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Effects of different fertilizers on methane emissions and methanogenic community structures in paddy rhizosphere soil

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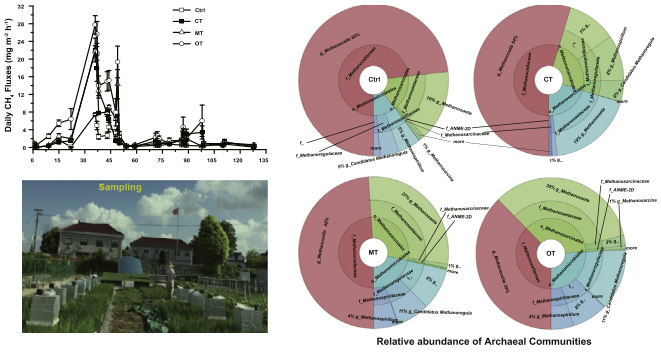
HIGHLIGHTS

- CH_4 emissions were positively related to mcrA/pmoA.
- Soil potassium, oxalate, acetate and succinate significantly affect dominant methanogens.
- Organic fertilizer increased the relative abundance of acetoclastic *Methanosaeta*.
- Organic fertilizer decreased the relative abundance of hydrogenotrophic *Methanocella*.

GRAPHICAL ABSTRACT



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ABSTRACT

Paddy soil accounts for 10% of global atmospheric methane (CH_4) emissions. Many types of fertilizers may enhance CH_4 emissions, especially organic fertilizer. The aim of this study was to explore the effects of different fertilizers on CH_4 and methanogen patterns in paddy soil. This experiment involved four treatments: chemical fertilizer (CT), organic fertilizer (OT), mixed with chemical and organic fertilizer (MT), and no fertilizer (ctrl). The three fertilization treatments were applied with total nitrogen at the same rate of 300 kg N ha^{-1}. Paddy CH_4, soil physicochemical variables and methanogen communities were quantitatively analyzed. Rhizosphere soil *mcrA* and *pmoA* gene copy numbers were determined by qPCR. Methanogenic 16S rRNA genes were identified by MiSeq sequencing. The results indicated CH_4 emissions were significantly higher in OT (145.31 kg ha^{-1}) than MT (84.62 kg ha^{-1}), CT (77.88 kg ha^{-1}) or ctrl (32.19 kg ha^{-1}). Soil organic acids were also increased by organic fertilization. CH_4 effluxes were significantly and negatively related to *mcrA* and *pmoA* gene copy numbers, and positively related to *mcrA*/*pmoA*. Above all, hydrogenotrophic *Methanocella* and acetoclastic *Methanosaeta* were the predominant methanogenic communities; these communities were strictly associated with soil potassium, oxalate, acetate, and succinate. Application of organic fertilizer promoted the dominant acetoclastic methanogens, but suppressed the dominant hydrogenotrophic methanogens. The transformation in methanogenic community structure and enhanced availability of C substrates may explain the increased CH_4 production in OT compared to other treatments. Compared to OT, MT may partially mitigate CH_4 emissions while guaranteeing a high rice yield. On this basis, we recommend the local fertilization pattern should change from 300 N kg ha^{-1} of organic manure to the same level of mixed fertilization. Moreover, we suggest multiple combinations of mixed fertilization merit more investigation in the future.

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1. Introduction

Methane (CH_4) is considered the second most potent greenhouse gas in the atmosphere after carbon dioxide (CO_2), but has a global warming potential 25-fold higher than that of CO_2 over a 100-year period (Yvon-Durocher et al., 2014). Irrigated rice cultivation accounts for 51% of rice producing areas worldwide and is an important anthropogenic biological source of atmospheric CH_4, responsible for 7%–17% of global CH_4 emissions (Su et al., 2015). CH_4 is the final product of the degradation of organic matter by anaerobic bacteria and archaea (Bodelier, 2015). Energy metabolism in methanogenic archaea, a phylogenetically diverse group of strictly anaerobic *Euryarchaeota*, is constrained to formation of CH_4 from simple carbon compounds such as CO_2, acetate and formate (Conrad et al., 2014; Lu et al., 2005; Ma et al., 2012). Previous studies have indicated methanogens are distributed among the seven orders of *Euryarchaeota*: *Methanosarcinales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, *Methanopyrales*, *Methanocellales* and *Methanoplasmatales* (Hofmann et al., 2016).

