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Non-microbial methane formation in oxic soils

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Abstract. Methane plays an important role as a radiatively and chemically active gas in our atmosphere. Until recently, sources of atmospheric methane in the biosphere have been attributed to strictly anaerobic microbial processes during degradation of organic matter. However, a large fraction of methane produced in the anoxic soil layers does not reach the atmosphere due to methanotrophic consumption in the overlaying oxic soil. Although methane fluxes from aerobic soils have been observed, an alternative source other than methanogenesis has not been identified thus far.

Here we provide evidence for non-microbial methane formation in soils under oxic conditions. We found that soils release methane upon heating and other environmental factors like ultraviolet irradiation, and drying-rewetting cycles. We suggest that chemical formation of methane during degradation of soil organic matter may represent the missing soil source that is needed to fully understand the methane cycle in aerobic soils. Although the emission fluxes are relatively low when compared to those from wetlands, they may be important in warm and wet regions subjected to ultraviolet radiation. We suggest that this methane source is highly sensitive to global change.

1 Introduction

Traditionally, biogenic methane (CH_4) was thought to be formed only by methanogens under strictly anaerobic conditions in wetland soils and rice paddies, intestinal tracts of termites and ruminants, and human and agricultural waste. However, Keppler et al. (2006) demonstrated that plants produce CH_4 under aerobic conditions. Subsequently, this possibility has been critically debated (Dueck et al., 2007; Ferretti et al., 2007; Kirschbaum et al., 2007; Keppler and Röckmann, 2007; Vigano et al., 2008; Wang et al., 2008; Nisbet et al., 2009; Keppler et al., 2009; Beerling et al., 2008) and some researchers have suggested alternative explanations for the observed release of CH_4 from plants (Nisbet et al., 2009; Kirschbaum and Walcroft, 2008; Terazawa et al., 2007). Nevertheless, recent observations have provided unambiguous evidence for several pathways by which CH_4 is generated under aerobic conditions, independent of microbial activity (Wang et al., 2008; Keppler et al., 2008; McLeod et al., 2008; Cao et al., 2008; Bruhn et al., 2009; Messenger et al., 2009; Brüggemann et al., 2009; Qaderi and Reid, 2009; Althoff et al., 2010). Although details of the mechanism(s) are still unknown, methoxy groups of plant pectin have been identified in several studies as a precursor compound of aerobic CH_4 emission from detached plant matter (Vigano et al., 2008; Keppler et al., 2008; McLeod et al., 2008) (Bruhn et al., 2009). Furthermore, temperature and UV-light have been confirmed as environmental factors that control CH_4 emission from dried plant matter (Vigano et al., 2008; Keppler et al., 2008). Next to plants, saprotrophic fungi were also recently found to produce CH_4 in their own metabolism and without assistance of methanogenic archaea (Lenhart et al., 2012).

1.1 Previous observations of methane formation in aerobic soils

Whilst aerobic soils are considered to be net CH_4 sinks due to methanotrophic oxidation of CH_4, it has been shown that oxic upland forest soils produce CH_4. Although observations of CH_4 production in oxic soil are numerous (Megonigal and Guenther, 2008; Hao et al., 1988; Andersen et al., 1998; von Fischer and Hedin, 2007), all have been attributed

Table 1. Organic carbon content, pH value and CH_4 emissions from dry and wetted samples heated at 30 and 40 °C and under UV irradiation of different soils and soil components at 30 °C.

Sample			Methane emission				
			$[ng\,g^{-1}\,(dw)\,h^{-1}]$				$[\mu g\,m^{-2}\,h^{-1}]$
	pH	C_{org} [% (dw)]	Dry (30 °C)	Dry (40 °C)	Wet (30 °C)	Wet (40 °C)	UVB radiation ($2\,W\,m^{-2}$)
Sphagnum peat (PH)	3.7	49.2 %	0.05 ± 0.02[a]	0.05 ± 0.00[a]	0.19 ± 0.01	0.41 ± 0.01[a]	0.76 ± 0.24
Sphagnum peat, sterile (PHS)	3.7	49.2 %	0.11 ± 0.15	0.03 ± 0.02	0.32 ± 0.09	0.52 ± 0.03	n.m.
Deciduous forest soil O_h (SW)	7.4	23.4 %	n.d.	n.d.	0.23 ± 0.02	0.24 ± 0.06	0.25 ± 0.13
Coniferous forest soil A_h (SG)	7.2	5.0 %	n.d.	n.d.	n.d.	0.04 ± 0.01	1.73 ± 0.41
Deciduous forest soil A_h (SL)	4.4	4.0 %	n.d.	0.09 ± 0.02	0.06 ± 0.01	0.10 ± 0.04	4.92 ± 1.46
Deciduous forest soil A_h (SHA)	6.7	5.8 %	n.d.	0.08 ± 0.03[a]	n.d.	0.20 ± 0.05[a]	0.50 ± 0.13
Humic acid (HA)	5.5	43.5 %	0.06 ± 0.02	0.82 ± 0.06	0.18 ± 0.03	3.10 ± 0.34	0.80 ± 0.17
Lignin (LN)	9.6	49.5 %	0.1 ± 0.01[a]	0.33 ± 0.01[a]	0.65 ± 0.02	1.89 ± 0.20[a]	0.40 ± 0.11
Lignin sterile (LNS)	9.6	49.5 %	n.d.	0.39 ± 0.03	1.45 ± 0.48	2.70 ± 0.57	n.m.

