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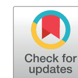
Response of the denitrifier community and its relationship with multiple N_2O emission peaks after mature compost addition into dairy manure compost with forced aeration



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HIGHLIGHTS

- Multiple N_2O emission peaks during temperature transition in early stage of manure composting was investigated.
- Nitrate in the bulking agent was consumed and partly converted into N_2O.
- Bacterial community shifted significantly, orders *Flavobacteriales*, *Burkholderiales* and *Xanthomonadales* increased.
- Increase of Pseudomonas-like denitrification genes partly explain these N_2O emission peaks.

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ABSTRACT

Animal manure is a source of the greenhouse gas nitrous oxide (N_2O), therefore understanding the mechanisms underlying its production is essential for developing mitigating strategies and sustainable livestock production system. In this study, microbial communities potentially involved in multiple emission peaks during initial stage of laboratory-scale dairy manure composting with forced aeration system were investigated. Mature compost was used for the bulking agent. Change of overall bacterial community and nitrification-denitrification gene abundance were monitored by using 16S rRNA gene *amoA*, *nirS*, *nirK* or *nosZ* genes, respectively. Three N_2O emission peaks were observed when the temperature reached at 45, 60 and 72 °C, at the same timing of oxygen consumption peaks. The maximum N_2O emission peak was 3.86 mg h^{-1} kg^{-1} TS when the temperature reached at 60 °C. The shift of bacterial community among these experimental periods was significant, orders *Flavobacteriales*, *Burkholderiales* and *Xanthomonadales* increased, while orders belong to *Bacillales*, *Lactobacillales*, *Clostridiales* and *Bacteroidales* decreased. In addition, abundance of two denitrification genes (*nirS* and *nosZ*) significantly increased during this period. Clone library analysis of these genes showed that significantly increased sequences belonged to *Pseudomonas*-like clusters for both genes, indicates that denitrifiers possesses these genes are involved for these N_2O emission peaks caused by mature compost addition.

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1. Introduction

Composting is a traditional animal manure treatment technology which is widely used in Japanese dairy industry. The fresh manure is mixed with the bulking agent such as bedding materials or other agricultural residues like rice straw. The organic substrate in the manure can be degraded aerobically mainly by bacteria and

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converted into CO_2, H_2O, ammonia or heat, and this heat production can arise the temperature over 70 °C in the compost core zone (Bernal et al., 2009). Part of nitrogen in the fresh manure can be converted into greenhouse gas N_2O during the composting (Maeda et al., 2011). Because N_2O is also known to cause the ozone depletion problem (Ravishankara et al., 2009), its mitigation is desired from the point of the view of the environmental protection.

Previously we found that two N_2O emission peaks can be observed in the laboratory scale small composting reactor (3 L of effective volume) within first 24 h (Miyatake et al., 2011), which could not be observed from the large-scale compost. These N_2O emission occurs in the initial stage of the composting, when the temperature reached about 40 °C, it drops around 50 °C and increase again around 60 °C. However, the detail why we have these emission peaks are largely unknown, thus we need to elucidate its mechanisms in order to establish effective mitigation strategy.

N_2O emission can be occurred both in nitrification and denitrification (Ni et al., 2011; Law et al., 2012), which can be performed by various microbes including bacteria, archaea, fungi or other eukaryotes (Piña-Ochoa et al., 2010; Shoun et al., 2012; Zumft, 1997). Previous study shows that denitrification is relatively more important for N_2O emission from the large scale compost (Maeda et al., 2010b). Nitrification genes and denitrification genes can be used as molecular markers to understand the ecology of the microbes which are responsible for these inorganic nitrogen transformations in the environmental samples (Ishii et al., 2011, Yoshida et al., 2010, Bru et al., 2010, Philippot et al., 2009).

In this study, we investigated the changes in microbial communities in relation to N_2O emission peaks during temperature transition. Especially, we focused on the first 24 h in the composting since it accounted most of N_2O emission in the previous study (Miyatake et al., 2011). For this purpose, samples were collected periodically during the temperature transition, which enable us to track the shift of bacterial community during these N_2O emission peaks. Mature compost was chosen for the bulking agent, since it promotes denitrification and N_2O production (Maeda et al., 2010a), and typical bulking agent such as rice straw or sawdust is being less available because of the conflict with use for biomass energy. Nitrifiers and denitrifiers were quantified using qPCR, and their composition was studied by clone library sequencing to understand their potential role in these N_2O peaks during this transition of the compost temperature.

