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Cattle manure composting: Shifts in the methanogenic community structure, chemical composition, and consequences on methane production potential in a rice paddy

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ABSTRACT

Compost application has been considered to be one of the most promising methods for sustaining soil quality and crop productivity and possibly attenuates the impact of methane (CH_4) emission in rice paddies. The physico-chemical and biological characteristics of manure may vary, depending on the maturity of the compost. Therefore, compost obtained from different stages of maturation could differentially influence CH_4 production in rice paddies following input into the fields. In composting samples, we investigated the effect of composting on alterations in physico-chemical characteristics and changes in methanogenic abundance and community structure in manure during the maturation process using a conventional static chamber method. Thereafter, CH_4 production potential was monitored in soil samples amended with compost obtained from different composting stages (0 as a starting material, 7, 14, 21, 28, and 35 days after installation) via an incubation experiment. The results show that manure composting significantly decreased the methanogenic abundance and altered the methanogenic community structure by qPCR and T-RFLP analyses, respectively. The physico-chemical properties, such as labile carbon (C) and nitrogen (N) availabilities in manure, were gradually changed due to high temperature and oxygen supplement. These changes result in decreasing CH_4 production in a rice field soil amended with composts. Our results suggest that composting is a promising approach to mitigate the impact of CH_4 emissions in rice paddy fields with manure. In conclusion, composting should be indispensable for mitigating the impact of CH_4 emission in manure-amended paddy soil during cultivation.

1. Introduction

Methane (CH_4) is a potent greenhouse gas similar to carbon dioxide (CO_2) but with 34-fold higher global-warming potential than CO_2 over a 100-year period (IPCC, 2013). Global CH_4 concentration in the atmosphere has been increasing from a pre-industrial level of 0.715 ppm to 1.824 ppm in 2013 (WMO, 2014). Among anthropogenic CH_4 sources, rice paddies contribute approximately 31–112 Tg CH_4 yr^{-1} corresponding to 19% of the total CH_4 emission globally (Forster et al., 2007). It is well-known that CH_4 is the end product of anaerobic organic matter decomposition by the methanogenic archaea, simultaneously influencing CH_4 dynamics and turnover of soil organic matter in flooded rice paddy soils.

Soil organic matter (SOM) plays a crucial role in maintaining soil productivity and fertility in arable lands (Thangarajan et al., 2013). Livestock manure has been considered to be an excellent source of SOM and has been widely used as an organic soil additive in agricultural fields. However, the addition of livestock manure significantly stimulates CH_4 emission, providing a readily available carbon source for the methanogens in flooded ecosystems (Ho et al., 2015a; Qin et al., 2010; Singh et al., 2012). Moreover, by adding manure in rice fields, manure-derived methanogens are inoculated into the soil, possibly leading to higher CH_4 emissions in the long term (Gattinger et al., 2007; Ho et al., 2015a; Kim et al., 2014a; Radl et al., 2007). Therefore, strategies to mitigate CH_4 emission in rice soils amended with livestock manure are urgently needed.

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Compost application as an organic amendment has been proposed as a promising approach to sustain soil quality and mitigate CH_4 emissions in rice paddies (Chen et al., 2011; Ho et al., 2015b; Kim et al., 2014b; Ma et al., 2009; Pramanik and Kim, 2014). Recently, composted manure application was shown to decrease CH_4 emissions by up to 20% compared to un-composted manure (Pramanik and Kim, 2014). Moreover, amendment with composted manure resulted in a higher stable organic C fraction, although the same amounts of total C were added using both manures (composted and un-composted) in a rice field soil (Kim et al., 2014b). Anaerobic decomposition of organic matter in rice field soils is largely regulated by their chemical composition (Tian et al., 1993) and the methanogenic community composition (Singh et al., 2012).

Composting is well-known as an aerobic process for rapid stabilization and humification of organic materials as well as an eco-friendly and economically promising way for treating solid organic waste from agro-environments (Huang et al., 2006). There are four stages in the composting process (Keener et al., 2000; Bernal et al., 2009): (1) the initial mesophilic phase, where mesophilic bacteria and fungi degrade simple organic compounds such as sugars, amino acids, proteins, etc., by increasing the temperature rapidly; (2) the thermophilic phase, when the composting material reaches its maximum temperature (> 40 °C), which is most rapid stage of decomposition process; (3) cooling phase, a decrease of the temperature due to the reduction of the microbial activity, which is associated with the consumption of degradable organic substrate; (4) the maturation phase, a lengthy period of stabilization for producing a good quality of compost which is stabilized, matured and humified. The composting process can be influenced by many environmental factors including oxygen content, moisture, biochemical composition of the manure, pH, and temperature, which ultimately affect the quality of end product of composting and the composting efficiency.

Biological and chemical properties in livestock manure can be significantly altered by the composting process, primarily due to the high temperature (60–65 °C) and oxygen supplement. Microbial activities related to organic matter decomposition decline rapidly between 60–65 °C (Bernal et al., 2009), and most of the methanogenic archaea are sensitive to oxygen exposure. Therefore, composting could be a promising approach to reduce methanogenic activity in manure. Recently, an interest in organic farming has been increasing around the world; thus, organic farming-based rice cultivation is expected to be substantially expanded in the near future (Singh et al., 2012).

