Agriculture, Ecosystems and Environment 258 (2018) 121–128

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Effects of continuous manure application on methanogenic and methanotrophic communities and methane production potentials in rice paddy soil

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ARTICLE INFO

Keywords:
Manure
Methanogens
Methanotrophs
Methane production potentials
Soil nutritional status
Paddy soil

ABSTRACT

Livestock manures are broadly used in agriculture to improve soil productivity; however, the impact of continuous manure application on the behaviors of methanogens (*mcrA*) and methanotrophs (*pmoA*) is poorly understood. The objective of this study was to determine the influence of continuous pig manure application on methanogenic and methanotrophic communities and the methane production potentials (MPPs) of rice paddy soil. The results show that adding manure induced significantly higher *mcrA* gene abundance than chemical fertilizer treatments, and that manure led to higher *mcrA* gene abundance when it was applied together with full NPK fertilizers than it did when applied with N or NP fertilizers. However, there were no obvious effects of continuous manure application on *pmoA* gene abundance. The community structures of *mcrA* and *pmoA* were distinctly altered by continuous manure application, and their variation was closely associated with fertilizer-induced changes in dissolved organic C, total P, and available P and K in the soil. We also observed that manure application along with full NPK fertilizers caused significantly higher MPPs compared to chemical fertilization alone, while manure application with N or NP fertilizers had no obvious effect on MPPs. Moreover, MPPs were positively correlated with *mcrA* gene abundance, suggesting that continuous manure application may enhance methane emissions by stimulating methane production. Therefore, long-term manure application can affect both the abundance and composition of methanogens, and thus, enhance methane production. This effect is largely dependent on soil nutritional status.

1. Introduction

Methane (CH_4) is the second most important greenhouse gas after carbon dioxide (CO_2) and has 25 times more global warming potential than CO_2 (Forster et al., 2007); therefore, small changes of CH_4 in the atmosphere could significantly contribute to global warming (Bridgham et al., 2013). Rice fields are an important anthropogenic biological source of atmospheric CH_4, which account for approximately 10% of the global CH_4 emission and will probably increase in the future due to the demand for rice production to feed the increasing human population (Van Nguyen and Ferrero, 2006).

CH_4 emissions depend on the net balance between the activities of methanogens and methanotrophs. In rice fields, methane is produced by two major physiological guilds, the acetotrophic and hydrogenotrophic methanogens. Acetotrophic methanogens convert acetate to CH_4 and CO_2, while hydrogenotrophic methanogens reduce CO_2 with H_2 to CH_4 (Conrad, 2007). Generally, more than 67% of the CH_4 produced is acetotrophically generated (Kruger et al., 2005). Methanotrophs are gram-negative bacteria that utilize methane as their source of carbon and energy. They play an important role in regulating CH_4 emissions from rice paddy fields and can oxidize up to 90% of the CH_4 produced by methanogens (Krause et al., 2010; Ma et al., 2013). Given the critical roles of methanogenic and methanotrophic microorganisms in the methane cycling of rice fields, a growing body of research is targeted at defining the behaviors of methanogens and methanotrophs and their responses to environmental factors (Le Mer and Roger, 2001; Dumont et al., 2006; Kolb, 2009; Bodelier, 2011; Zheng et al., 2013).

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https://doi.org/10.1016/j.agee.2018.02.018
Received 21 September 2017; Received in revised form 8 February 2018; Accepted 12 February 2018

Livestock manures are widely used in agricultural systems to improve soil fertility and crop yields effectively and to sequester carbon (C) in soils (Thangarajan et al., 2013; Hou et al., 2015). Moreover, long-term field investigations have demonstrated that addition of livestock manure is more effective than crop residue supplementation or the application of chemical fertilizers as a means of offsetting soil organic carbon decline (Kapkiyai et al., 1999). However, a number of studies have reported that manure addition can significantly enhance CH_4 emissions in flooded paddy ecosystems (Yan et al., 2005; Kim et al., 2014; Ho et al., 2015). Kim et al. (2014) reported that cattle manure application could significantly increase CH_4 emissions due to the increased C substrates for methanogenic bacteria and nutrient availability in rice paddy soil during cultivation. Additionally, based on a statistical model, organic supplementation is one of the top two variables that control CH_4 emissions (Yan et al., 2005). Therefore, due to the trade-off between improved crop yields and soil health versus greenhouse gas emissions, the environmental effects of manure application in paddy fields should be evaluated.

In China, the use of agricultural organic wastes, such as animal manure and straws, has increased with the rapid development of modern agriculture. In 2010, the total amount of animal manure used was approximately 23 million tons (Chinese Animal Husbandry Yearbook, 2010), and untreated animal manures have become a huge environmental challenge (Li, 2009). In particular, animal manures are applied to crops to enrich the organic C and cropland nutrients, specifically nitrogen (N) and phosphorus (P), in the soil (Lv et al., 2011). However, inappropriate application of manure increases in soil nutrients while risking surface- and groundwater contamination (Sharpley and Moyer, 2000; Parvage et al., 2013) and global warming (Kim et al., 2014; Zhou et al., 2016). Soil fertility varies greatly in the arable lands of China, and many of the croplands suffer from shortages of phosphate (P) and potassium (K), which are needed to enhance soil fertility and increase crop yields to the levels demanded by rapid population growth. Hence, rational application of manure is an effective management practice for croplands with low soil fertility. Although manure application stimulates paddy CH_4 emissions, it is not clear how continuous manure application affects the behaviors of methanogens and methanotrophs. The main objectives of this study were to investigate the responses of methanogenic and methanotrophic communities to continuous pig manure application to paddy soils with varied soil nutritional statuses induced by long-term fertilization after 30 years, and to evaluate the effects of continuous manure amendments on methane production potentials during the rice growing season.

