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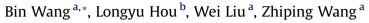
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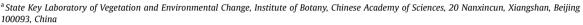
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Non-microbial methane emissions from soils





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HIGHLIGHTS

- Non-microbial CH₄ emissions both from oxic and anoxic soils were confirmed.
- The emissions were sensitive to various environmental factors.
- The emissions were inhibited or enhanced by anoxia contingent on hydrogen peroxide.
- Macroaggregates contributed more to the emissions from forest soils.

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ABSTRACT

Traditionally, methane (CH₄) is an erobically formed by methanogenic archaea. However, non-microbial CH₄ can also be produced from geologic processes, biomass burning, animals, plants, and recently identified soils. Recognition of non-microbial CH₄ emissions from soils remains inadequate. To better understand this phenomenon, a series of laboratory incubations were conducted to examine effects of temperature, water, and hydrogen peroxide (H2O2) on CH4 emissions under both aerobic and anaerobic conditions using autoclaved $(30 \text{ min}, 121 \,^{\circ}\text{C})$ soils and aggregates (>2000 μm , A1; 2000–250 μm , A2; 250–53 μm , M1; and <53 μm , M2). Results show that applying autoclaving to pre-treat soils is effective to inhibit methanogenic activity, ensuring the CH₄ emitted being non-microbial. Responses of non-microbial CH₄ emissions to temperature, water, and H₂O₂ were almost identical between aerobic and anaerobic conditions. Increasing temperature, water of proper amount, and H₂O₂ could significantly enhance CH₄ emissions. However, the emission rates were inhibited and enhanced by anaerobic conditions without and with the existence of H₂O₂, respectively. As regards the aggregates, aggregate-based emission presented an order of M1 > A2 > A1 > M2 and C-based emission an order of M2 > M1 > A1 > A2, demonstrating that both organic carbon quantity and property are responsible for CH₄ emissions from soils at the scale of aggregate. Whole soil-based order of A2 > A1 > M1 > M2 suggests that non-microbial CH_4 release from forest soils is majorly contributed by macro-aggregates (i.e., >250 µm). The underlying mechanism is that organic matter through thermal treatment, photolysis, or reactions with free radicals produce CH₄, which, in essence, is identical with mechanisms of other non-microbial sources, indicating that non-microbial CH₄ production may be a widespread phenomenon in nature. This work further elucidates the importance of non-microbial CH4 formation which should be distinguished from the well-known microbial CH_4 formation in order to define both roles in the atmospheric CH₄ global budget.

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1. Introduction

Methane (CH₄), as a potent greenhouse gas and the most reduced organic molecule in the atmosphere, plays a considerable role in global warming and atmospheric chemistry. Traditionally, CH₄ is generated through anaerobic degradation of organic matter

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by methanogenic archaea. Non-microbial CH₄ production, however, has also been validated in several distinctive sources. For example, non-microbial CH₄ can be produced within the Earth's crust (Etiope and Klusman, 2002) and during biomass burning (Andreae and Merlet, 2001). CH₄ production through an alternative route, which is different from methanogenesis in gastrointestinal tract of ruminants, has also been detected in animal cells (Ghyczy and Boros, 2001; Ghyczy et al., 2003, 2008). Non-microbial CH₄ is not limited to the animal kingdom. In recent years, lots of studies have corroborated that living plants and dry plant materials can emit non-microbial CH₄ under both aerobic (Keppler et al., 2006; Wang et al., 2008; Cao et al., 2008; McLeod et al., 2008; Vigano et al., 2008; Bowling et al., 2009; Brüggemann et al., 2009; Keppler et al., 2009; Messenger et al., 2009; Vigano et al., 2009; Wishkerman et al., 2011) and anaerobic conditions (Wang et al., 2011a, 2011b).

Very recently, two successive studies by Hurkuck et al. (2012) and Jugold et al. (2012) confirmed the release of non-microbial CH₄ from oxic soils. However, to further understand non-microbial CH₄ emissions from soils, we have investigated the following points: 1) Release of non-microbial CH₄ under anaerobic conditions; 2) Differences between CH₄ emissions under aerobic and anaerobic conditions; and 3) Differences in non-microbial CH₄ emissions among soil aggregates. We firstly found an effective method to pre-treat soils and ensure the exclusion of any microbial CH₄ contamination. Next, effects of temperature, water, and hydrogen peroxide ($\rm H_2O_2$) on CH₄ emissions were examined under both aerobic and anaerobic conditions. Finally, we physically separated the soil into four different aggregate-size groups into which CH₄ emissions were also investigated.

2. Materials and methods

2.1. Soil sterilization experiment

Wetland surface (0–5 cm) soils (silt clay, with 1.56% organic carbon) from Cuihu Wetland Park (40°11′N, 116°2′E), Beijing, China were sampled. Visible plant debris and rocks were thrown away. Totally, five treatments were set up: (1) lyophilization, (2) airdrying, (3) chloroform (CHCl₃) fumigation, (4) autoclaving with only one cycle (30 min, 121 °C) and (5) with three cycles. Posttreated soil samples were immediately lyophilized, passed through a 2 mm sieve, and uniformly referred to as dry soil.