Numerous studies have determined the impact of composting on CH_4 dynamics, as well as its effect on the microbial community and/or changes in the chemical properties of manure during composting (Cahyani et al., 2004; Chen et al., 2014, 2011; Jäckel et al., 2005; Thummes et al., 2007a,b; Tiquia et al., 2002; Zhang et al., 2011). However, the effects of maturing manure compost on the methanogenic abundance and community composition, as well as the physico-chemical properties, particularly the labile organic C and N characteristics, during composting are less known.

We hypothesized that alterations in methanogenic communities and physico-chemical properties in manure during composting may exert an effect on CH_4 production potential in a rice field soil amended with composts from different maturing stages. Therefore, the effect of manure amendment obtained from different stages of composting on CH_4 production potential was investigated in a rice paddy soil. In addition, changes in methanogenic abundance and community composition were monitored in the manure at various stages of the composting process using quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP), respectively. Moreover, the chemical properties, with a specific focus being placed on the labile C and N pools, were characterized to evaluate changes in substrate and nutrient availabilities for the methanogenic archaea during manure composting.

2. Materials and methods

2.1. Experimental setup

An in situ composting experiment was performed in triplicate based on a conventional static chamber method described in Fig. S1. Fresh cattle manure was obtained from an agricultural cattle farm. During composting, fresh manure was mixed with sawdust, and the moisture content was maintained within the range of optimum values 50–60% (Gajalakshmi and Abbasi, 2008). The dimensions of the composting chamber were 0.9 m width × 0.6 m length × 0.6 m height (0.32 m^3). A ventilation port was installed at the bottom of the chamber to circulate the air throughout the compost pile. The compost pile was turned weekly for aeration and sampled during the composting process. Sampling was performed on days 0 (starting material), 7, 14, 21, 28, and 35 days after installation (DAI). At each sampling point, compost was collected randomly at a depth of approximately 0.3 m after thorough manual mixing and subsequently stored at 4 °C or lyophilized for further analyses. Temperature in the piles (depth: 0.3 m) was monitored using a thermometer (Digital thermometer, SK-1250MC IIIa, SATO, Japan) before mixing.

2.2. Measurement of CH_4 production potential

The potential for methane production was determined in triplicate using laboratory-scale incubations. Rice paddy soil was collected at the same sampling site as described before (Kim et al., 2016), and physico-chemical properties of the soil is given in Table S1. In the slurry incubation, 5 g of air-dried rice paddy soil was used. Autoclaved deionized water (10 ml) was placed in a sterile glass bottle (120 ml) amended with manure (0.15% wt wt^{-1} based on dry weight, approximately 8 mg dried manure per each bottle) at different stages of composting, corresponding to approximately 5 Mg d.w ha^{-1} manure input in a rice field. Soil incubation without manure was performed to compare the effect of manure addition on CH_4 production potential. The bottles were capped with butyl rubber stoppers, shaken for 30 min using a rotary shaking machine, and flushed with N_2 for 1 h. All bottles were incubated for 4 weeks at 25 °C in the dark. The gas samples were collected weekly during incubation. CH_4 concentrations in the headspace in the collected gas samples were measured by gas chromatography (Shimadzu, GC-2010, Japan) packed with Porapak NQ column (Q 80–100 mesh) and a flame ionization detector (FID). The temperatures of the column, injector and detector were adjusted at 80 °C, 100 °C, and 110 °C, respectively. Helium and H_2 gases were used as the carrier and burning gases, respectively.

2.3. DNA extraction and quantitative PCR formcrA gene

Genomic DNA was extracted from the lyophilized manure samples using the Fast DNA SPIN Kit for soil (MP Biomedical, Santa Ana, CA, USA) in accordance with the manufacturer's instructions. Quantity of nucleic acids was measured by a NanoDrop (NanoDrop 1000 spectrophotometer, Thermo Scientific, USA) before quantitative PCR (qPCR). The copy numbers of the *mcr*A gene were determined using the primers *mcr*A forward 5'-GGTGGTGTMGGATTCACACARTAYGCWACAGC-3' and *mcr*A reverse 5'-TTCATTGCRTAGTTWGGRTAGTT-3' (Luton et al., 2002). The qPCR was performed using a BioRad CFX96 real-time thermocycler (BioRad Laboratories, Hercules, CA, USA). The experimental conditions (i.e., PCR thermal profile and fluorescence signal acquisition) were described previously by Ball et al. (2004). Briefly, the reaction mixtures (SYBR Green Realtime PCR Master Mix, Toyobo, Japan) were composed of 10 pmol of each primer, 1 µl template DNA, 2.0 µl bovine serum albumin (BSA) (5 mg ml^{-1}; Sigma-Aldrich, USA), and sterilized distilled water was added for a final volume of 50 µl. The amplification was carried out at 95 °C for 10 min followed by 40 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s. The DNA standard was