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Microbial versus non-microbial methane releases from fresh soils at different temperatures

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ABSTRACT

Methane (CH_4) production in soils can occur by microbial and non-microbial processes. We postulated that there exist the mixed microbial and non-microbial CH_4 emissions from fresh soils in nature. To test both emissions and their importance, this study examined CH_4 releases from fresh soils of forest, orchard, croplands, grasslands, and wetland. By designing the treatments with or without inhibitor(s) in the laboratory conditions, we used inhibition method to compare/distinguish microbial and non-microbial CH_4 releases from fresh soils at a series of temperatures. Microbial CH_4 release occurred mainly in wetland soils and moist upland soils, with the peak rates of 10^1–10^3 ng gdw^{-1} h^{-1} around 40 °C. Non-microbial CH_4 release occurred mainly in upland soils and usually increased with temperature, showing negligible rates at ambient temperatures of 0–40 °C and detectable rates of approximately 0.2–0.7 ng gdw^{-1} h^{-1} at high temperatures of 50–70 °C. Microbial CH_4 release was much more important than non-microbial CH_4 release from fresh soils at different temperatures, when all land uses were considered together. In nature, soils are frequently exposed to various forms of environmental stress. Besides temperature fluctuation examined in the present study, solar ultraviolet radiation, soil water deficit and flooding, hypoxia and hyperoxia, tillage, and herbicide may also affect non-microbial CH_4 production. Thus, more measurements are required for understanding the contribution of non-microbial CH_4 emission to the total from soils.

1. Introduction

Methane (CH_4) is traditionally thought to originate from organic matter degradation via complex microbial processes. The microbes involved are a limited group of obligate prokaryotes called methanogenic archaea that thrive under anoxic condition and are phylogenetically distinct from bacteria and eukarya (Conrad, 1996, 2005). Microbial CH_4 production and emission have widely been observed in soils over the past decades (Conrad, 2009). Non-microbial CH_4 is produced from organic compounds by instant reactions under no enzymatic catalysis of methanogenic archaea (Wang et al., 2013a) and has also widely been observed in nature (Bousquet et al., 2006; Denman et al., 2007; Etiope, 2012). The global CH_4 emission was estimated at 582 Tg yr^{-1} over the 2000–2004 period (Denman et al., 2007), of which microbial and non-microbial CH_4 emissions would account for approximately 60% and 40%, respectively (Wang et al., 2013a). Besides the known sources of non-microbial CH_4 such as energy usage, biomass burning, and geological events, its production and emission have recently been observed from plants (Althoff et al., 2014; Bruhn et al., 2014; Keppler et al., 2006; Wang et al., 2008; Wang et al., 2011a, b), animals (Ghyczy et al., 2003, 2008), fungi (Lenhart et al., 2012), cryptogamic covers (Lenhart et al., 2015), and soils (Hurkuck et al., 2012; Jugold et al., 2012; Wang et al., 2013b). However, current estimates on non-microbial CH_4 emission in terrestrial ecosystems are highly uncertain (Wang et al., 2013a) and could be negligible.

In order to study non-microbial CH_4 in soils, previous studies have used sterilization such as autoclaving and/or ultraviolet (UV) radiation to ensure the CH_4 released be non-microbial (Hurkuck et al., 2012; Jugold et al., 2012; Wang et al., 2013b). However, sterilized soils do not occur in nature while the results obtained from sterilized soils cannot be extended to fresh soils in the field. Sterilization method cannot be used to compare/distinguish microbial and non-microbial CH_4 releases and evaluate their relative importance in soils. In this study, we used inhibition method as a new approach to test the importance of non-microbial CH_4 relative to microbial CH_4.

In soils microbial CH_4 production rate usually shows an exponential relationship with temperature, with the rate peak corresponding to the temperatures of 25–30 °C (Dunfield et al., 1993). Plant enzymes are generally denatured above the threshold of around 50 °C (Berry and Raison, 1981). The upper temperature for enzymatic metabolisms of methanogenic archaea in terrestrial ecosystems may be assumed around 50 °C, exceeding which enzymes would be denatured. Previous studies found that non-microbial CH_4 production increased with

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temperature between 30 and 70/90 °C in soils (Hurkuck et al., 2012; Jugold et al., 2012; Wang et al., 2013b). Soils as an important component of terrestrial ecosystems may provide a case study for testing the responses of microbial and non-microbial CH_4 releases to temperature in laboratory conditions and further understanding the coexistence and/or alternation of both emissions in the field. We hypothesized that microbial and non-microbial CH_4 releases in response to temperature in soils would roughly follow the following pattern: no CH_4 release at/below freezing temperature, microbial CH_4 release between 0 and 30 °C, coexistence of microbial and non-microbial CH_4 releases between 30 and 50 °C, and non-microbial CH_4 release above 50 °C.

The objective of this study is to compare/distinguish microbial and non-microbial CH_4 releases from fresh soils of various land uses. We would address such questions: how much are microbial and non-microbial CH_4 releases from fresh soils at different temperatures? Is non-microbial CH_4 release from fresh soils more or less important relative to microbial release, when all land uses are considered together?