The procedure of CHCl₃ fumigation was referred to Jenkinson and Powlson (1976) and Trevors (1996). Briefly, together with two 50 ml beakers with 2/3 alcohol-free CHCl₃ (plus anti-bumping chips) and full of sodium hydroxide (NaOH) solution, respectively, fresh soil samples were placed into a desiccator. Then, it was pumped to reach the boiling point of chloroform for 5 min, after which the desiccator was incubated in the dark under 25 °C for 24 h. Finally, the desiccator was repeatedly evacuated for six times to fully clear out the chloroform absorbed by soils.

To testify which treatment was the most effective one to inhibit microbial CH₄ production, experiments of microbial CH₄ emission kinetics with time were further conducted. Dry samples of the wetland soil subjected to the five distinctive treatments were put into 120 ml serum bottles, and sterilized ultra pure water was added until saturation. Then, they were incubated in dark under anaerobic conditions with a specific temperature of 25 °C. Meanwhile, both blank controls with equivalent volume of water and controls with fresh soil samples were set up. Headspace CH₄ concentration was continuously measured at different time intervals. Details of incubation method were referred to the 4th part of this section—Incubation experiment.

2.2. Soil sampling and preparation for non-microbial CH_4 experiments

Another three different types of soil were sampled from northern China for non-microbial CH₄ incubation experiments. One was peat that was purchased from local market (produced by peat from Northeast China, with 16.56% organic carbon). Another one was sampled from a temperate *Leymus chinensis* steppe which was fenced since 1999 and located near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), China (43°38′N, 116°42′E;

sandy loam with 3.71% organic carbon). The third soil was sampled from the broad-leaved Korean pine mixed forests in Changbai Mountain, Northeast China (42°23′N, 128°5′E; loam with 17.98% organic carbon) which belongs to a typical temperate forest ecosystem. The latter two soils were both taken to a depth of 5 cm, transferred intact to polyethylene bags, and kept intact during shipping. Except for the Changbai Mountain soil, of which subsamples were hand crumbled to pass a 8-mm sieve and then airdried for aggregate separation next (Elliott et al., 1991), all the three soils were autoclaved (30 min, 121 °C), lyophilized, and homogenized for subsequent incubation experiments. Large roots, litter fragments, and rocks were removed during these processes.

2.3. Soil aggregate separation

According to the wet sieving method (Cambardella and Elliott, 1993; Six et al., 1998, 2002), the Changbai Mountain soil was separated into four aggregate groups: large macroaggregates (>2 mm, A1), small macroaggregates (2-0.25 mm, A2), large microaggregates (0.25-0.053 mm, M1), and silt & clay fractions (<0.053 mm, M2). Each time, a 100 g subsample was firstly submerged for 5 min in room temperature deionized water on top of the 2 mm sieve. The separation was achieved by manually moving the sieve up and down 3 cm with 50 repetitions during a period of 2 min. After the 2 min cycle, the stable aggregates (>2 mm) were gently backwashed off the sieve into an aluminum pan. Floating organic material (>2 mm) was decanted and discarded because this large organic material is, by definition, not considered SOM (soil organic matter). Water plus soil that went through the sieve were poured onto the next sieve and the sieving was repeated, but floating material was retained. Totally, this process was repeated eight times in order to obtain necessary amount of each size aggregates for incubation experiments and organic carbon analysis. During sieving, both aggregates and sand particles of the same size as the aggregates were retained on the sieves, which would dilute the organic matter content of the aggregate size fractions (Elliott et al., 1991). Therefore, subsamples of each fraction were ovendried (50 °C), weighted, and analyzed for sand content and organic carbon content. The remaining of each fraction was autoclaved (30 min, 121 °C), lyophilized, weighted, and finally homogenized for subsequent incubation experiment. Sand contents (>53 µm) of all aggregate size fractions were determined by dispersing 5 g of each fraction in 20 ml of 5 g L⁻¹sodium hexametaphosphate (Elliott et al., 1991; Six et al., 2000). The organic carbon content was measured with a digestion method (Liu, 1996). Both aggregate percentage and organic carbon concentration of each fraction were sand-corrected.

2.4. Incubation experiment

Autoclaved (30 min, 121 °C), lyophilized, and homogenized soil samples (including whole soils and aggregates) were placed into 120 ml serum bottles, sealed with butyl rubber stoppers (diameter 20 mm), and incubated in the dark. Aerobic and anaerobic conditions were established by flushing compressed CH₄-free air and nitrogen, respectively (Wang et al., 2009). Sterilized ultra pure water and H₂O₂ solution were added into soils before and after bottle sealing, respectively, through sterilized syringes. Different incubation temperatures were achieved by an oven chamber. All incubations lasted for 3 h except the H₂O₂-related experiments for which the incubation only lasted 1 h. The methods of oxia—anoxia cycling experiments were referred to Wang et al. (2011a). Blank controls, without water for dry samples and with equivalent volume of water or H₂O₂ solution for wet samples, were set up to examine whether the background CH₄ concentration changed (if

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