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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).