2. Materials and methods

2.1. Soil sampling

Soils were collected from forest, orchard, croplands, grasslands, and wetland in northern China in the summer 2014. The sampling sites are located in the semiarid temperate climatic zone. Specifically, forest soils were sampled in the Beijing Forest Ecosystem Research Station, where dominant plant species were deciduous broad-leaved trees. Orchard soils were sampled in a vineyard of the Institute of Botany, Chinese Academy of Sciences. Cropland soils were sampled in wheat-corn rotation fields in Beijing and Hebei. Grassland and wetland soils were sampled in Inner Mongolia. A detailed description on the sampling sites and their plant species and soil characteristics is listed in Table 1.

In nature, the 0–5 cm surface soils exposed to the atmosphere are generally susceptible to temperature. Accordingly, fresh surface soils collected from all land uses were examined (Table 1). However, wetlands are an important land use for the global CH_4 budget and thus subsurface soils of 5–15 cm were also collected from the mire in IMGERS (Inner Mongolia Grassland Ecosystem Research Station) to compare CH_4 releases between two soil layers (Table 1). For each land use, soils were randomly sampled using a stainless steel corer (3.5 cm in diameter) in ten locations and then mixed to form a composite sample for each layer. The soils in each upland site were sampled within an area with about 100 m in diameter, whereas the sampling area in the mire site was adjusted to adopt the specific landform. Soils in all land uses were briefly processed in the field such as breaking cores and removing gravels and litter, and then put into polyethylene bags and taken to laboratory. In the laboratory, soils were further processed to remove small gravels and litter via sieving through a 2 mm mesh and then stored in polyethylene bags at 0–4 °C refrigerator in the dark prior to analysis. Accordingly, no intact soil cores were used for assays. Laboratory incubation and measurements were accomplished within two weeks since soil collection in each site.

2.2. Experimental treatments

Two groups of microbes, methanogenic archaea and methanotrophic bacteria, are important in determining net CH_4 releases from fresh soils. As a structural analog of coenzyme M ($HSCH_2CH_2SO_3^-$), BES (2-bromoethanesulfonate) as specific inhibitor has widely been used for inhibiting microbial CH_4 production (Conrad et al., 2000; Liu et al., 2011). Halogenated aliphatic hydrocarbons, such as chloroform ($CHCl_3$), fluoroacetate (FCH_2COO^-) and methyl fluoride (CH_3F), are nonspecific inhibitors but can also effectively inhibit microbial CH_4 production (see Liu et al., 2011). Gaseous chloromethane (CH_3Cl) is a halogenated hydrocarbon and was previously proved to be effective in inhibiting microbial CH_4 production in a landfill cover soil (Chan and Parkin, 2000) and a wetland soil (Wang et al., 2011a). Gaseous difluoromethane (CH_2F_2) may be employed as inhibitor to inhibit microbial CH_4 oxidation (Miller et al., 1998). The inhibited effects of CH_3Cl and CH_2F_2 respectively on the production and oxidation of microbial CH_4 were realized via their disturbance on enzymatic metabolisms of the microbes (Bédard and Knowles, 1989; Oremland and Capone, 1988). However, non-microbial CH_4 is produced under no enzymatic metabolisms of the microbes (Wang et al., 2013a). Previous study also suggested that the inhibitors did not influence non-microbial CH_4 production in soils (Jugold et al., 2012). In this study, we attempted to use gaseous CH_3Cl and CH_2F_2 as inhibitors. By designing the treatments with or without inhibitor(s) incubated in a series of temperatures, we used inhibition method to compare/distinguish microbial and non-microbial CH_4 releases from fresh soils of various land uses. Eight temperatures may be classified into ambient (0, 10, 20, 30, and 40 °C) and high (50, 60, and 70 °C) levels.

Specifically, we designed the following treatments (T): parallel blank for determining whether background CH_4 concentrations in the serum bottles changed in absence of soil sample and inhibitor (T0), soils for measuring net CH_4 release (T1), soils + CH_2F_2 for measuring gross microbial and non-microbial CH_4 releases by inhibiting microbial CH_4 oxidation (T2), soils + CH_3Cl for measuring net non-microbial CH_4 release by inhibiting microbial CH_4 production (T3), and soils + CH_3Cl + CH_2F_2 for measuring gross non-microbial CH_4 release by inhibiting both production and oxidation of microbial CH_4 (T4). Generally, T0 showed undetectable change in CH_4 concentrations during incubation and was omitted for clarity purpose.

When microbial CH_4 release was negligible at the temperatures of 20–40 °C, it was assumed to be negligible at the other temperatures, since microbial CH_4 production is generally maximal at ambient temperatures favoring methanogenic archaea. When microbial CH_4 oxidation was negligible and not considered statistically different from zero, gross microbial/non-microbial CH_4 release was assumed to be equal to net microbial/non-microbial CH_4 release. When non-microbial CH_4

Table 1
Plant species and soil characteristics in the sampling sites.