2. Methods and materials

2.1. Experimental site

The sampling site was located at the Nanhu experimental station of the Hubei Academy of Agricultural Sciences, China (30°28′N, 114°25′E; elevation 20 m above mean sea level). The climate is subtropical humid monsoon with a mean annual precipitation of 1, 300 mm and evaporation of 1, 500 mm. The average annual accumulated temperature above 10 °C is 5, 190 °C, and the nonfrost period is about 230–300 d. The soil at the experimental site is *stagnic anthrosols* developed from yellow-brown soil with 27.34 g kg^{-1} soil organic matter, 1.80 g kg^{-1} total N, 1.00 g kg^{-1} total P, 30.22 g kg^{-1} total K, 5.0 mg kg^{-1} available P, 98.50 mg kg^{-1} available K, and 6.3 pH (H_2O).

The experiment started in 1981 with a rotation of rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum* L.). The plot size was 8 m × 5 m. Nine treatments were arranged in a completely randomized block design with three replicates as follows: CK (no fertilizers); N (150 kg N ha^{-1} only); NP (150 kg N ha^{-1} plus 75 kg P_2O_5 ha^{-1}); NPK (150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1}); M (6, 975 kg pig manure ha^{-1} dw); MN (6, 975 kg pig manure ha^{-1} dw and 150 kg N ha^{-1}); MNP (6, 975 kg pig manure ha^{-1} dw, 150 kg N ha^{-1} and 75 kg P_2O_5 ha^{-1}); MNPK (6, 975 kg pig manure ha^{-1} dw, 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1}); M′NPK(11, 648 kg pig manure ha^{-1} dw, 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1}).The fertilization strategies were as follows: 60% of chemical fertilizers were applied to rice directly, and the rest were applied to winter wheat. Pig manure was collected and composted two weeks before its application, and an equal amount of composted pig manure was applied to both rice and wheat. All P (superphosphate) and K (potassium chloride) fertilizers and pig manure were applied as basal fertilization before rice transplantation or wheat sowing. For N fertilizer (urea), 40% was applied as basal fertilizer, 40% as tillering fertilizer, and 20% as panicle fertilizer in the rice season; 50% was applied as basal fertilizer, 25% as tillering fertilizer, and 25% as jointing fertilizer in the winter wheat season.

2.2. Soil sampling and chemical analyses

Soil samples were collected in July 2011, when the rice was at the tillering stage, because high CH_4 emissions were observed at this stage due to flooding conditions, application of tillering fertilizer, and more root exudates (Jia et al., 2006). Composite soil samples were taken from the plough layer (0–15 cm depth) at five randomly selected points in each plot with a soil auger. Each sample was divided into two aliquots; one was frozen immediately in liquid nitrogen and stored at −80 °C for molecular analysis, and the other was collected and brought to the laboratory in a sealed polybag for methane production potential and soil chemical property analyses. Soil pH was determined with a soil-to-water ratio of 1:2.5 using a glass electrode. Soil organic C was determined by wet digestion according to the Turin method (Nelson and Sommers, 1982). Dissolved organic C (DOC) was determined using a procedure described by Jones and Willett (2006). Total N was measured using the Kjeldahl method (Bremner and Mulvaney, 1982). Total P and available P were extracted with HF-HNO_3-$HClO_4$ and measured by the molybdenum-blue method. Available K was extracted with ammonium acetate and determined by a flame photometry. Grain yields were measured at the physiological maturity stage by hand-harvesting each plot.

2.3. Methane production potential measurement

Methane production potential (MPP) was detected as described by Singh et al. (2012) with minor modifications. Briefly, to monitor methane production, anoxic sterile water was added to 50 g fresh soil samples in 250 mL flasks to provide a 10 mm standing water layer above the soil surface. The headspace was purged with O_2-free N_2 gas for 3 min with constant shaking to ensure an anaerobic environment. The flasks (in triplicate) were dark-incubated statically at 30 °C for 5 d. Methane in the headspace was measured using a gas chromatograph (Agilent 7890A, USA) equipped with a Flame Ionization Detector and pora PLOT Q column (3 m × 2 mm). The methane production potential of each soil sample was calculated as the slope of the methane concentration in the headspace at intervals of 1, 60, and 120 h. The production potentials are given in μg CH_4 per g dry weight per day. The water content of fresh soil samples was approximately 34.6% ± 4.

2.4. Soil microbial DNA preparation

Soil microbial DNA was extracted according to the method described by Chen et al. (2010) with minor modifications. Briefly, after the addition of the lysing solution, an MP FastPrep-24 (MP Biomedicals, Santa Ana, CA, USA) was used instead of a vortex to mix the lysate, followed by a 15 min incubation in a 68 °C water bath. DNA concentration and quality were measured using a NanoDrop NA-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).