing of endogenous gases through the soils is widespread (Chiodini et al., 2005) and in such environment soils are a source rather than a sink for CH_4 (Castaldi and Tedesco, 2005). This is due both to the emission of CH_4 among the geogenic gases and to the fact that chemico-physical conditions in the soils of volcanic/geothermal areas are not suitable for methanotrophic oxidation. The O₂ content is too low, temperature and proton activity are too high (Bender and Conrad, 1995). In recent times it has been demonstrated that methanotrophic consumption in soils occurs also under such harsh conditions. Extremely acidophilic and thermophilic methanotrophic bacteria of the phylum Verrucomicrobia have been isolated both at Campi Flegrei, Italy (Pol et al., 2007) and at Hell's Gate, New Zealand (Dunfield et al., 2007).

Previous studies (D'Alessandro et al. 2006) determined an CO₂ gas output from the Sousaki geothermal system of about 20,000 t/a almost exclusively from diffuse soil emission. The same authors made also a preliminary estimate of the CH₄ output cross-correlating the measured CO₂ soil fluxes with the CH₄/CO₂ mass ratio measured in the soil gases at 50 cm depth. The obtained value was about 36 t/a. But as evidenced at the volcanic/geothermal system of Pantelleria (D'Alessandro et al., 2009) such method is prone to overestimation of the CH₄ output because it disregards possible CH₄ consumption by methanotrophic microorganisms within the soil. In the present paper we present the results of two CH_4 flux measurement campaigns made in October 2009 and in June 2009 on a total of 38 measurement sites. During the June 2009 campaign soil samples were also collected at 9 sites. These samples were used for laboratory incubation experiments to highlight possible methanotrophic activity.

2. Study area and Methods

The Sousaki area (Fig. 1) is located about 65 km west from Athens, near the Isthmus of Corinth and represents the NW end of the active Aegean volcanic arc. Here, sparse outcrops of dacitic rocks are the remnants of late-Pliocene to Quaternary volcanic activity (4.0–2.3 Ma - Pe-Piper and Hatzipanagiotou 1997), while widespread fumarolic alteration and warm (35–45 °C) gas emissions are still recognizable. Drilling exploration assessed the presence of a low enthalpy geothermal field, revealing two permeable formations at shallow depth (<200 m) and one at deeper levels (500–1100 m). All geothermal waters are of Na-Cl type and display temperatures in the range 50–80 °C and salinities in the range 39–49 g/l (Fytikas et al., 1995).

Sampling sites for CH_4 flux measurements were selected on the basis of previous CO_2 flux meas-



Fig. 1. (a) Location of the Sousaki geothermal system with respect to the south Aegean volcanic arc (volcanoes with historical activity are evidenced with a triangle); (b) Area of the Isthmus of Corinth; (c) Study area with methane flux measurements points and main gas vents (stars). Symbols as follows: circles = sampling campaign of October 2008; squares = sampling campaign of June 2009; white symbols = negative fluxes; grey symbols = $0 - 1000 \text{ mg m}^{-2} \text{ d}^{-1}$; black symbols = > 1000 mg m⁻² d⁻¹; (d) enlargement of the high flux area.

