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Research Paper

Physicochemical characteristics of stored cattle manure affect methane emissions by inducing divergence of methanogens that have different interactions with bacteria

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ABSTRACT

Greenhouse gas (GHG) emissions from manure management are an environmental concern that hinders the livestock industry. Methane (CH_4) is the primary non-CO_2 GHG emitted from outdoor manure storage facilities. Understanding the relationship between the microbial community and manure physicochemical characteristics, together with their contributions to CH_4 emission during storage are of importance for mitigation and ecological significance. In this study, the archaeal and bacterial communities in manure were investigated using high-throughput sequencing, revealing that manure physicochemical characteristics have a major influence on the distribution and enrichment of methanogenic taxa as well as CH_4 emission. Moisture and total phosphorus (TP) were positively correlated with *Methanocorpusculum* abundance in cow manure with high CH_4 emission, while they were negatively correlated with *Methanobacterium* abundance in heifer manure with low CH_4 emission at the species level. Quantitative PCR analysis of transcript abundance of alpha subunit of Methyl coenzyme-M reductase (mcrA) gene in cow manure disclosed relatively strong activity of *Methanocorpusculum*. sPLS regression and network analyses of microbial taxa revealed that different bacteria–methanogen patterns are associated with CH_4 emission. Our data indicates that the manure physicochemical characteristics influence CH_4 emissions by altering the divergence of methanogens that differ in transcriptional efficiency of mcrA gene and are correlated with some bacterial taxa, providing insights into the mechanisms of CH_4 emission during manure storage.

1. Introduction

Animal manure is a nutrient resource containing most of the essential elements required for plant growth. Moreover, manure is a significant source of N in both intensive and subsistence production systems. The increase in livestock population, particularly ruminants, is a considerable source of GHG emissions occurring from both the enteric environment and manure management (Johnson and Johnson, 1995; Patra, 2012). In ruminant production systems, manure is the second largest contributor to GHG emissions, following enteric emission. The contribution of manure management to global GHG emissions has been estimated at 2.2 Gt warming potential equivalents (CO_2-e) per year (Steinfeld et al., 2006), with CH_4 accounting for the greatest proportion of total GHGs from manure, with a global warming potential of 25 kg CO_2-e kg^{-1} (IPCC, 2007). The outdoor storage of manure, prior to its application on a farm or for organic fertilizer production, is one of the most important aspects of manure management worldwide (Montes et al., 2013). Global CH_4 emissions from manure storage were estimated as 470 Mt CO_2-e $year^{-1}$ in 2010, with an expected 11% increase by 2020 (U.S. Environmental Protection Agency, 2006). However, the output of CH_4 emissions from manure storage are more complicated given the influence of various factors. According to the report by Holly et al. (2017), CH_4 emissions from dairy manure vary from 185 to 278 mg kg^{-1} manure at temperatures between 1 and 18 °C during a 6-month storage period. In comparison, in another study of short-term dairy manure storage reported by Külling et al. (2002), CH_4 emissions varied from 400 to 600 mg $^{-1}$ d^{-1} kg^{-1} manure during a period of 49-day storage at 20 °C. These studies suggest that manure storage

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significantly contributes to total CH_4 emission from dairy husbandry and the temperature influenced the potential of CH_4 emissions more than storage time. In China, the majority of districts are located in temperate or subtropical zones with higher than average temperatures, and therefore, though usually stacked for a short period before used as fertilizers, the possibility of greater potential CH_4 generation from manure in these regions compared to other districts in moderate temperature zones cannot be overlooked (Amon et al., 2006; Holly et al., 2017). Given the above, it is necessary to explore the effects of biotic and abiotic factors on CH_4 emissions from manure storage.

Methane emissions result from the activity of complex interactions of anaerobic bacteria together with methanogenic archaea, which utilize formate, acetate, methylamine, carbon dioxide, and hydrogen as substrates to produce CH_4 (Zinder, 1993; Garcia et al., 2000; Thauer et al., 2008). Methane emissions from stored manure are derived from methanogenic activity in the anaerobic environment (Montes et al., 2013). The complex biochemical process of methanogenesis depends on the interaction of environmental factors, physicochemical characteristics of samples and microbial communities (Da Silva et al., 2015). The influence of several environmental factors and manure characteristics on CH_4 emission has been comprehensively analyzed (Zeman et al., 2002; Hao et al., 2004; Li and Xin, 2010; Kim et al., 2014), but the relationship between the microbial community and manure characteristics remains to be established. Barret et al. (2013) reported spatio-temporal variations in manure characteristics and their impact on methanogenic activity on swine manure in its liquid state in laboratory-scale anaerobic incubation experiment, but their study did not focus on both methanogens and bacteria. In addition, solid manure contains less moisture during storage and may be absolutely different from stored liquid manure in terms of the relationship between microbial community and physicochemical characteristics and their combined effects on CH_4 emission (Rico et al., 2007, 2012). To elucidate the mechanisms underlying CH_4 emission from manure during storage, further research is essential that takes into account changes in the microbial community and physicochemical characteristics of manure, together with their association with CH_4 emission.