Subscript h indicates soil horizon, C_{org} is organic carbon content, PH is peat Hille, Germany; SW is soil Häverstädt, Wiehen Mountains, Germany; SG is soil Gonsenheim, Germany; SL is soil Lerchenberg, Germany; SHA is soil Hainich, Germany; n.d. is not detectable (rate cannot be provided as increase in headspace CH_4 was less than 0.02 ppm); n.m. is not measured; [a] data from Hurkuck et al. (2012). Data show mean value ± SD ($n = 3$–5).

to methanogenesis. Methane production by oxic eubacteria (Rimbault et al., 1988) and anaerobic microsites, a refuge for methanogens (Peters and Conrad, 1995), were offered as possible explanations even though CH_4 production from eubacteria could only be detected in trace quantities. In experiments by Kammann et al. (2009), soil cores emitted up to $4.58\,\mu g\,kg^{-1}\,d^{-1}\,CH_4$ per core even after homogenization, which may be expected to lead to the destruction of anoxic microsites. Von Fisher and Hedin (2007), using stable carbon isotope studies, showed that our understanding of CH_4 formation in oxic soils is incomplete and discussed that methanogens as the sole source for CH_4 in oxic soils should be critically reviewed.

1.1.1 Possibility of non-microbial methane formation in soil

In this study we tested the previously postulated hypothesis that non-microbial CH_4 formation occurs in soils (Jugold and Keppler, 2009; Hurkuck et al., 2012). Following preliminary observations, we undertook a series of experiments measuring CH_4 formation from soils (see Table 1 and Methods section) as a function of temperature, water content and UV-B irradiation. We used five different soils, including one highly organic soil (referred to as peat, Table 1), which had been lyophilised and homogenized prior to the experiments. Humic acid and lignin were used as alternatives for soil organic matter. Additionally, subsamples of peat and lignin, sterilised using gamma radiation, were also used in our investigations. Finally, inhibitors of methanogenic microorganisms were tested in order to further prove the hypothesis of non-microbial CH_4 formation in soil.

1.2 Materials and methods

1.2.1 Origin of samples and preparation

Four soils and one peat type were used. If present, stones and larger wood particles were removed from the samples before they were lyophilised and then milled using an electronic coffee grinder (Elta UM105).

Soil SL was sampled at the Lerchenberg forest south of Mainz, Germany (49° 57′ 47″ N 8° 11′ 01″ E). The sampling site is a deciduous forest dominated by beech trees (*Fagus sylvatica*), featuring few oaks and nearly no undergrowth. The sample was collected from the surface after brushing away the layer of leaf litter.

For soil SG the upper 10 cm of a pine forest soil was sampled at Mainz-Gonsenheim, Germany (50° 0′ 24.4″ N, 8° 11′ 50.3″ E). The soil in this area is rich in medium to coarse sand and powdery clay particles. It also contains rotting wood debris, pine twigs and is densely rooted.

Soil SHA was topsoil of a *terra fusca* sampled at the *Nationalpark Hainich*, Germany (51° 04′ 46″ N, 10° 27′ 08″ E). The sampling site is a deciduous forest dominated by beech trees.

Soil SW was collected from the organic rich O-horizon of a deciduous forest soil. The vegetation is dominated by beech trees. The sampling site is situated south of Minden, Germany (52° 15′ 17.4″ N, 8° 52′ 29.5″ E).

Peat PH was sampled at the peat bog *Großes Torfmoor* near Hille, Germany (52° 19′ 23.7″ N 8° 42′ 34.7″ E). The top 10 cm of *sphagnum* peat was collected as a bulk sample. A subsample was sterilised using gamma irradiation.

1.3 Exposure to γ-radiation

Sterilisation of the soil samples was performed by exposure to γ-radiation using a ^{60}Co source (dose, 25 kGy; dose rate, 2.2 kGy h^{-1}; temperature, 4 °C).

1.4 Reaction vials

Samples were incubated in glass vials (360 ml); made in-house by modification of a 300 ml Erlenmeyer-flask (Duran group) fitted with the neck of a 40 ml screw top vial (Supelco) sealed with a hole type screw cap (Supelco) containing a PTFE/silicone septum (Supelco). The UV reaction chambers were also custom built; 200 ml glass chambers with a quartz glass lid and a septa sealed side port for headspace sampling. The irradiated surface was 19.63 cm^2.

1.5 Determination of organic carbon

Organic carbon content of the samples was determined with an SC Analyser (SC-144 DR, LECO) by combustion of 0.1–0.5 g of sample material at 1300 °C. The carbon content was calculated by comparison to a calcium carbonate standard. For soil SW, the organic carbon content was determined by loss on ignition. Therefore the weight loss after two hours at 600 °C was determined. Half of the loss was assigned to carbon combustion.

1.6 Methane measurements

Headspace above samples in the sealed vials were sampled (5 ml) with a Hamilton gas syringe and analysed using a gas chromatograph (Shimadzu GC-14B) with flame ionization detector (GC-FID). Two reference CH_4 standards (containing 8.905 ppm and 1.736 ppm) were used.

1.7 Statistical methods

The statistical comparison of different samples was examined with the software package SPSS version 20 (Chicago, IL, USA). The Student's t-test was employed to evaluate statistical difference in CH_4 content between the various inhibitor treatments. Levels of significances were defined as follows: $P < 0.001$ highly significant and $P > 0.05$ non-significant.