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		position		CH ₄ CO ₂		CH ₄ CC	
site	date	easting northing		concent	ration	flux	
		UTM W	'GS84	ppı	n	mg m ⁻² day ⁻¹	g m ⁻² day ⁻¹
SOU1	9/10/2008	683400	4200670	7035	947400	7818	3290
SOU2	9/10/2008	683430	4200671	776	860100	-10.2	182
SOU3	9/10/2008	683447	4200704	2655	202500	1182	1690
SOU4	9/10/2008	683443	4200735	5751	470700	11870	2970
SOU5	9/10/2008	683433	4200740	7449	699400	16310	3930
SOU6	9/10/2008	683441	4200776	18	66900	42.5	509
SOU7	9/10/2008	683448	4200798	7676	581100	14540	4090
SOU8	9/10/2008	683427	4200821	10900	840100	17480	5350
SOU9	9/10/2008	683417	4200843	7549	568800	11720	3670
SOU10	9/10/2008	683418	4200870	5481	889000	-8.5	970
SOU11	9/10/2008	683418	4200887	12500	912600	29150	6700
SOU12	9/10/2008	683417	4200905	11700	899900	5780	2590
SOU13	9/10/2008	683424	4200919	8378	782800	-18.7	334
SOU14	9/10/2008	683428	4200945	60	247200	-15.3	58.3
SOU15	9/10/2008	683415	4200934	12700	916200	10980	2650
SOU16	9/10/2008	683405	4200922	12600	912100	18690	4540
SOU17	9/10/2008	683396	4200673	6017	796900	-6.8	378
SOU18	9/10/2008	683384	4200660	6.5	381000	-47.6	173
SOU19	9/10/2008	683347	4200626	2.5	194200	-5.9	60.7
SOU20	9/10/2008	683357	4200607	10.8	32100	-1.7	2.3
SOU21	9/10/2008	683448	4200474	2.5	59600	-3.4	77
SOU22	9/10/2008	683445	4200462	1.4	7300	-11.9	2.4
SOU23	9/10/2008	683464	4200485	2.2	651200	-2.55	383
SOU24	9/10/2008	683473	4200469	51	915300	-0.85	331
SOU25	13/10/2008	684076	4200449	0.1	44800	0	23.3
SOU26	13/10/2008	684069	4200460	0.3	7800	-0.85	2.7
SOU27	13/10/2008	684053	4200468	1	344100	68	553
SOU28	13/10/2008	684056	4200482	1985	872900	-0.85	84
SOU29	21/6/2009	683403	4200672	6510	934400	8020	3350
SOU30	21/6/2009	683425	4200665	4534	900200	11490	6950
SOU31	21/6/2009	683439	4200683	4112	812200	723	4150
SOU32	21/6/2009	683445	4200707	6034	886900	8140	4220
SOU33	21/6/2009	683434	4200744	10800	886900	18910	4810
SOU34	21/6/2009	683449	4200760	2126	737300	-8.5	42
SOU35	21/6/2009	683448	4200791	11700	880400	6640	2590
SOU36	21/6/2009	683428	4200821	11700	885100	8390	2870
SOU37	21/6/2009	683423	4200652	38	160400	-27.2	4.7
SOU38	21/6/2009	683471	4200289	15	16700	-14.4	8.2

Table 1. CH₄ and CO₂ concentrations in and fluxes from the soils of Sousaki.

urement campaigns (D'Alessandro et al. 2006). Most of the 38 CH₄ flux measurements were made within the CO₂ anomalous degassing area which extends for about 0.015 km². A few CH₄ flux measurements were made also outside the main degassing area and one farer away in an abandoned olive tree plot to get insight on background CH₄ fluxes in the area (Fig. 1).

Measurements were made with the accumulation chamber method (Livingstone and Hutchinson, 1995). The flux chamber has cross section area of 0.07 m^2 and height of 10 cm. The chamber top has

two fixed capillary tubes, one is used to collect chamber air samples and the other is used to balance the pressure between the inside and outside. Three gas samples were drawn from the headspace in the chamber at fixed intervals after the deployment (5, 10 and 15 min). The 20 mL samples are collected using a syringe and injected through a three-way valve and a needle into a 10 mL preevacuated sampling vial (Exetainer[®], Labco Ltd.). The overpressured vials were sent to the laboratory for CH₄ and CO₂ analysis.

The flux of CO₂ and CH₄ from the soil can be cal-

culated as the rate of concentration increases in the chamber:

$$\Phi = dC/dt \times V/A \ (1)$$

where Φ is the flux of a gas, V is the volume of air in the chamber (m³), A is the area covered by the chamber (m²), C is the chamber concentration of a gas and dC/dt is the rate of concentration change in the chamber air for each gas. Volumetric concentrations are converted to mass concentrations accounting for atmospheric pressure and temperature. Flux values are expressed as g m⁻² d⁻¹ for CO₂ and as mg m⁻² d⁻¹ for CH₄.

Ground temperature measurements were taken at 10 and 50 cm depth using thermal probes and a digital thermometer. Samples of soil gas were collected at each site at a depth of 50 cm through a Teflon tube of 5 mm ID using a syringe. At ten sites soil gases were collected through a special sampling device with three 2 mm ID tubes tapping soil gases at 13, 25 and 50 cm depth. Soil gas samples were collected and stored for subsequent laboratory analyses in the same way as gases from the flux chamber.

Gas concentrations were measured using the GC Perkin Elmer Clarus 500 equipped with Carboxen 1000 columns, HWD and FID detectors with methanizer. The gas samples were injected through an automated injection valve with a 1000 μ L loop. Calibration was made with certified gas mixtures. Analytical precision (±1 σ) was always better than ±5%. The detection limit for CH₄ was about 0.1 ppm.