Land type	Land use	Sampling date	Sampling site (coordinate)	Dominant plant species	Soil depth (cm)	Soil moisture (%)	pH	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)
Wetland	Mire in IMGERS	July 20	43°37.0′N, 116°42.0′E	*Carex dahurica, Glyceria spiculosa*	0–5	30.4	5.5	15.4	1.7
					5–15	34.4	6.2	12.5	1.2
Upland	Forest	June 11	43°56.0′N, 115°50.4′E	*Larix leptolepis, Populus tomentosa*	0–5	13.0	6.9	48.4	4.0
	Orchard	May 10	39°59.8′N, 116°12.4′E	*Vitis vinifera*	0–5	16.9	6.6	11.8	1.3
	Cropland in Cuihu	July 5	40°06.4′N, 116°10.5′E	*Triticum aestivum, Zea mays*	0–5	5.2	7.3	9.6	1.1
	Cropland in Guan	July 13	39°21.0′N, 116°19.0′E	*Triticum aestivum, Zea mays*	0–5	10.9	7.6	10.1	1.2
	Grassland in Keyouzhongqi	June 13	44°33.0′N, 122°04.4′E	*Leymus chinensis, Koeleria cristata*	0–5	20.9	7.2	25.8	2.7
	Grassland in IMGERS	July 19	43°33.0′N, 116°40.0′E	*Leymus chinensis, Stipa grandis*	0–5	7.2	6.8	26.4	2.7

The pH, organic carbon and total nitrogen were measured by the use of air-dried soils. For the purpose of clarity, abbreviations are used for soil organic carbon (SOC) and total nitrogen (TN).
IMGERS is the Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences (see the description on IMGERS in Wang et al., 2008).

release was negligible and not considered statistically different from zero, furthermore, the T1 and T2 were considered as microbial CH_4 release. For most of fresh soils at the temperatures of 0–40 °C, the T1 and T2 represented (net) microbial CH_4 release while the T3 and T4 represented (net) non-microbial CH_4 release. At high temperatures of 50–70 °C, however, enzymatic denature of the microbes occurred and accordingly detectable CH_4 released in the T1, T2, T3, and T4 were assumed to be non-microbial.

2.3. Laboratory incubation

About 10 g of fresh soils were transferred to a 120-mL serum bottle, which was then sealed with a high temperature resistant butyl rubber stopper (diameter 20 mm). Flushing is an effective way for obtaining anoxic condition in the serum bottle (Wang and Ineson, 2003; Wang et al., 2011a). Soils sealed in serum bottles were flushed with CH_4-free nitrogen (0% O_2) at a rate of about 500 mL min^{-1} for 6 min using 'inlet-outlet' needles inserted through the stoppers. Accordingly, initial CH_4 concentrations were regarded as zero in anoxic treatments. In contrast, initial CH_4 concentrations were about 2.0 μL L^{-1} in oxic treatments that were not flushed. CH_3Cl and CH_2F_2 as inhibitors were gaseous and can rapidly diffuse into soil pore space. Diffusion of CH_3Cl and CH_2F_2 could be slowed when soil moisture is high, but the slowed diffusion would be still much shorter relative to incubation time. CH_3Cl and/or CH_2F_2 were injected into the sealed serum bottles using a syringe immediately prior to incubation. Previous study observed that CH_4 uptake by upland soils was completely inhibited by 0.003%–0.03% (v/v) of CH_2F_2 while CH_4 release from wetland soils was mostly inhibited by 0.1% (v/v) of CH_3Cl (Wang et al., 2011a). In this study, the 0.8% or 2.4% (v/v) of CH_3Cl and/or CH_2F_2 employed were assumed to completely inhibit microbial CH_4 production and/or oxidation. At least purities of 99.9% CH_3Cl and 99.5% CH_2F_2 were obtained from the Beijing AP-BAIF Gases Industry Co., Ltd. The samples were incubated in growth chambers at different temperatures in the dark. Gas samples were extracted from the serum bottles after appropriately 0, 24, and 48 h incubation for the measurements of CH_4 concentration. At the end of the incubation, soil dry weight was determined by oven drying at 105 °C to a constant weight.

2.4. CH_4 concentration measurement

The CH_4 concentrations in gas samples were measured using a Hewlett-Packard 5890 Series II Gas Chromatograph. The running conditions of the GC were described in Wang et al. (2009). Certified CH_4 standard in 2.0 μL L^{-1} (the Beijing AP-BAIF Gases Industry Co., Ltd) was used for calibration.

2.5. Soil characteristics measurement

Air-dried soils were sieved through a 2 mm mesh and ground to fine powder (mesh number 100) with a pestle and mortar. Air-dried soil characteristics were determined by the use of standard procedures (Liu, 1996). Soil pH was measured by shaking 5 g of soils in 25 mL deionized water, using a combination glass electrode (Sartorius PB-10). Soil organic carbon (SOC) was determined using the combustion method. First, 5 mL 0.8-M $K_2Cr_2O_7$ solution and 5 mL H_2SO_4 were added into the weighed soil and then boiled at 170–180 °C for 5 min, and then the remaining $K_2Cr_2O_7$ was titrated using 0.2-M $FeSO_4$. Total nitrogen (TN) was determined by the semi-micro Kjeldahl method (FOSS-2200).

