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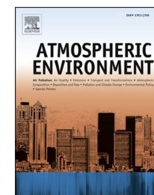

Influence of elevated ozone concentration on methanotrophic bacterial communities in soil under field condition

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HIGHLIGHTS

- O_3 stress can influence the potential methane oxidation rate (PMOR).
- O_3 influenced the potential methane production rate (PMPR) in soil.
- O_3 and the soil depth affected the gene *pmoA* of total methanotrophic bacteria.
- O_3 and the soil depth affected the type I and type II methanotrophic bacteria.
- O_3 influenced the community structure of the methanotrophic bacteria in soil.

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ABSTRACT

The open top chamber (OTC) method was used in combination with real-time quantitative PCR and terminal restriction fragment length polymorphism (T-RFLP) techniques in the wheat field to study the influence of different levels of O_3 concentrations (ambient air filtered by activated carbons, 40 ppb, 80 ppb and 120 ppb) on the quantity and community structure of methanotrophic bacteria. O_3 stress can influence the potential methane oxidation rate (PMOR) and potential methane production rate (PMPR) in the farmland soil. O_3 treatment of 40 ppb improved significantly the 16S rRNA gene copy number in the total methanotrophic bacteria *pmoA*, and type I and type II methanotrophic bacteria in the soil depth of 0 −20 cm. When the O_3 concentration reached 120 ppb, the 16S rRNA gene copy number in the total methanotrophic bacteria *pmoA* and type I methanotrophic bacteria decreased significantly as compared to the control treatment in 10–20 cm layer. The 16s rRNA gene copy number of total methanotrophic bacteria *pmoA* and type I and type II methanotrophic bacteria were influenced by different O_3 concentration and soil depth. The T-RFLP analysis indicated that O_3 stress influenced significantly the community structure of the methanotrophic bacteria in soil, causing potential threat to the diversity of methanotrophic bacteria. It seems to imply that the rise of O_3 concentration could produce an impact on the carbon cycling and the methane emission of the wheat field soil by changing the community structure and diversity of methanotrophic bacteria, which then influences the global climate change.

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1. Introduction

Methane is an important greenhouse gas, with a contribution of about 25% to the global warming (Thompson et al., 1992). The methane concentration in the global atmosphere has increased from 715 ppb before the industrialization to the present 1800 ppb (Shindell et al., 2009). The microbes are involved in the methane cycling in two ways, methanogenesis and methane oxidation (Edwards et al., 1998; Raghoebarsing et al., 2005). In natural soil, methanogens and other bacteria form a special syntrophism, by which the biomass is continuously degraded and the terminal electrons are accepted to produce methane. A large part of the methane is oxidized by the methanotrophic bacteria before being released to the atmosphere from soil and water (Le Mer and Roger, 2001).

Methanotrophic bacteria are a group of microbes that rely on methane as the sole carbon source and energy. It oxidizes methane into methanol under the action of methane monooxygenase

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(MMO), and the latter is subsequently oxidized into formaldehyde by methanoldehydrogenase (MDH). Formaldehyde is oxidized into formic acid by formaldehyde dehydrogenase (FADH), and under the effects of formate dehydrogenase (FDH), formic acid is oxidized into CO_2 (Hanson and Hanson, 1996; Madigan et al., 2009). The methanotrophic bacteria that participate in this oxidation process are divided into three types: type I, type II and type X methanotrophic bacteria (Hanson and Hanson, 1996). The methane consumed by the oxidation by methanotrophic bacteria in the soil takes up approximately 10% of the total methane consumption in the atmosphere (Duxbury and Mosier, 1993). Therefore, the methanotrophic bacteria play a crucial role in the carbon cycling of terrestrial ecosystems and in the global atmospheric methane balance (Duxbury and Mosier, 1993).

In the molecular ecological studies of methanotrophic bacteria, the available target genes include the 16S rRNA gene and some functional genes encoding the important enzymes involved in the methane oxidation (Zheng, 2009). Gene *pmoA*, which encodes particulate methane monooxygenase (pMMO), can be used to detect all the methanotrophic bacteria other than Methylocella; gene *mmoX* specifically encodes soluble methane monooxygenase (sMMO); gene *mxaF* is the gene encoding methanol dehydrogenase present in most Gram-negative methylotrophic bacteria. The 16S rRNA gene can be used to distinguish type I from type II methanotrophic bacteria.