After collection of gas samples for CH₄ flux determination, soil samples for CH₄ consumption experiments were collected at 9 of the sites in June 2009. Samples collected at 25 cm depth were stored in sterilised PP bottles. In the laboratory the soils were sieved at 2mm mesh and pH was determined in a 1:2.5 w/w soil/water suspension. To determine CH₄ consumption, soil samples (15g) were incubated in 160ml glass jars closed with neoprene rubber stoppers, with enriched atmospheric air (about 10 ppm CH₄) at nearly constant ambient temperature (22-25 °C). Gas samples, withdrawn immediately after closure and 24, 48 and 72 hours after closure, were analysed as previously described. All analyses were performed on duplicate. One of the samples did not show any detectable CH₄ after 24 hours therefore, only on this soil sample, the experiment was repeated withdrawing the gas sample 2, 4, 6 and 8 hours after closure.

The biological origin of the measured fluxes was also tested by measuring CH_4 oxidation rates on sterilized soil samples. For this purpose, soil samples in closed jars were held overnight in an oven at 110 °C. The experiments were performed on 9 soil samples and starting CH_4 concentrations were about 10 ppm CH_4 . Obtained values were expressed in pmol CH_4 h⁻¹ g⁻¹ of soil (dry weight) and negative sign indicated CH_4 consumption.

3. Results and discussion

3.1. Geographical distribution and total output

Flux values range from -47.6 to 29,150 mg m⁻² d⁻¹ for CH₄ and from 2.3 to 6950 g m⁻² d⁻¹ for CO₂. Methane fluxes showed a strongly bimodal distribution (Fig. 2) with about 45% of the sites showing negative fluxes $(-47.6 - 0 \text{ mg m}^{-2} \text{ d}^{-1})$ and as much samples showing very high values (1000 -29.150 mg m⁻² d⁻¹) and only few samples show intermediate values. The first modal population, which displays negative values, is typical of soils of the Mediterranean climate sustaining normal methanotrophic activity (Castaldi and Fierro, 2005). A few sites show extremely negative value and they are found at the periphery of the anomalous methane degassing area and close to the main gas manifestations. Maybe the peculiar environmental conditions of the area, specifically the higher than normal atmospheric methane concentrations, sustain a bacterial community capable of high methane consumption rates like those found in landfill cover soils.



Fig. 2. Percent frequency distribution of methane flux values.

The second modal population, characterized by very high values, can be referred to anomalous endogenous methane degassing. Values above 10,000 mg $m^{-2}d^{-1}$ have never been measured in volcanic/geothermal areas (Cardellini et al., 2003) but are sometimes found in regions where surface

manifestations of hydrocarbon reservoirs are found. Such high values are typical of areas in which mud volcanoes are present (Etiope et al., 2002).

The high flux areas are geographically related to the geothermal system of Sousaki. As highlighted in figure 1, the anomalous sites are close to the two main gas vents and the nearby high CO₂-flux area (D'Alessandro et al., 2006). Two minor anomalous CO₂ degassing areas display negative or low positive CH₄ fluxes. Gases emitted by the gas vents and in the soils of the anomalous degassing area have CH₄ concentrations of about 1% (D'Alessandro et al., 2006) and its isotopic composition (δ^{13} C -20.5‰ and δ D -98‰ – Jens Fiebig personal communication) is compatible with an abiogenic geothermal origin (Fiebig et al., 2009).

The total methane output of the geothermal system of Sousaki has been estimated multiplying the geometric mean of the high flux population (5300 mg m⁻² d⁻¹) by the area enclosing the most anomalous degassing sites (10,000 m²). The obtained value 19 t/a of CH₄ is lower than that estimated by D'Alessandro et al. 2006 (36 t/a) considering the measured CO₂-flux and CO₂/CH₄ concentrations in the soil. Such a difference could be explained by the methanotrophic activity within the soil that has previously been disregarded because it was considered improbable in soils affected by geothermal activity.

3.2. Methanotrophic activity within the soil

CH₄ and CO₂ fluxes display a fairly positive correlation (Fig. 3) but most of the sites display a CH₄ flux value that is lower than that expected if the CO_2/CH_4 ratio of the main gas vents, considered to be representative of the gas output of the geothermal system, would be maintained until the gas is diffusely emitted from the soil. Figure 4 shows that soil gases measured in sites characterised by high gas fluxes have a CO_2/CH_4 ratio very close to that of the main vents while where low or negative CH₄ fluxes were measured such ratio increases abruptly. The best explanation of such pattern is methanotrophic activity, which is more effective at sites where the lower flux allows a longer interaction of the gases with the microbial community within the soil.