2.6. Statistical analysis

CH_4 concentration was adjusted for prevailing temperature and atmospheric pressure according to the ideal gas law. When CH_4 concentrations linearly changed over approximately 48 h in various treatments of an experiment, CH_4 release rates were calculated by linear regressions of CH_4 concentrations at 3 time points of approximately 0, 24 and 48 h ($R^2 \geq 0.9$). When CH_4 release rates were negligible or unreadable, it is difficult to obtain statistically significant linear regressions and thus R^2 values were not used as judge parameter, where negligible release rates only had symbolic significance. In fresh soils of some land uses, however, CH_4 release would not follow linear change over time. For instance, CH_4 release from fresh soils of wetland showed exponential kinetics, presumably as the methanogens multiplied with time (Wang et al., 2011a). When CH_4 concentrations non-linearly changed over approximately 48 h, CH_4 release rates were calculated directly by the use of CH_4 concentrations at approximately 24 and 48 h. Value is mean ± 1 standard deviation ($n = 4$). A positive value indicates a net CH_4 release while a negative value represents a net CH_4 uptake.

Statistical analysis was performed by the use of a Statistical Analysis System program (SAS Institute, 1999). Duncan's multiple range test was employed to compare CH_4 release rates among each group of treatments at some temperature ($P < 0.05$). The different letters indicated significant differences among each group of treatments. When there was no statistically significant difference among each group of treatments, no letters were marked for the purpose of clarity. When CH_4 release rates shown in the figures were negligible and unreadable,

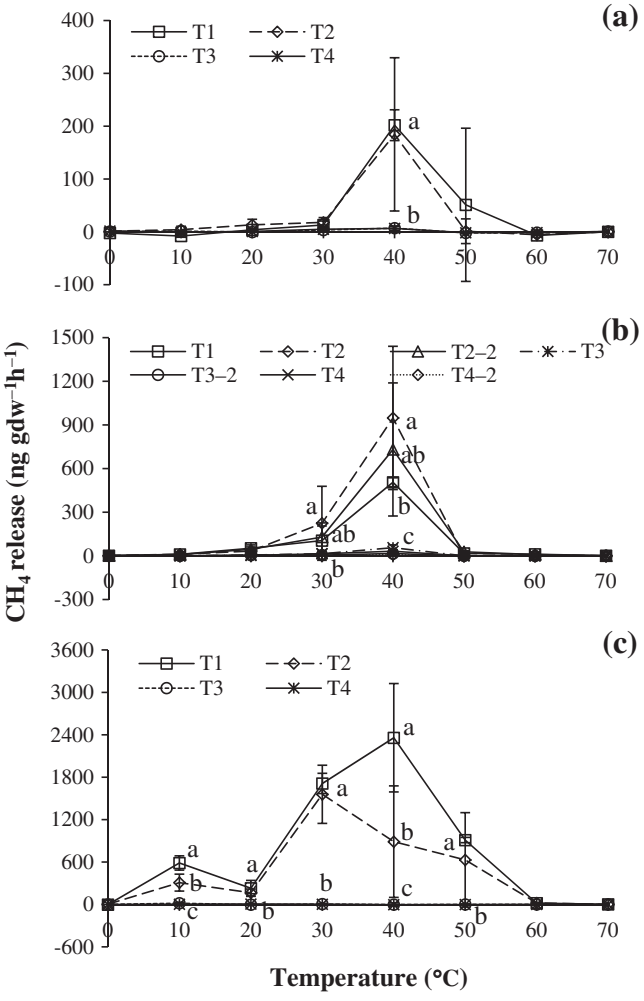

Fig. 1. CH_4 releases in fresh soils of wetland incubated under oxic or anoxic condition. (a) Oxic, surface soils, (b) anoxic, surface soils, and (c) anoxic, subsurface soils. Four treatments, T1, T2, T3, and T4, were described in the text. However, T2–2, T3–2, and T4–2 (b) were similar treatments as T2, T3, and T4, respectively, but had CH_3Cl concentration of 2.4% (v/v). The CH_4 release rate is mean ± 1 standard deviation ($n = 4$). The different letters indicate statistically significant differences ($P < 0.05$) in each group of treatments. When two or multiple treatments had no significant difference and were not easily separated, one same letter was marked.

statistical test was not conducted even if the release rates would have statistically significant differences among each group of treatments.

3. Results

3.1. Microbial CH_4 release

Microbial CH_4 release occurred mainly in the T1 and T2 of wetland soils, with peak rates around 40 °C, whereas it was completely inhibited by CH_3Cl in the T3 and T4 (Fig. 1). Specifically, microbial CH_4 release reached approximately 200 ng gdw^{-1} h^{-1} from fresh surface soils of wetland under oxic condition. Microbial CH_4 had similar release pattern under oxic and anoxic conditions and was much stronger release from subsurface than surface soils under anoxic condition. However, microbial CH_4 release was negligible at 0, 60, and 70 °C.

Soil moisture was markedly different in various land uses; particularly, grassland soils in Keyouzhongqi had the highest moisture of 20.9% among upland soils (Table 1). Because the mire in IMGERS was sandy, its SOC was much lower when compared with that in the forest (Table 1). Microbial CH_4 release was observed in the T1 and T2 in moist surface soils of upland grassland in Keyouzhongqi, with the peak rate of 5–6 ng gdw^{-1} h^{-1} around 40 °C (Fig. 2b), whereas detectable microbial CH_4 release was not observed in surface soils of upland grassland with water content of 7.2% (Table 1) in IMGERS (Fig. 2a). Microbial CH_4 release was also undetectable in fresh soils of the other uplands (Figs. 3–4). Accordingly, microbial CH_4 release occurred mainly in moist soils.