1.8 Experimental setups

1.8.1 Temperature dependence

Sets of non-sterile and sterile peat samples (PH, 5 g per 360 ml screw cap vial, $n = 5$) as well as non-sterile sets of each soil sample were incubated for 24 h at temperatures ranging from 30 to 90 °C at 10 °C intervals. At the end of the incubation period, a sample of the vial headspace was analysed for CH_4 content.

1.8.2 Drying-rewetting cycles

Peat PH (5 g in 360 ml screw cap vials, $n = 5$) was incubated for 24 h at either 30, 40 or 50 °C. Another set of samples was incubated under the same conditions but supplemented with 5 ml of double distilled water. After incubation a sample of the headspace was analysed for CH_4 content. The samples were frozen and lyophilised again directly after measurements. After being rewetted and incubated again, headspace samples were analysed again for CH_4. This cycle was repeated five times.

In a further experiment, dependence of CH_4 release on the water-sample-ratio was investigated. For this, samples of peat PH (5 g in 360 mL screw cap vials, $n = 5$) were supplemented with 1, 5 and 10 ml double distilled water.

1.8.3 Inhibition of methanogenic microorganisms

Non-sterile peat (PH) and lignin samples (5 g per 360 ml screw cap vial, $n = 3$) were used with the inhibitors 2-bromoethanesulfonate, (BES) and chloromethane (CH_3Cl) of methanogenic microorganisms (Chidthaisong and Conrad, 2000; Chan and Parkin, 2000). Five ml of a 10 mm BES aqueous solution was added to the sample so that the water content was 50 %. This concentration has been shown to completely inhibit methanogenesis or acetate metabolism in both pure culture of microorganisms and in environmental samples (Oremland and Capone, 1988).

Approximately 3 ml of gaseous CH_3Cl was added to the sample so that the mixing ratio in the vial was around 0.8 %. Chan and Parkin (2000) reported that at a mixing ratio of 0.1 % CH_3Cl inhibited soil methanogenesis by 89 %. The samples were incubated for 24 h at 50 °C and then a sample of the vial headspace was analysed for CH_4 content.

1.8.4 Activity of methanogenesis

For enrichment of possible methanogenic microorganisms in the soils samples, aliquots of the peat and lignin were incubated in defined, anaerobically prepared bicarbonate-buffered, sodium sulfide-reduced methanogenic mineral media (Widdel and Bak, 1992). As substrates, sterile solutions of methanol (50 mm) and acetate (10 mm) and sterile hydrogen gas (80 %) were added to each vial (100 ml). The headspace (approximately 50 ml) of the sealed vial was flushed with N_2-CO_2 to remove oxygen. Afterwards the vials were incubated for 10 days at 25 °C. The control was prepared in the same way except that sterile water was added instead of the enrichment culture.

1.8.5 Experiments with H_2O_2

Samples PH or SHA (5 g in 360 ml vials, $n = 3$) and 10 ml aqueous solution with varying concentrations of H_2O_2 (0–25 mm) were added and vials immediately sealed. The samples were incubated for 24 h at 30 ° C, after which a sample

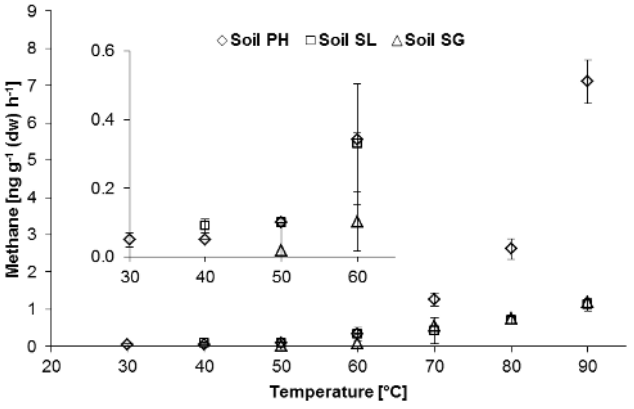

Fig. 1. Formation of CH_4 from soil with increasing temperature. Temperature dependence of CH_4 emissions from peat PH, soil SL and soil SG. Data show mean value ± SD ($n = 5$). Inset shows magnified area between 30 and 60 °C.

of the vial headspace was analysed for CH_4 content. The experiment was also repeated for lignin and humic acid with 25 mm H_2O_2.

1.8.6 UV irradiation experiments

An Osram Ultra-Vitalux lamp (300 W) served as UV source. The radiation of this lamp shows an UV-A/UV-B content comparable to solar radiation when the source is located at the appropriate distance. The total unweighted UV-B radiation was determined with a UV radiometer (UVlog, sglux, Berlin, Germany) precalibrated for the used lamp type. For more details of the lamp characteristics we refer to Vigano et al. (2008). The UV lamp was placed above the leak-tight UV reaction chambers. The height was adjusted so as to set the UV-B intensities to the desired value between 1 and 4 W m^{-2}. To exclude undesired UV-C radiation, the quartz glass lids were covered with a 95 nm film of cellulose diacetate. Two fans were employed in order to keep the temperatures in the chambers at 30 °C (±2 °C). Temperature was monitored with a thermocouple. All experiments were conducted with 2–5 g of sample material but the data is presented based on irradiated area rather than sample weight. Methane concentrations in the headspace were measured after 0, 24 and 48 h. The difference between 0 and 24 h was used to calculate emission rates.

The emissions induced solely by UV-B were calculated by subtracting the CH_4 concentration measured for the control samples from that measured for the UV irradiated samples so as to eliminate the temperature effect. The temperature monitored in the vials during UV experiments ranged from 28 to 32 °C. The control samples, which were also placed under the UV lamp, but covered with UV-opaque glass, showed emissions (transferred to ng g^{-1} (dw) h^{-1}) comparable to those observed for the temperature experiments which were incubated in the dark at similar temperatures.