Methane flux measurements with the closed chamber method are labour intensive procedure. Therefore data on volcanic/geothermal areas are very scarce and the assessment of the methane emis-



Fig. 3. Binary plot CH_4 fluxes vs. CO_2 fluxes. The grey line represents the CO_2/CH_4 concentration ratio in the main gas vents.

sions from these areas have been estimated crosscorrelating carbon dioxide or water vapour output data and the respectively CO_2/CH_4 or H_2O/CH_4 ratios of the gaseous manifestations which are available for many areas (Etiope et al. 2007). But such calculation is prone to overestimation because it does not consider organic methane oxidation processes within the soil.

If we apply the same method to our sampling sites the calculated methane flux values are always higher than the measured ones (Fig. 5a). Recently D'Alessandro et al. (2006) applied a similar extrapolation to the measured CO₂ soil fluxes to obtain CH₄ flux estimations. In this case instead of the CO₂/CH₄ ratio of the main gas vents the authors used the CO₂/CH₄ ratios measured at 50 cm depth in the soil. But as can be seen in figure 5b also the methane flux values calculated with this method are overestimated. This is probably due to the fact that methanotrophic activity is generally highest in the first 15 cm of the soil profile (Koschorreck and Conrad, 1993; Kruse et al., 1996).

If we use the methane flux values calculated with the two above methods we obtain methane output



Fig. 4. Binary plot CH_4 fluxes vs. CO_2/CH_4 concentration ratio in the soils at 50 cm depth. The grey area represents the range of CO_2/CH_4 concentration ratios in the main gas vents.



Fig. 5. Binary plot of measured vs. calculated CH_4 fluxes. (a) Fluxes calculated multiplying measured CO_2 fluxes by the CO_2/CH_4 ratios in the main gas vents. (b) Fluxes calculated multiplying measured CO_2 fluxes by the corresponding CO_2/CH_4 ratios in the soils at 50 cm depth.

values respectively of 52 and 47 t/a. These values are more than a factor 2 higher that the value obtained with the measured values highlighting the importance of methanotrophic activity within the soil also in geothermal areas.

3.3. Laboratory incubation experiments

Methanotrophic activity has long been considered impossible in geothermal areas because environmental conditions are sometimes inadequate or even lethal for "normal" methanotrophic microorganisms. Many study, in fact, show that methanotrophic activity decreases until it ceases with increasing temperature, acidity or sulphur gas content (Bender and Conrad, 1995). Such conditions are widespread in geothermal areas. But notwithstanding this harsh conditions methanotrophic activity has been evidenced for the first time at the geothermal area of Solfatara di Pozzuoli (Castaldi and Tedesco, 2005). The microorganism responsible for methanotrophic activity was attributed to the phylum of Verrucomicrobia (Pol et al., 2007). Methanotrophy attributed to microorganisms of the same phylum has been evidenced also at Hell's Gate geothermal system in New Zealand (Dunfield et al., 2007) highlighting their global distribution.

To ascertain the presence of methane consuming microorganisms in the soils of Sousaki laboratory incubation experiments were made on soil samples collected at 9 methane flux measurement points. All collected soil samples except one evidenced methanotrophic activity (tab. 2). Methane consumption rates ranged generally from -4.9 to -38.9 pmolCH₄ h⁻¹ g⁻¹. Such values are of the same order of magnitude as those measured in soils in temper-

ate climates (Smith et al., 2000). One soil sample (SOU34) displays a much higher consumption rate (-478 pmolCH₄ h⁻¹ g⁻¹) while only one (SOU29) displays a positive value (5.5 pmolCH₄ h⁻¹ g⁻¹) indicating methane production within the soil. All samples, after they have been autoclaved at 110 °C, showed no measurable methane consumption confirming the biological origin of this process. The only exception is sample SOU29 which shows the same positive value as the non autoclaved aliquots indicating that the methane production process is probably inorganic.

Consumption rates do not show significant relations with other measured soil parameters at Sousaki (CH₄ flux or concentration, temperature or pH). Methanotrophic activity in the soils displays generally a maximum when the soil pH is close to 7 and decreases going towards higher and lower values being normally suppressed at $pH(H_2O)$ val-



Fig. 6. Binary plot of the methane consumption rate vs. soil $pH(H_2O)$. Inset: same plot with enlarged y-axis. Error bar is shown for the consumption rates. Where not shown the error bar is less than the dimension of the symbol.

ues above 10 and below 4 (Bender and Conrad, 1995). But in the soils of Sousaki such pattern cannot be seen and significant consumption rates have been measured also in soil displaying $pH(H_2O)$ values as low as 1.56 (Fig. 6).