The main objective of this study was to explore temporal variations in the microbial community and physicochemical characteristics in relation to CH_4 emission in dairy cattle manure. The physicochemical characteristics of manure samples from milk cows and heifers differ significantly owing to variations in diet and digestion ability (Agle et al., 2009; Grandl et al., 2016), which, in turn, exert variable effects on the methanogen community and CH_4 emission. To comprehensively investigate the effects of manure characteristics on CH_4 emissions as well as the specific microbial patterns contributing to gas production, we compared samples from manure storages of lactating cows and heifers, with the aim of providing insights into the roles of microbial taxa, key influencing factors and their contributions to CH_4 emission. Data from this microbial community analysis should aid in improving our understanding of the mechanisms underlying CH_4 production by methanogens, and be useful for CH_4 mitigation in manure management.

2. Materials and methods

2.1. Ethical approval

The study was performed on an experimental farm with the permission of the farm administrators. The experimental protocol was approved by the Animal Care and Use Committee of the Chinese Academy of Agricultural Sciences, and care of experimental animals provided in accordance with Chinese standards.

2.2. Experimental design, sampling and physicochemical characteristics

Twelve healthy, non-antibiotic-treated Holstein lactating cows (three to four years of age, 690 kg body weight) and 12 heifers (8–9 months old, 320 kg body weight) were divided into two groups and fed the same total mixed ratio diet based on corn silage (Table S1). All animals had free access to food and water. Manure from lactating cows and heifers was collected from the floor at 06:00 and 18:00 each day; after mixing, 50 kg samples were taken and stored at 4 °C. After four consecutive days of collection, manure from cows and heifers was vigorously mixed once more and transferred into vessels for storage. Six duplicates of manure incubation per treatment group were analyzed with 20 kg manure in each vessel. The temperature difference between day and night was within 5 °C, and temperatures of the environment and inner stack were recorded using a HOBO detecting thermometer (U23, Onset, Bourne, MA, USA). Sampling was conducted on days 0, 20 and 33 for chemical and microbial analyses. For each vessel, five samples were extracted in a diagonal line sampling method, with one point at the intersection site and four points at the edge sites. Each sample (~100 g) was sampled using a cylindrical sampler penetrating the stack surface 10 cm deep, and all five samples were vigorously mixed as the final subsample for further analysis. As fresh manure was mixed thoroughly before storage, we collected a mixed sample on day 0 in each treatment group prepared for further MiSeq sequencing.

For chemical composition measurements, manure samples were dried, ground and sieved, and indices of organic matter (OM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), volatile solid (VS) and ammonia-N were measured based on dry matter. OM was measured using the potassium dichromate method, TP using ammonium molybdate tetrahydrate spectrophotometry, VS with burning at 550 °C, and TC and TN with the element analyzer (Elementar, Heraeus, Hanau, Germany). OM, TP, VS and ammonia-N were measured according to protocols of the China National or Industrial Standards (NY525–2012, NY/T2541–2014, GB/T6435–2006 and GB/T3600–2000). TC and TN were measured according to the manufacturer's protocol.

2.3. CH_4 emission monitoring

A dynamic vessel was used for monitoring gas emission from stored manure of cattle (Fig. S1). The cylindrical vessels had a diameter of 30 cm and height of 100 cm and were made of Plexiglass. The initial volume of manure in each vessel was approximately 35 L for cows group and 30 L for heifers group, and the headspace left was at least half volume of the vessel. The dynamic monitoring system was supplied with air using an air compressor, which drove air into the vessels equally through rotor flow meters that were calibrated before use. Air flow into the vessel was set to 4 L min^{-1}, and the air outlet connected to a photoacoustic multi-gas analyzer (Innova 1412, Ballerup, Denmark) together with a multi-channel sampler for analysis of CH_4 concentrations (Mulbrya and Ahn, 2014). The multi-gas analyzer conducted sampling every 2 min, with the first four times of gas sampling for washing the air channel and the last sampling for analysis. Gas emission flux was calculated using the formula:

$$\mathrm{EF} = (C_O - C_i) \times \frac{Q}{1000^{*}V}$$

$$\mathrm{EF_{avg}} = \frac{\sum_1^n (C_0 - \mathrm{C}_i) \times \frac{\mathrm{Q}}{\mathrm{v}}}{1000^{*}N} \times 24$$

where EF represents instant gas flux (g m^{-3} h^{-1}), EF_{avg} the daily average gas emission flux (g m^{-3} d^{-1}), C_0 and C_i the gas concentrations of outlet and inlet (mg m^{-3}), respectively, Q the gas flux into the vessel (mg m^{-3} h^{-1}), N the data collection time for each vessel per day, and V the volume of stored manure (m^3).

2.4. DNA extraction and 16S rRNA gene sequencing

Total microbial DNA was extracted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the