Many researchers have indicated that CH_4 emission dynamics and methanogenic community structure can be naturally influenced by the application of fertilizer to anaerobic soil (Bao et al., 2016; Chen et al., 2014; Nguyen et al., 2015; Win et al., 2011; Zhou et al., 2016). In general, fertilization is a primary anthropogenic factor that leads to CH_4 emission, particularly in rice cultivation (Adviento-Borbe and Linquist, 2016; Fan et al., 2016; Kim et al., 2015; Xie et al., 2009). However, experiments have shown chemical fertilization with ammonia-based or nitrate-based nitrogenous fertilizers can either inhibit or stimulate CH_4 efflux (Bodelier and Laanbroek, 2004; Frolking et al., 2004; Kim et al., 2015). Ke et al. (2014) indicated increased N fertilization also enhances the relative abundance of methanogens, with *Methanosarcinaceae* and *Methanocellales* the dominant methanogens in paddy rhizosphere soil. Zhou et al. (2016) found that the abundance of *Methanosarcina* and *Methanosaeta* at the rice tillering stage decreased rapidly after application of composted manure compared to non-composted manure treatment. Furthermore, a decrease in *Methanosarcina,* but increase in *Methanobrevibacter,* was observed in the composted treatment in contrast to the control treatment. Varied types or fertilization could have varied effects on CH_4 emissions and methanogens from paddy soil (Serrano-Silva et al., 2014). Application of organic fertilizers (such as animal manure, sewage sludge, crop residues) greatly enhances soil nutrient availability and microbial activity and biodiversity (Jannoura et al., 2014). Consequently, the increased availability of carbon after application of organic fertilizer increases CH_4 emissions and leads to a clear shift in the dominant methanogens in paddy soil (Chen et al., 2014; Pramanik and Kim, 2014; Zhou et al., 2016). However, the potential diversity of methanogenic archaeal species in soil is enormous (Singh et al., 2012). It is still difficult to demonstrate how methanogen diversity affects production of soil methane. A summary of 33 publications on dominant methanogens in rice soil is presented in Table 1. Methanogenic community structure varied between studies due to the different paddy soils and varied experimental treatments. Overall, 19 reports stated *Methanosarcinaceae* were the predominant methanogens in soil. The other leading common dominant methanogens were *Methanomicrobiales* and *Methanosarcinales*.

In a review, Alpana et al. (2017) stated that CH_4 emissions from flooded paddy soil could be governed by soil properties (including soil water content, oxygen availability, soil Eh, organic matter, pH, soil texture and mineralogy, chemical properties), climate factors (such as temperature, precipitation, humidity) and cultural practices (like water management, fertilization, rice cultivar). Ultimately, CH_4 emission from paddy soil is the result of soil microbial processes, including its production in flooded anaerobic and its consumption (oxidation) in aerobic microsites (Pump et al., 2015). Microbial communities involved in various biogeochemical processes of irrigated rice fields shift markedly during the rice cropping cycle. Understanding how the composition and abundance of methanogen communities are affected by soil management remains a fundamental question (Alpana et al., 2017). Our previous study demonstrated that both CH_4 emission and soil fertility were significantly increased by multiyear organic fertilization in paddy soil (Yuan et al., 2017; Zhao et al., 2015). We hypothesized the dominant methanogen community structure in paddy rhizosphere soil, and consequently, the potential for producing CH_4, may also be significantly affected by organic fertilization. In this study, CH_4 fluxes and emissions were continually monitored. Soil physicochemical variables, including soil organic acids, were also investigated throughout all stages of rice cultivation. Quantitative PCR and Illumina MiSeq sequencing were employed to explore methanogenic community structure.

2. Materials and methods

2.1. Experimental site

The field experimental site was established in 2009 at the Irrigation Technology Extension Station of Qingpu, Shanghai (121.12° E, 31.15° N). The site experiences a subtropical humid monsoon climate, with an annual average daily temperature of 14.1–17.5°C, annual average rainfall of 1100–1400 mm, and 1200 to 1800 h of sunshine per year. Before transplanting, the soil pH, EC, available nitrogen, phosphorus and potassium levels of the site were 7.70, 0.09 mS cm^{-1}, 1.29 g kg^{-1}, 0.38 g kg^{-1}, and 0.55 g kg^{-1}, respectively. Rotation between winter wheat cv. Yangmai-5 and rice cv. Huayou-14 has been conducted since the site was established. Winter wheat is cultivated from November to May; rice seedlings were transplanted into the field in June and harvested in November. The growth of rice was classified into seven stages: regreening, tillering, jointing, booting, heading, filling and maturing.

2.2. Experimental design and management

The experiment was conducted with four treatments: (1) no fertilizer (ctrl); (2) chemical fertilizer treatment (CT); (3) mixed with chemical and organic fertilizer treatment (MT); and (4) organic fertilizer treatment (OT). The three fertilization plots were treated with total N at the same rate of 300 kg N ha^{-1}, in line with the levels applied by local farmers in Shanghai district (Yuan et al., 2017; Zhao et al., 2015). Urea, the chemical N fertilizer, was applied on three occasions at a ratio of 6:2:2. Chicken manure compost was the organic fertilizer and applied only once, at pre-transplantation. The mixed fertilizer was 80% chemical fertilizer and 20% organic fertilizer, and was applied as shown in Table 2. Each treatment had three replicates. Twelve field blocks (3 m × 2 m) were randomly arranged within the experimental site. The time-line, dates of fertilization and major farming managements are listed in Table 3.

2.3. Sampling and measurements

2.3.1. CH_4

Field gas samples were collected from each of the three replicates per treatment using dark static chambers once a week between 8:00–9:00 a.m. from Jun. 13 to Oct. 20, 2016. The surface of static chamber was covered with tinfoil papers. The size of chamber was of 50 cm length, 50 cm width, and 100 cm height. Two fans were installed in the chamber to mix the gases (Yuan et al., 2017). A stainless base frame was buried into the soil to a depth of 20 cm before rice transplanting, and remained on the soil during the entire rice season. During the sampling period, the static chambers were placed on the base frame and sealed with water. Gas samples were collected with a 100-ml plastic injection syringe (Zhao et al., 2015). Agilent 6890D gas chromatograph (CA, USA) was used to analyze CH_4 concentration. The sampling frequency was increased to once a day when fertilizer was