1.8.7 Isotopic data

$\delta^{13}C$ sample analysis was carried out using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) which consisted of a cryogenic pre-concentration unit directly coupled to an HP 6890 N gas chromatograph (Agilent, Santa Clara, USA), which was connected to a Delta^{PLUS}XL isotope ratio mass spectrometer (ThermoQuest Finnigan, Bremen, Germany) via an oxidation reactor (ceramic tube (Al_2O_3), length 320 mm, 0.5 mm i.d., with oxygen activated Cu/Ni/Pt wires inside, reactor temperature 960 °C) and a GC Combustion III Interface (ThermoQuest Finnigan, Bremen, Germany). The gas chromatograph (GC) was fitted with a GS-Carbonplot capillary column (30 m × 0.32 mm i.d., d_f 1.5 µm; Agilent, Santa Clara, USA) and a PoraPlot capillary column (25 m × 0.25 mm i.d., d_f 8 µm; Varian, Lake Forest, USA). Both columns were coupled using a press fit connector.

A tank of high-purity carbon dioxide (carbon dioxide 4.5, Messer Griesheim, Frankfurt, Germany) with a known $\delta^{13}C$ value of –23.6 ‰(VPDB) was used as the working reference gas. All $\delta^{13}C$ values obtained from analysis of methane were corrected using three CH_4 working standards (isometric instruments, Victoria, Canada) calibrated against IAEA and NIST reference substances. The calibrated $\delta^{13}C$ values of the three working standards in ‰ vs. VPDB were –23.9 0.2 ‰ , –38.3 0.2 ‰ and –54.5 ± 0.2 ‰.

All $^{13}C/^{12}C$ -isotope ratios are expressed in the conventional δ notation in per mil versus VPDB, defined as (Eq. 1):

$$\delta^{13}C = (({}^{13}C/{}^{12}C)_{sample}/({}^{13}C/{}^{12}C)_{standard}) - 1 \quad (1)$$

2 Results

2.1 Temperature dependence

The first experiment was designed to determine the temperature dependence and the required activation energy of CH_4 formation in a deciduous forest soil (SL), a coniferous forest soil (SG) and a sphagnum peat sample (PH). Samples were incubated at temperatures ranging from 30 to 90 °C. Methane emissions reached 7.11 ± 0.59 ng g^{-1} (dw) h^{-1}, 1.19 ± 0.15 ng g^{-1} (dw) h^{-1} and 1.12 ± 0.16 ng g^{-1} (dw) h^{-1} at 90 °C for PH, SG and SL, respectively (Fig. 1). Whereas CH_4 release could be observed for PH and SL at 30 °C and 40 °C respectively (Table 1), CH_4 release from SG was only measurable above 50 °C. Soil SHA which had a similar organic carbon content to soils SL and SG (Table 1) was also investigated, and CH_4 emissions of 0.45 ± 0.02 ng g^{-1} (dw) h^{-1} at 70 °C were observed. For all samples the temperature curves showed an exponential increase of CH_4 emissions with temperature. Interestingly, the results found for the soil and peat samples (Fig. 1) showed a similar pattern to those reported by Keppler et al. (2006)

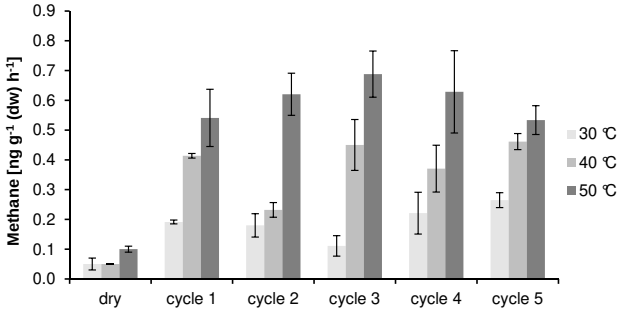

Fig. 2. Methane formation from wetted and dry peat samples. Effect of repeated wetting and drying cycles on CH_4 release from peat PH at 30, 40 and 50 °C. Data show mean value ± SD ($n = 5$).

and Vigano et al. (2008) for heated plant matter. Whereas biotically mediated reactions usually have their optimum temperatures between 25 and 40 °C (Dunfield et al., 1993) the observed strong increase in CH_4 emissions over the whole temperature range from 30 to 90 °C supports a chemically driven process. Furthermore, sterile peat samples (exposed to γ-radiation) showed similar or slightly higher emissions of CH_4 when compared to untreated peat samples. The fact that the emissions were not reduced in the sterile sample is further evidence for a non-microbial pathway. The slightly higher emissions observed for some of the sterile samples may possibly be ascribed to CH_4 production during the sterilisation process.

Since humic substances are usually the main constituents of organic-rich soils, commercially available lignin and humic acid were investigated for CH_4 release. These substances, with an organic carbon content of 49.5 % and 43.5 % respectively, when similarly heated up to 90 °C, showed even higher CH_4 emissions (at 30 °C 0.1 ± 0.01 ng g^{-1} (dw) h^{-1} for lignin and at 90 °C 18.3 ± 0.4 and 6.6 ± 0.9 ng g^{-1} (dw) h^{-1} for lignin and humic acid, respectively) than the organic-rich soil PH. The similar dependence of CH_4 formation in soils and organic soil components on temperature strongly suggests that the organic soil fraction is the source of CH_4 thermally produced in soils.