Due to rapid population growth, economic development and energy demand, people have to recklessly exploit and utilize fossil fuels and nitrogen fertilizers, which increases the O_3 concentration in the surface layer. The surface O_3 pollution has become one of the most challenging environmental issues (Selin et al., 2009). The average O_3 concentration in the troposphere rose from 38 ppb before the industrial revolution to 50 ppb in 2000. It is predicted that by 2050, the average O_3 concentration in the surface layer will be as high as 70 ppb, and will reach 80 ppb by 2100, much higher than 40 ppb, which is the critical value of damage to plants (Fiscus et al., 2005; Sitch et al., 2007). In the Yangtze River Delta region and Beijing, the maximum surface O_3 concentration has reached over 150 ppb (Shao et al., 2006; Zhang, 2010).

The O_3 pollution has damaging effects to plants (Avnery et al., 2013), causing the reduction of crop yield and huge economic loss (Feng et al., 2003; Zhu et al., 2011). The influence of O_3 stress on terrestrial ecosystems is not limited to the aboveground part. O_3 stress also affects the reactions and underground carbon cycling. It is reported that the rise of atmospheric O_3 concentration inhibits the growth of plant roots, reduces the root biomass and the microbial activity (Kasurinen et al., 2005; Chen et al., 2009). Reddy et al. (1995) studied the impact of O_3 stress on the soil enzymes of *Pinus taeda*. They found that when the O_3 concentration was 160 ppb, it inhibited significantly the activity of the phosphatase in the soil; when the concentration reached 240 ppb, it significantly inhibited the activity of soil arylsulfatase. The high O_3 concentration reduced significantly the microbial biomass carbon in the farmland soil, but had little influence on the soil organic carbon content (Islam et al., 2000).

Recently, it was found that the O_3 stress reduced significantly the methane emission in the farmland soil (Bhatia et al., 2011; Zheng et al., 2011), however the microbial mechanics that regulates the soil methane emission under O_3 stress has not been reported. Our hypothesis is that the O_3 stress influences the soil methane emission by exerting impact on the structure and functions of the microbial communities that participate in the soil carbon cycling in the farmland (such as methanotrophic bacteria). This study investigated the influence of O_3 concentration increase on the structure and functions of soil methanotrophic bacteria by the open top chamber (OTC) method in the winter wheat field. The molecular biotechnologies such as real-time quantitative PCR and T-RLFP were used to reveal the changing characteristics of the soil methanotrophic bacteria and the mechanism driving the dynamics of the methane emission by microbes under the O_3 stress in the field.

2. Materials and methods

2.1. Experiment site

The experiment site was located in the Seed Management Station of Changping, Beijing (40°12′N, 116°8′E). The station is located in the northwest Beijing with continental monsoon climate and four distinct seasons. The annual average rainfall is 550.3 mm. The annual average temperature is 11.8 °C. The fundamental physicochemical properties of soil are as follows: organic matter contents 16.4 g kg^{-1}; total nitrogen 0.9 g kg^{-1}; available phosphorus 38.1 mg kg^{-1}; available potassium 102.1 mg kg^{-1}; pH value 8.3.

2.2. Plant material

The variety used in the experiment was *Triticum aestivum* L. Beinong 9549, provided by Beijing Agriculture College. The seeds were sowed on September 28, 2009. Before sowing, the compost was applied. On April 26, 2010, urea was applied (225 kg ha^{-1}). The field management was coherent to that of the local farms during the entire growth season of winter wheat.

2.3. Ozone fumigation

In-situ ozone fumigation experiment was carried out on winter wheat with the self-made open-top fumigation system (Fig. 1, Huang et al., 2012). Four O_3 treatments were set up: the ambient air filtered by activated carbon (CK), 40 ppb, 80 ppb and 120 ppb. 3 replicates were set for each treatment. O_3 fumigation on winter wheat began from April 5, 2010. The fumigation lasted for 9 h (8:00–17:00) every day and stopped on June 12. The duration was 50 days. On June 13, 2010 (during the grain-filling stage of the winter wheat), the soil samples were collected. The 5-point mixing method was used for sampling. In every experimental plot, the fresh soil at the depths of 0–10, 10–20 and 20–40 cm was collected respectively with an earth auger 2 cm in diameter. The gravels and root residues were removed. The samples were mixed separately, put into sterile sampling bags for cold storage and taken back to the laboratory. The samples were sieved through the filter of 2.0 mm. Part of the soil samples were preserved in refrigerator at −80 °C and 4 °C. The test of soil properties and total DNA extraction were conducted. The remaining soil samples were freeze dried and

Fig. 1. Simulation experiment of ozone stress on winter wheat grown in the field.

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