3.2. Non-microbial CH_4 release

Non-microbial CH_4 release occurred mainly in fresh soils of uplands, generally increased with temperature and was higher under anoxic than oxic conditions (Figs. 2a and 3–4). CH_4 oxidation was detectable

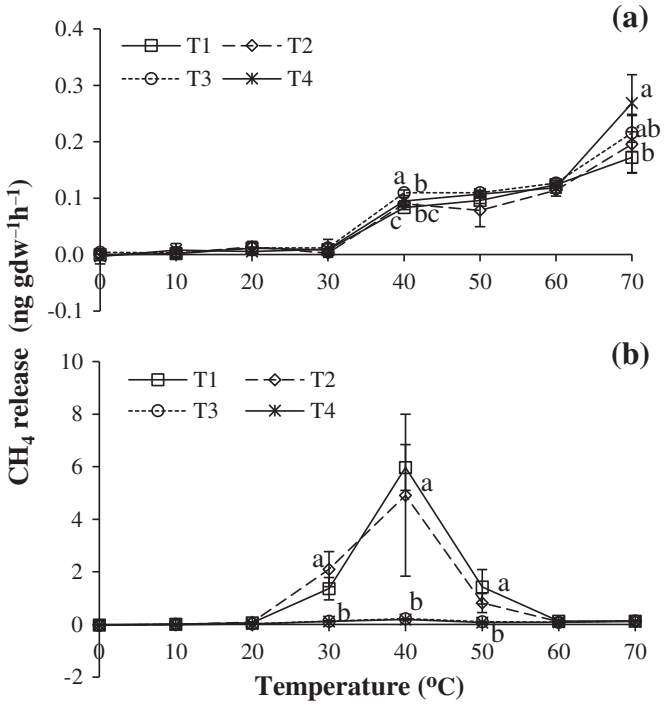

Fig. 2. CH_4 releases in fresh surface soils of upland grassland under oxic condition. (a) Grassland soils sampled in the IMGERS (Inner Mongolia Grassland Ecosystem Research Station) and (b) grassland soils sampled in Keyouzhongqi, Inner Mongolia. Four treatments, T1, T2, T3, and T4, were described in the text. The CH_4 release rate is mean ± 1 standard deviation ($n = 4$). The different letters indicate statistically significant differences ($P < 0.05$) in each group of treatments. When two treatments had no significant difference and were not easily separated, one same letter was marked.