The soil sample SOU34 (Tab. 2), whose pH(H₂O) is 3.63, displays a consumption rate which is more than one order of magnitude higher (-478 pmolCH₄ h^{-1} g⁻¹). This value was obtained in a single experiment but is supported by other two experiments in which no measurable CH₄ was present within the vials after 24 h incubation, pointing to consumption rates of at least –224 pmolCH₄ h^{-1} g⁻¹. Furthermore a fourth experiment on an aliquot of the same soil with a starting CH₄ concentration of about 400 ppm showed a consumption rate of nearly –33,000 pmolCH₄ h^{-1} g⁻¹. Such a very high value could indicate that high methane flux areas, where this gas reaches elevated concentrations, could sustain intense methanotrophic activity.

4. Conclusions

Flux measurements at Sousaki confirmed that this geothermal system is diffusively degassing significant amounts of methane (19 t/a) through the soils. This study further confirms that volcanic/geothermal areas are non-negligible source of methane to the atmosphere. But on the other hand the present study also confirms that previous estimations of this source has been somewhat overestimated because methanotrophic activity within the soils was disregarded. Almost all previous estimation were made cross-correlating carbon dioxide or water vapour output data and the respectively CO_2/CH_4 or H_2O/CH_4 ratios of their gaseous manifestations. Such method was based on the correct assumption that normal methanotrophic microorganisms could not survive the harsh conditions (high temperature, acidity and anoxia) existing within the soils in volcanic/geothermal areas. But recent studies revealed the existence of acidophilic

Table 2. Soil parameters and results of the CH₄ consumption experiments.

	depth	CH_4	CO_2	- т°С	лЦ	CH_4 consumption (pmol g ⁻¹ h ⁻¹)	
	(cm)	concentra	ation (ppm)	- 10	pm	average	±σ
SOU29	-13	6232	932800	29.2	3.30	n.m.	
	-25	6356	929400	n.m.	5.71	5.5	2.1
	-50	6510	934400	29.6	n.m.	n.m.	
SOU30	-13	4432	889400	27.8	2.26	n.m.	
	-25	4517	914000	n.m.	2.70	-4.9	1.0
	-50	4534	900200	28.1	n.m.	n.m.	
	-13	1831	402400	29.5	7.40	n.m.	
SOU31	-25	2364	694100	n.m.	7.49	-20.9	26.5
	-50	4112	812200	31.4	n.m.	n.m.	
SOU32	-13	436	54300	32.4	7.44	n.m.	
	-25	5604	792800	n.m.	7.44	-11.8	14.0
	-50	6034	886900	33.7	n.m.	n.m.	
SOU33	-13	5054	499500	37.4	1.54	n.m.	
	-25	9870	802300	n.m.	1.56	-4.9	0.6
	-50	10800	886900	37.8	n.m.	n.m.	
	-13	424	456300	36.2	3.54	n.m.	
SOU34	-25	715	617700	n.m.	3.63	-478	
	-50	2126	737300	33.6	n.m.	n.m.	
SOU35	-13	10500	829100	33.4	7.33	n.m.	
	-25	11700	879900	n.m.	7.22	-18.5	0.02
	-50	11700	880400	29.4	n.m.	n.m.	
SOU36	-13	6104	825200	33.8	7.24	n.m.	
	-25	10800	877000	n.m.	7.30	-22.9	0.6
	-50	11700	885100	33.2	n.m.	n.m.	
SOU37	-13	33	86000	36.9	7.99	n.m.	
	-25	609	99900	n.m.	8.08	n.m.	
	-50	38	160400	29.9	n.m.	n.m.	
SOU38	-13	2.6	5800	34.8	8.32	n.m.	
	-25	3.7	6300	n.m.	8.43	-38.9	50.6
	-50	15	16700	29	n.m.	n.m.	

and thermophilic methanotrophic bacteria of a different phylum in such environments (Dunfield et al., 2007; Pol et al., 2007). In the present study, laboratory incubation experiments, confirmed the methanotrophic activity also within the soils of the geothermal system of Sousaki with sometimes very high consumption rates. Preliminary estimates indicate that methanotrophic activity within the soils at Sousaki decreases the total methane output of the system by at least a factor 2.

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