2. Materials and methods

2.1. Laboratory scale composting reactor and outlet gas measurement system

Laboratory scale composting reactor (10 L in effective volume) with forced aeration system was used in this study (Fig. 1). The temperature of the compost was measured with thermocouples, and the temperature inside of the chamber was controlled to track the compost temperature (±1 °C) to mimic the full-scale compost piles. These temperature data were recorded every 10 min. The air was introduced from the reactor bottom after the flow control (0.62 L/min/kg dry matter). Exhaust air was passed with ammonia trap (H_2SO_4 solution) and water trap, and O_2 concentration was measured every 10 min with oxygen sensor (OS-3SD, New Cosmos Denki, Japan). Greenhouse gas (N_2O, CH_4 and CO_2) concentrations of the outlet air were determined by infrared photoacoustic detector (INNOVA 1412-5, Lumasense Technologies, USA) every 10 min.

2.2. Composting experiment

Fresh dairy manure corrected at Obihiro university farm were used in this study. The moisture content was adjusted at 70% by drying for 15 h, and mature compost corrected at the same farm (moisture content; 32.2%) were added (200 g dry weight/kg fresh compost) as the bulking agent. About 1.3 kg of the mixed samples was used for 24 h composting experiment using the laboratory scale reactor described above. The subsamples (20 g each) were taken when the temperature reached at 30, 40, 50, 60 and 70 °C during the process and stored at −20 °C for chemical analysis and DNA extraction.

2.3. Chemical analysis of the compost

Fresh compost samples were used to measure total solids, inorganic-N and pH. Total solids (TS) were measured after drying the samples overnight at 105 °C. To measure inorganic-N and pH, 5 g of fresh compost was placed into a 50 ml tube with 40 ml of deionized water, then shaken (200 rpm, 30 min) and centrifuged (3000 g, 20 min). The supernatant was collected and NH_4^+, NO_2^--N and NO_3^--N were measured using ion chromatography (ICS-1600; Dionex, USA); pH was determined with calibrated electrodes (Horiba, Japan).

The values were analyzed by ANOVA using the general linear model procedure described by SAS (SAS Institute, 2001). Tukey's multiple range comparison tests were used to separate the means. A value of $P < 0.05$ was considered statistically significant.

2.4. Microbial community structure analysis

DNA extraction from the compost samples was performed using the commercially available DNA extraction kit Isofecal (Nippon Gene, Japan). The extraction was done according to the manufacturer's instructions, and the concentrations of DNA samples were measured by BioSpec-nano (Shimadzu, Japan). The purified DNA samples were stored at −80 °C until further analysis.

Microbial community analysis was performed using the PCR-DGGE method targeting the 16S rRNA gene. The nested PCR procedure was used to obtain a highly specific PCR product. To amplify the 16S rRNA gene fragment, we performed nested PCR. For the PCR amplification, the primer set used was 27F/907R for the first PCR and 341F and 517R for the second PCR. The reaction mixture was prepared with template DNA (ca. 20 ng), 5 μM of each primer, 5 × PCR buffer for PrimeSTAR (included in the kit), 0.2 mM of each dNTP, and 0.5 U of PrimeSTAR DNA polymerase, in a final volume of 20 μl. The thermal profile for the first PCR was as follows: initial denaturation at 98 °C for 5 min; 25 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 5 s, and extension at 72 °C for 1 min; final extension at 72 °C for 7 min; and cooling at 4 °C. The thermal profile for the second PCR was as follows: initial denaturation at 98 °C for 5 min; 30 cycles of denaturation at 95 °C for 10 s, annealing at 65 °C for 5 s, and extension at 72 °C for 1 min; final extension at 72 °C for 7 min; and cooling at 4 °C. The PCR product was purified using the commercial kit Monofas (GL Science, Japan) and then used for the second PCR. A GC clamp was attached to the 5′ end of the forward primer to improve the separation of the PCR fragments. DGGE analysis of the amplified bacterial 16S rRNA gene was performed on the DCode Universal Mutation Detection System (Bio-Rad, USA) according to the instruction manual. The polyacrylamide gels (7% w/v) containing a linear formamide/urea gradient ranging from 25% to 65% denaturant were used. The gels

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