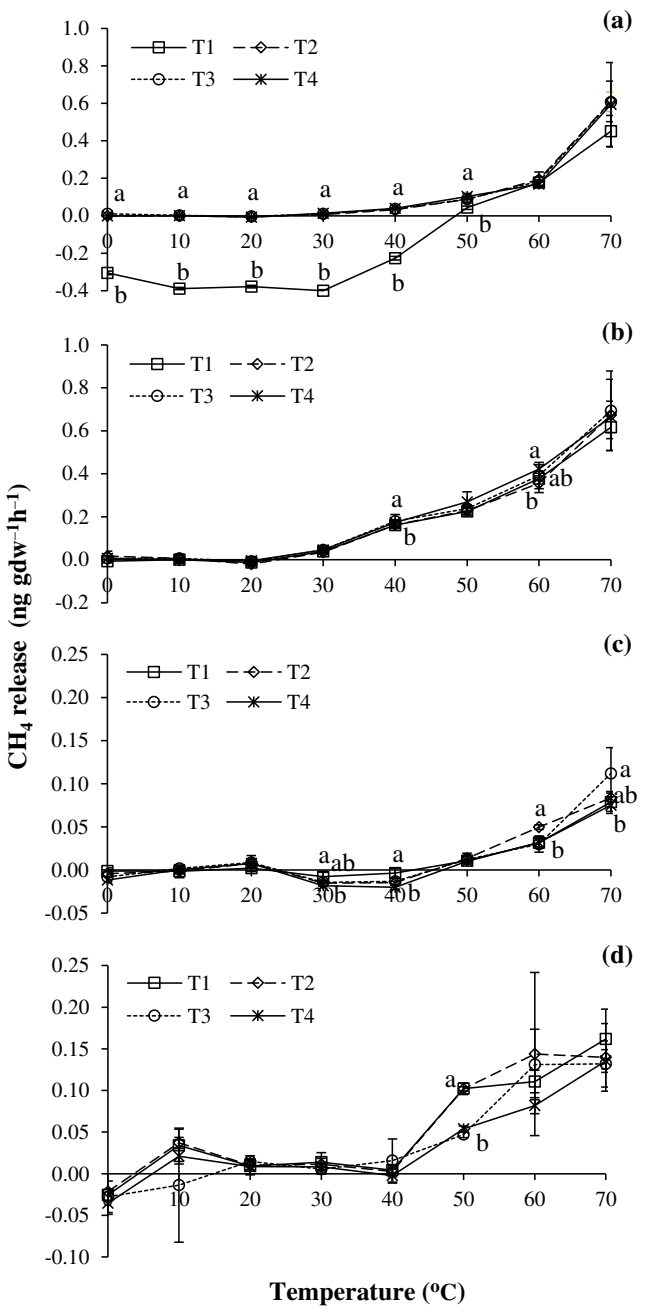

Fig. 3. CH_4 releases in fresh surface soils of upland forest and orchard incubated under oxic or anoxic condition. (a) Oxic, forest, (b) anoxic, forest, (c) oxic, orchard, and (d) anoxic, orchard. Four treatments, T1, T2, T3, and T4, were described in the text. The CH_4 release rate is mean ± 1 standard deviation ($n = 4$). The different letters indicate statistically significant differences ($P < 0.05$) in each group of treatments. When two or multiple treatments had no significant difference and were not easily separated, one same letter was marked.

in oxic surface soils of forest at 0–40 °C but undetectable at 50–70 °C, indicating that methanotrophic bacteria were killed at high temperatures. CH_4 oxidation was inhibited by CH_2F_2 in the T2 and T4. Non-microbial CH_4 release was detectable at 50–70 °C but negligible at 0–40 °C (Fig. 3a). Surface soils of forest under anoxic condition had neither CH_4 production nor CH_4 oxidation at 0–20 °C but showed detectable release at subsequent increasing temperatures (Fig. 3b). Non-microbial CH_4 releases ranged in approximately 0–0.2 and 0.1–0.7 ng gdw^{-1} h^{-1} from orchard and forest soils at 50–70 °C (Fig. 3). Generally speaking, non-microbial CH_4 release was detectable and had no significant differences ($P > 0.05$) among the T1, T2, T3, and T4 at high temperatures.

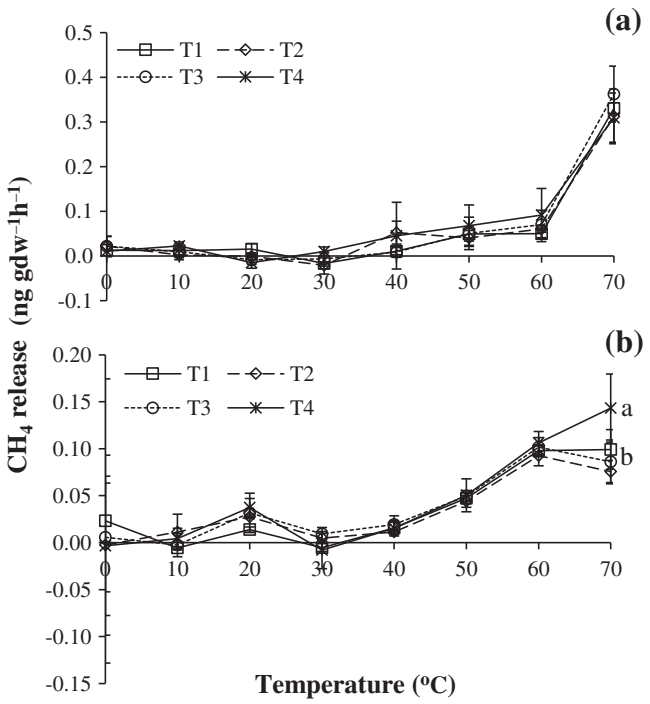

Fig. 4. CH_4 releases in fresh surface soils of upland croplands under oxic condition. (a) Cropland soils sampled in Cuihu and (b) cropland soils sampled in Guan. Four treatments, T1, T2, T3, and T4, were described in the text. The CH_4 release rate is mean ± 1 standard deviation ($n = 4$). The different letters indicate statistically significant differences ($P < 0.05$) in each group of treatments. When multiple treatments had no significant difference and were not easily separated, one same letter was marked.

Non-microbial CH_4 release from fresh soils of upland grassland in the IMGERS had similar response to temperature as the releases from forest and orchard soils. Non-microbial CH_4 release was detected at 40–70 °C, ranging in approximately 0.1–0.3 ng gdw^{-1} h^{-1} (Fig. 2a), but it was negligible in fresh soils of upland grassland in Keyouzhongqi (Fig. 2b). Accordingly, non-microbial CH_4 release generally had no significant differences ($P > 0.05$) among the T1, T2, T3, and T4 at 50–70 °C (Fig. 2).

We also found similar responses of non-microbial CH_4 release from two cropland soils to temperature as the releases from upland soils described above. Almost neither microbial CH_4 production nor microbial CH_4 oxidation was found in oxic surface soils of croplands. Non-microbial CH_4 release, ranging in approximately 0.1–0.3 ng gdw^{-1} h^{-1}, generally had no significant differences ($P > 0.05$) among the T1, T2, T3, and T4 at 50–70 °C but was higher from cropland soils incubated at 70 °C in Cuihu than in Guan (Fig. 4).