The experimental data obtained from samples SL, SG and PH were used to draw Arrhenius plots for CH_4 formation (Supplementary Fig. S1). The activation energies (E_a) for CH_4 formation, calculated from these plots, yielded values of 50.1 kJ mol^{-1} for SL, 77.5 kJ mol^{-1} for SG and 79.2 kJ mol^{-1} for PH. These activation energies, being higher than 50 kJ mol^{-1}, provide supportive evidence of an abiotic process (Schönknecht et al., 2008). Since adsorption/desorption processes of CH_4 can occur with organic materials, it was considered that in this instance, desorption might explain the observed emissions upon heating of the soil samples. Therefore, a series of experiments were performed to test such a possibility. From these it was found that a desorption process did not give rise to significant CH_4 fluxes from any of the soil samples employed in this study except when exceptionally high levels of CH_4 were added (12 500 ppm, see Supplementary Information). These results are in accordance with the findings of Kirschbaum and Walcroft (2008) who reported no significant desorption of CH_4 from plant matter and concluded that desorption is not a quantitatively important artefact contributing to observed aerobic CH_4 fluxes in dry plant leaves.

2.2 Effect of wetting and drying

Many surface soils and sediments are frequently subjected to changing precipitation and evaporation conditions and as a consequence undergo changes in water content. In extreme cases these conditions range from droughts to flooding events, including anthropogenic influences on the water budget like damming rivers or drainages for land reclamation. It is therefore important to study the effect of sample water content on the release of CH_4. This was investigated in an experiment where soil samples were exposed to repeated cycles of wetting and drying. The sample PH emitted up to five times more CH_4 after the addition of water, compared to the dried sample when incubated at the same temperature (Fig. 2). Interestingly, this increase appeared to be independent of the amount of water added, when the water content of the sample was in the range of 17 to 67 %. In a succession of five wetting-drying cycles, no decline in CH_4 release rate was observed. A highly significant rise in emissions was noted with increasing temperature ($p < 0.001$). Emissions from dry samples doubled when the temperature was increased from 30 to 50 °C and a similarly strong effect was also observed for the wetted samples at these temperatures.

2.3 Influence of methanotrophic and methanogenic microorganisms on CH_4 formation

To rule out the influence of CH_4 consuming bacteria on our findings, a selection of measurements was repeated after the addition of difluoromethane (DFM) (Miller et al., 1998) as described in the supplementary section. No differences were observed between samples with and without added DFM. Considerable CH_4 emissions could also be detected after wetting samples of lignin and humic acid, where, respectively, 1.9 ± 0.2 and 3.1 ± 0.3 ng g^{-1} (dw) h^{-1} were released (Table 1).

Although some experiments were conducted with soils that were sterilised by γ-radiation, we cannot fully exclude that methanogens contributed to CH_4 formation in the dry and wet soil samples. As discussed by Brock (1978) it is very difficult to prepare sterile soil samples. Thus we conducted further experiments to test for the possibility of methanogenic activity in the dry and wet peat and lignin samples. We added BES and CH_3Cl compounds that are known to strongly inhibit methanogenic activity in soils (Chan and Parkin, 2000; Wang et al., 2011, Chidthaisong and

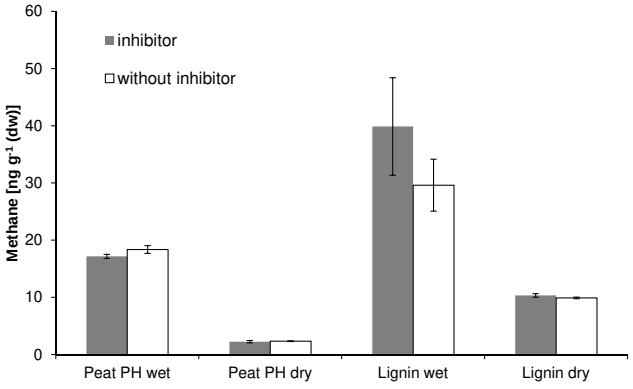

Fig. 3. Methane formation from wetted and dry peat and lignin samples treated with inhibitors of methanogenic microorganisms. Inhibitors for wet and dry samples were BES and CH_3Cl, respectively. No significant difference in CH_4 formation was found between samples treated with or without inhibitors (p ranging from >0.1 to 0.5). Data show mean value ± SD ($n = 3$). Incubation: 23 h at 50 °C.

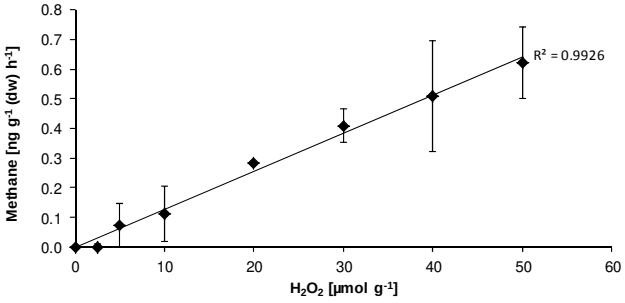

Fig. 4. Relationship between CH_4 emission from peat PH and added amount of H_2O_2. Data show mean value ±SD ($n = 3$, except 20 µmol ($n = 1$)). Incubation: 24 h at 30 °C.