4. Discussion

4.1. Microbial and non-microbial CH_4 releases from fresh soils

Microbial CH_4 production and oxidation are highly heterogenic in wetland soils (Bartlett and Harriss, 1993). Difference in microbial CH_4 releases between the T1 and T2 of wet soils (Figs. 1 and 2b) could be due to the heterogeneities of microbial CH_4 production and oxidation in soil samples. Furthermore, microbial CH_4 release was significantly higher ($P < 0.05$) in the T1 than T2 at 40 °C (Fig. 1c), which may be explained by no CH_4 oxidation and/or large heterogeneity in microbial CH_4 production.

In general, oxic soils of uplands are thought to be net CH_4 sink due to CH_4 oxidation by methanotrophic bacteria. However, previous studies had widely observed net CH_4 emissions from oxic soils (Hao et al., 1988; Kammann et al., 2009; Megonigal and Guenther, 2008; Von Fischer and Hedin, 2007). Oxic eubacteria (Rimbault et al., 1988) and anoxic micro-sites as the refuge of methanogenic archaea (Peters and Conrad, 1995) may be offered as reasonable explanation for CH_4 emissions from oxic soils. Non-microbial CH_4 production by saprophytic fungi (Lenhart et al., 2012), cryptogamic covers (Lenhart et al., 2015), and/or micro-seepage of geological CH_4 (Etiope and Klusman, 2010) may be offered as co- or alternate-explanation for CH_4 emitted from oxic soils. Recent studies found that CH_4 may also be produced in oxic soils by non-microbial mechanism (Hurkuck et al., 2012; Jugold et al., 2012; Wang et al., 2013b). This study further observed non-microbial CH_4 release from soils. Thus, net CH_4 emission may occur in oxic soils of uplands, to which non-microbial CH_4 production contributed a fraction.

The CH_4 release of <0.2 ng gdw^{-1} h^{-1} was not considered statistically different from zero. Previous study summarized that CH_4 release of <0.2 ng gdw^{-1} h^{-1} from fresh plant leaves under ambient conditions was considered as negligible (Wang et al., 2011a). For keeping with this definition, in this study CH_4 release of <0.2 ng gdw^{-1} h^{-1} from fresh soils was also defined as negligible but CH_4 release of >0.2 ng gdw^{-1} h^{-1} was defined as detectable. Non-microbial CH_4 release from fresh soils of uplands was negligible at ambient temperatures of 0–40 °C and detectable at high temperatures of 50–70 °C; the detectable release ranged in approximately 0.2–0.7 ng gdw^{-1} h^{-1} (Figs. 2a and 3–4). Non-microbial CH_4 release from fresh wet soils was considered to be negligible when compared with microbial CH_4 release (Figs. 1 and 2b). Thus, non-microbial CH_4 release from oxic soils of uplands occurred mainly at 50–70 °C. Our non-microbial CH_4 release was usually located within or lower than those observed by Hurkuck et al. (2012) and Jugold et al. (2012), who observed non-microbial CH_4 release of 0.0–6.2 ng gdw^{-1} h^{-1} from soils under experimental conditions and 7.1, 1.2, and 1.1 ng gdw^{-1} h^{-1} respectively from a sphagnum peat, a coniferous forest soil, and a deciduous forest soil when incubated at 90 °C. The differences in non-microbial CH_4 release between this study and previous studies may be explained by the use of soil dependence and/or different incubation conditions.

In nature, the 0–5 cm surface soils of uplands are exposed to the atmosphere and are usually oxic. Microbial CH_4 is traditionally thought to be produced under anoxic condition (Conrad, 1996, 2005). Accordingly, microbial CH_4 production should not occur in oxic surface soils. This study indicates that almost no microbial CH_4 was produced in oxic surface soils of uplands (Figs. 2a and 3–4). On the other hand, surface soils were unsuitable for the growth and activity of methanotrophic bacteria probably because of strong fluctuations of environmental factors (Wang and Ineson, 2003). In this study, surface soils had almost no CH_4 oxidation with the exception of forest soils under oxic condition (Fig. 3a).

Non-microbial CH_4 release from fresh soils was slightly higher under anoxic than oxic conditions (Fig. 3). This is consistent with those observed by the use of fresh plant tissues (Wang et al., 2009, 2011a, b) where non-microbial CH_4 release was enhanced by anoxic condition. However, non-microbial CH_4 release from autoclaved soils was higher under oxic than anoxic conditions (Wang et al., 2013b). Furthermore, non-microbial CH_4 release from dried plant leaves incubated at rising temperature was plant species dependent, with three categories of response to oxygen levels: enhanced by oxic condition, similar between oxic and anoxic conditions, and enhanced by anoxic condition (Wang et al., 2011b). The differences in the response of non-microbial CH_4 release to oxygen levels in soils could be due to different soils where organic matter was originated from different plant species.

4.2. The importance of microbial versus non-microbial CH_4 emissions

Theoretically, the global non-microbial CH_4 emission from soils driven by temperature may be estimated using emission rates, soil parameters, and meteorological data. The time distribution of temperature throughout a year indicates that high air and soil temperatures of ≥50 °C rarely occur in nature. When surface soil temperature was ≥50 °C, for instance, its time accounted for only 1.3% throughout the

Table 2
Distributed proportions of air and surface soil temperatures in 2013 at IMGERS, Inner Mongolia.

Temperature range	Air temperature		Soil temperature at 0 surface	
	Time (h)	%[a]	Time (h)[b]	%[a]
T < 0	4192.5	47.9	4032	46.2
0 ≤ T < 10	2144.3	24.5	1390	15.9
10 ≤ T < 20	1923.7	22	1632	18.7
20 ≤ T < 30	499.5	5.7	782	9.0
30 ≤ T < 40	0	0	471	5.4
40 ≤ T < 50	0	0	317	3.6
50 ≤ T < 60	0	0	104	1.2
60 ≤ T < 70	0	0	8	0.1
T ≥ 70	0	0	0	0
Sum	8760	100	8736	100

The site observed is Inner Mongolia Grassland Ecosystem Research Station (IMGERS), Chinese Academy of Sciences.

[a] The percentage refers to the percent of hours in the year that reached specific temperature range.

[b] There were 365 days in 2013. However, original soil temperature on August 17th was missed. Accordingly, available days were 364 for soil temperature.

year 2013 at the IMGERS (Table 2). Assuming that non-microbial CH_4 production occurred mainly in the 0–5 cm surface soils at different temperatures and a mean soil bulk density was 1.5 g cm^{-3}, non-microbial CH_4 release rates of 0.2–0.7 ng gdw^{-1} h^{-1} at high temperatures of ≥50 °C (Figs. 1–4) can be roughly recalculated as an emission rate of 15.0–52.5 μg m^{-2} yr^{-1} throughout the year 2013 at the IMGERS. Non-microbial CH_4 release was negligible from fresh soils at ambient temperatures (Figs. 1–4). Even if this negligible release was added, non-microbial CH_4 release from fresh soils was very small when compared with mean microbial CH_4 emission of about 17–18 kg m^{-2} yr^{-1} from wetlands (cf. Cao et al., 1998) and about 25–50 kg m^{-2} yr^{-1} from temperate wetlands (cf. Bartlett and Harriss, 1993). In this study, non-microbial CH_4 release from oxic soils of uplands was three to four orders of magnitude lower than microbial CH_4 release from anoxic wet soils (Figs. 1–4). This implies that in the field non-microbial CH_4 emission is very small when compared with microbial CH_4 emission. On the global scale, uplands cover much more areas than wetlands. Of the Earth's land surface, for instance, woodlands, savannas, shrublands, and grasslands cover about 40% (DeFries et al., 1998) whereas wetlands account for about 2–6% (cf. Whiting and Chanton, 2001). The proportion of uplands versus wetlands are not likely to be large enough to change our conclusion that non-microbial CH_4 release from fresh soils at different temperatures is small in contributing to the total. On the global scale, thus, CH_4 produced in soils at different temperatures is mainly microbial in origin.

Previous studies reported that soil moisture greatly affected the productions of microbial CH_4 (Davidson et al., 2004; McLain and Martens, 2006; Shoemaker et al., 2014) and non-microbial CH_4 (Hurkuck et al., 2012; Jugold et al., 2012). Water addition enhanced non-microbial CH_4 release up to 8-fold those observed in the dried soils (Hurkuck et al., 2012). However, soil moisture would not always drive non-microbial CH_4 production under oxic or anoxic condition (Wang et al., 2013b). In this study, soil moisture was markedly different in various land uses (Table 1). Microbial and non-microbial CH_4 were mainly produced in moist and dry soils of uplands, respectively (Figs. 2–4), where 20% of soil moisture may be assumed as a dividing line between moist and dry soils (Table 1). Only 3 mm precipitation can make soil moisture be further increased 20% in the 0–5 cm soil layer when soil bulk density is 1.5 g cm^{-3}. For uplands, microbial CH_4 release rates in moist soils were much higher than non-microbial CH_4 release rates in dry soils (Figs. 2–4). Methanogenic archaea can survive in oxic dry soils for a long period (Ueki et al., 1997). When soils are wetted, the methanogens can recover and produce CH_4. We assumed the 10 mm of precipitation may make upland soil moisture maintain at least 20% for a few days when microbial CH_4 production and emission would occur. For instance, there were 13 days with daily precipitation of ≥10 mm in June–September 2003 at the IMGERS (Wang et al., 2007). For upland soils, thus, non-microbial CH_4 was produced on most temporal and spatial scales while microbial CH_4 production was transitory and patchy. In the field, however, total microbial CH_4 emission within a few days can be higher than total non-microbial CH_4 emission during most of the year. Even if in uplands, thus, on annual scale the CH_4 emitted is mainly produced by microbial mechanism.

In nature, soils are frequently exposed to environmental stresses, such as high temperature, UV radiation, drought, and flooding. Besides the high temperature examined in the present study, other environmental stresses can also affect non-microbial CH_4 production in soils. For instance, non-microbial CH_4 was produced in oxic soils by heating, UV radiation, and drying-rewetting cycles (Hurkuck et al., 2012; Jugold et al., 2012). Non-microbial CH_4 was produced from organic matter rather than mineral components and its release increased with organic carbon content in soils (Hurkuck et al., 2012). Soils are extensive and store a huge amount of organic matter. Currently, the global non-microbial CH_4 emission from soils under all categories of environmental stress is unknown. Thus, it is essential to conduct more measurements so as to improve our understandings of non-microbial CH_4 production in soils.

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