Conrad, 2000) to the peat and lignin sample (homogenized and lyophilised prior to the experiments). The samples containing BES were wet whereas gaseous CH_3Cl was added to the dry samples. For all peat and lignin samples there was no significant difference (p ranging from >0.1 to 0.5) of CH_4 formation when treated with or without the inhibitors BES or CH_3Cl (Fig. 3) at a temperature of 50 °C. Analogous to the results described above (Table 1 and paragraph 3.2 Effect of wetting and drying) similar differences between emission rates of CH_4 between wet and dry samples (factor 3 to 8) were observed.

In another experiment an aliquot of the sample PH or lignin was added to an enrichment culture known to enrich the growth of methanogenic archaea. When samples with or without enrichment culture were compared, no difference in CH_4 formation was measured after an incubation period of 4 days at a temperature of 25 °C. Moreover, no further increase in CH_4 formation was noted when samples were incubated for a longer time period.

These results provide strong support that neither methanotrophs nor methanogens were active in the soils investigated in this study and that CH_4 formation was solely driven by a chemical process.

2.4 Effect of hydrogen peroxide

Reactive oxygen species (ROS) such as hydroxyl radicals ($HO^{\bullet}$) have been suggested to play an important role in the release of CH_4 from pectin and might be the driving force in the CH_4 release during UV radiation of plant foliage (McLeod et al., 2008; Messenger et al., 2009). Hydrogen peroxide (H_2O_2) as a precursor of $HO^{\bullet}$ is an important reactant in many degradation processes in soils, being abundant due to its release by roots, soil bacteria and white rot fungi (Frahry and Schopfer, 1998; Kersten and Kirk, 1987). We therefore investigated the influence of H_2O_2 on CH_4 emissions from peat PH and soil SHA.

Interestingly, it was found that peat and soil responded rather differently following addition of H_2O_2. A strong increase in CH_4 emissions and a linear relationship ($R^2 = 0.99$) with increasing amounts of added H_2O_2 to sample PH (Fig. 4) was observed whereas for soil sample SHA no additional emissions were observed. It is not clear why the soil and peat samples behaved so differently to the addition of H_2O_2. One possible explanation might be related to the differences in the composition of soil SHA and peat PH. Peat consists mostly of organic matter and low mineral content, which might make it more prone to be attacked by ROS. Soil, on the other hand, contains other major components such as clay minerals and metal oxides that might more efficiently interact with H_2O_2.

Samples of lignin and humic acid were also treated with H_2O_2. Whereas increased CH_4 emissions were observed for humic acid, no elevated emissions were found for lignin. Thus it is evident that the structural composition of the organic matter in soil has a major impact on the CH_4 emissions.

2.5 Effect of ultraviolet radiation

Ultraviolet (UV) radiation has been shown to be an important factor for aerobic production of CH_4 from plant tissues and pectin. It was demonstrated that both UV-A (320–400 nm) and UV-B (280–320 nm) induce CH_4 emissions from plant tissue (Vigano et al., 2008; McLeod et al., 2008), with UV-B radiation showing a much stronger effect. Nevertheless, because average UV-A intensities are around 30-fold higher than UV-B values, UV-A is also an important component on a global level for UV-induced CH_4 emissions (Bruhn et al., 2009). Thus, the effect of UV radiation on the formation of CH_4 from soil was evaluated. For most experiments we used a total UV-B irradiance of 2 W m^{-2}, typical for mid-latitudes at the surface. In the tropics, where the UV-filtering ozone layer is thinner, ambient UV-B irradiances are about 3.7 W m^{-2} (Bernhard et al., 1997).

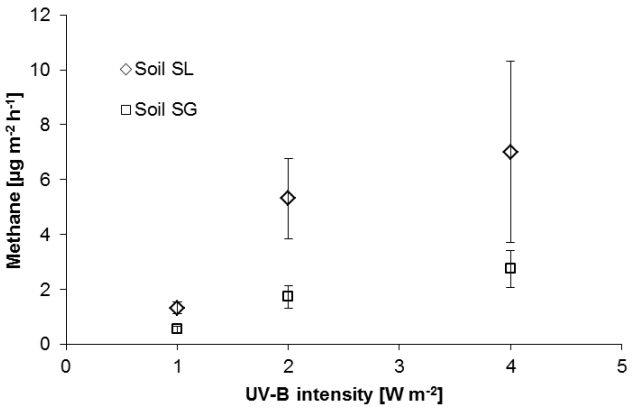

Fig. 5. Relationship between CH_4 emissions from soils SL and SG and UV-B intensity. Data show mean value ± SD ($n = 3$).

Measurements at 2 W m^{-2} UV-B and temperatures of 28 to 32 °C showed emissions of 0.25 to 4.92 µg m^{-2} h^{-1} (Table 1), which were linear over a two-day period. Methane emission rates were also found to be a function of UV-B intensity. With increasing intensities from 1 to 4 W m^{-2}, CH_4 emissions from soil SL increased linearly from 1.33 ± 0.22 to 7.28 ± 2.75 µg m^{-2} h^{-1}. Emissions from soil SG increased from 0.56 ± 0.12 to 2.75 ± 0.69 µg m^{-2} h^{-1} over the same intensity range (Fig. 5).

The combined emission rates under the influence of UV and temperature are similar to those reported for plant foliage (Vigano et al., 2008; Keppler et al., 2008). Interestingly, variations in CH_4 emissions under UV are not correlated to soil organic content (Table 1). However, the emission rates might be influenced by organic photo sensitizers, which have been shown to have a positive effect on CH_4 emissions from pectin (Messenger et al., 2009), or by clay minerals, often described as photo-catalysts (Katagi, 1990; Wu et al., 2008; Kibanova et al., 2011).

2.6 Stable carbon isotope composition of methane emitted from soil

In addition to CH_4 emission rates, the stable isotope composition ($\delta^{13}C$ values) of the released CH_4 from soil SHA, peat PH, humic acid and lignin were also measured. Heating experiments showed $\delta^{13}C$ values of –56 to –65 ‰ for lignin, –51 to –56 ‰ for PH and –42 to –52 ‰ for humic acid. Methane emitted from wet samples of lignin, humic acid and peat PH showed $\delta^{13}C$ values ranging from –53 to –69 ‰ with humic acid again being the substrate with the highest (less negative) CH_4 values (–53.2 ‰ ± 0.3 ‰). The $\delta^{13}C$ values measured for CH_4 emitted from humic acid and peat PH over a 24 h period following the addition of H_2O_2 were -54.9 ± 1.2 ‰ and -60.2 ± 4.5 ‰, respectively.

The $\delta^{13}C$ values measured for CH_4 emitted during 48 h under UV irradiation were -56.0 ± 6.0 ‰ for lignin, -63 ± 3.3 ‰ for SHA, -44.2 ± 1.4 ‰ for PH and -35.3 ± 9.4 ‰ for humic acid. In summary, the $\delta^{13}C$ values of CH_4 emitted from soil differed between substrates and experimental conditions and ranged from –35.5 to 69 ‰, whereas the $\delta^{13}C$ values for the organic matter of the bulk soil samples were in the range of –22 to –29 ‰. Thus, it appears that all treatments caused substantial fractionation between the precursor carbon and emitted CH_4. Similar $\delta^{13}C$ values and isotope fractionations have been reported for CH_4 emitted from plant foliage due to UV radiation or upon heating (Vigano et al., 2009). Both the isotopic values reported for the chemical formation of CH_4 from soil and vegetation are commonly also found for terrestrial biogenic sources (Vigano et al., 2009).

3 Conclusions and outlook

Our study shows that several hitherto unknown processes exist that produce CH_4 in soil and peat, which is clearly not related to methanogenic activity. Figure 6 summarizes our results regarding non-microbial CH_4 formation in the aerobic layers of soils and the environmental factors that might control emissions. From our findings we suggest that the abiotic formation of CH_4 through degradation of organic soil matter represents a thus far undiscovered pathway for CH_4 formation in oxic soils. Our results imply that there are at least two different mechanisms for non-microbial CH_4 formation in soils. This can be best distinguished by comparing thermal and UV-B induced CH_4 release. Samples that released only minor amounts of CH_4 when heated or wetted emitted significant amounts when irradiated with UV-B, and vice versa.

The amounts of CH_4 produced at ambient temperatures of 30 °C are small but increase considerably with increasing temperature. Wetted samples during the drying and rewetting cycle experiments showed much higher emissions than the dry sample itself at low temperatures. Assuming that the first five centimetres of the soil horizon account for most of the CH_4 production, the emission rates from dry and wet soil at 30 to 40 °C (Table 1) would correspond to emission rates of 0 to 18 µg m^{-2} h^{-1}, assuming a dry bulk density of 1.5 g cm^{-3} for soil and 0.1 g cm^{-3} for peat (Minkinnen and Laine, 1998). These emissions increase up to an order of magnitude when the soil surface temperature reaches 50 to 70 °C. Although these temperatures are often only observed at soil surfaces in tropical and savannah regions, when compared to field measurements from wetlands with observed CH_4 emissions up to 11.9 mg m^{-2} h^{-1} (286.5 mg m^{-2} d^{-1}) and calculated average emission rates of 2.1 mg m^2 h^{-1} (51 mg m^{-2} d^{-1}) (Morrissey and Livingston, 1992; Roulet et al., 1992; Cao et al., 1998), these are relatively minor emissions. The CH_4 emissions under UV light are consistent with findings by Vigano et al. (2008) and McLeod et al. (2008), who showed that UV irradiation drives CH_4 production from dried plant matter. Thus soil organic matter is most likely the precursor of CH_4 emissions observed in our studies. This is

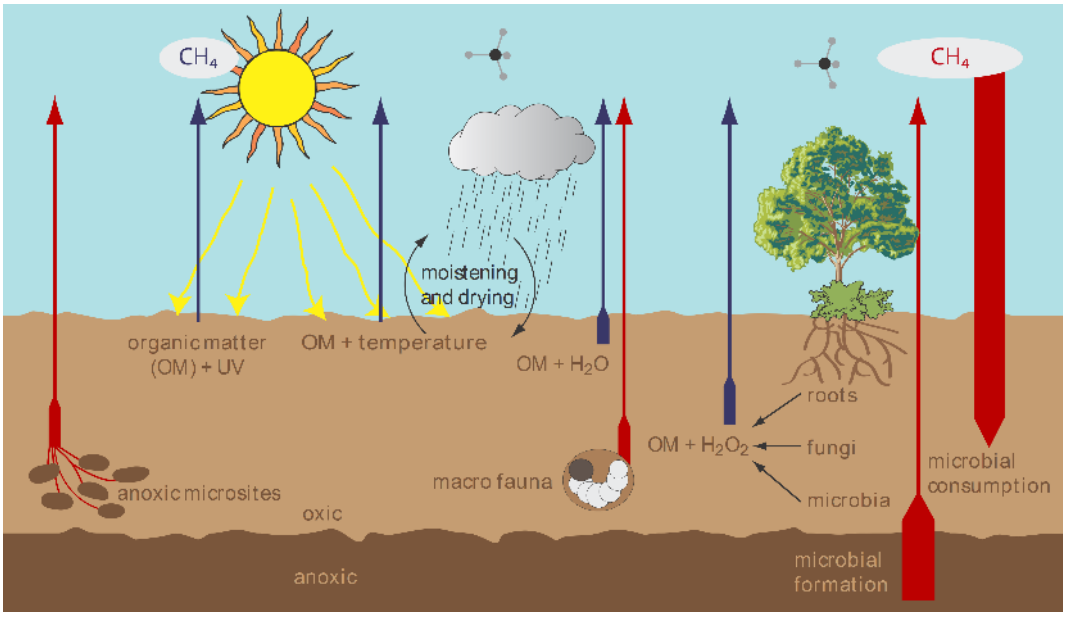

Fig. 6. Scheme of CH_4 cycling in soil including non-microbial (blue) and the previously known microbial sources (red). Environmental factors such as temperature, UV irradiation, drought/wet cycles and formation of hydrogen peroxide produced by biota might control chemical formation of CH_4 in soil.

supported by CH_4 emissions that were observed when lignin and humic acid were exposed to UV irradiation under the same conditions as that for the soil samples. However, it is interesting that under UV irradiation there was no apparent correlation between CH_4 production and the soil organic matter content. This indicates that other soil components also play a role in CH_4 formation. Organic photo-sensitizers such as tryptophan (Messenger et al., 2009) or the mineral soil fraction, e.g., clay minerals and metal oxides (Katagi, 1990; Wu et al., 2008; Kibanova et al., 2011) may catalyze surface reactions of organic matter leading to CH_4 formation. This would also be in agreement with the recent observation that meteoritic matter, such as carbonaceous chondrites, which contain only a few per cent organic matter, releases large amounts of CH_4 when exposed to UV irradiation (Keppler et al., 2012).

Methane emissions under UV radiation were found to be in the range of 0.25 to 7.28 $\mu g\, m^{-2}\, h^{-1}$ for various soils in the UV-B intensity range of 1 to 4 $W\, m^{-2}$. Again, these emission rates are considerably lower than emissions observed from natural wetlands (Morrissey and Livingston, 1992; Roulet et al., 1992; Cao et al., 1998). Further studies on samples collected from different vegetation zones, including subtropical and tropical regions, would be required to better estimate the global implications of our findings. A large fraction of the terrestrial surface is directly exposed to UV radiation, and this might even increase due to anthropogenic activities leading to deforestation and desertification. Interesting regions for on-site studies of UV-induced CH_4 release could then be steppes regions, newly deforested land, and freshly ploughed fields, whereas for water-mediated CH_4 release flooding plains and irrigation areas in dry climates would be relevant. However, it has to be considered that more than 90 % of CH_4 formed within soils is oxidised by methanotrophic bacteria before it reaches the atmosphere (King, 1990). Methane uptake into aerated temperate forest soils ranges from 10 to 204 $\mu g\, m^{-2}\, h^{-1}$, depending on soil type, temperature and water saturation (Born et al., 1990; Castro et al., 1995; King, 1997). Field measurements regarding the temperature and water-mediated CH_4 emissions may thus be impaired by methanotrophic consumption. In contrast, direct photolysis of soil organic matter will occur at the upper soil surface at maximum depths of 0.2 to 0.4 mm and indirect photolysis processes might affect the soil down to 2 mm depth (Hebert and Miller, 1990). Thus CH_4 formation induced by UV irradiation at the soil surface might lead to direct CH_4 emissions to the atmosphere.

Hydrogen peroxide was found to have a positive effect on CH_4 production from peat. Levels of H_2O_2 in soils are influenced by the activity of plant roots, fungi and bacteria (Schönknecht et al., 2008; Miller et al., 1998). As the release of H_2O_2 from living organisms is often a defence mechanism, the amount released might be affected by organism density in the soil and the level of stress applied by (changing) environmental factors.

The chemical CH_4 formation from organic soil components observed in this study might be only one of several CH_4 formation pathways that occur in aerated soils. Further sources involve the degradation of organic matter by saprophytic fungi (Lenhart et al., 2012), methanogenic archaea in anoxic microsites (Kammann et al., 2009), and

biological soil crusts (Angel et al., 2011). However, presently our knowledge on the (bio)chemical CH_4 formation processes behind all identified sources are limited, therefore it is much too early to speculate about the contribution of the various sources to the release of CH_4 to the atmosphere. The amount emitted by various sources to the atmosphere will be affected to a different extent by chemical, physical and biochemical environmental factors like UV radiation, temperature and moisture.

For example, soil moisture will not only affect the CH_4 release from chemical degradation of organic soil compounds and from fungi but will also affect oxygen concentration and therefore anoxic microsites where methanogenesis takes place. Thus, it will be a challenge to differentiate between the microbial and non-microbial sources of oxic soils in the field.

All effects shown to increase CH_4 production from oxic soils might gain importance in the course of climate change considering predicted changes in temperatures, precipitation levels and evaporation rates. Flood plains and other environments with strong fluctuations in the water budget might be of particular interest. Further investigations will be essential to fully understand the biogeochemical cycle of CH_4 in oxic soils and its relevance for the atmosphere and to gain further information on the chemical pathways involved. For the latter employing isotopically labelled precursor compounds would be beneficial. In particular identification of the differences between the pathways of thermal and photocatalytic CH_4 generation would be worthwhile for future investigations.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/5291/2012/bg-9-5291-2012-supplement.pdf.

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