JARQ 49 (1), 17 - 21 (2015) http://www.jircas.affrc.go.jp

REVIEW
Nitrous Oxide Emissions from Dairy Manure Compost

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Abstract

Nitrous oxide (N_2O), a strong greenhouse gas, is known to be emitted during the dairy manure composting process, so its emissions should be mitigated. Both pile-turning events and inappropriate use of mature compost containing NO_x^--N may cause N_2O emissions to soar, although using bulking agent appropriately can also reduce them significantly. Isotopomer analysis of emitted N_2O suggests that bacterial denitrification is the main process of N_2O production just after pile-turning events. Significant NO_x^--N is accumulated in the pile surface between the pile turnings, and β-proteobacterial ammonia-oxidizing bacteria is seemingly the main contributor to this accumulation. Because the amount of NO_x^--N accumulated in surface explains the N_2O production after mixing, denitrification of this accumulated NO_x^--N is the main source of significant production just after the turning events. Microbes in the pile core do not contribute to this N_2O production significantly, while denitrifiers in the compost surface seem to be the main producer of significant N_2O just after the pile-turning events. The bacterial community in the compost surface is always dominated by mesophiles, belonging to phylum *Bacteroidetes* and *Proteobacteria*. The future direction and mitigation strategy is discussed in detail based on these recent findings.

Discipline: Agricultural environment
Additional key words: bacterial community, denitrification, greenhouse gas, isotopomer

Introduction

Composting is a widely used method to treat solid animal manure. Large amounts of organic matter contained in fresh manure are degraded and converted to heat, CO_2 and H_2O, which leads to a considerable reduction in mass (Bernal et al. 2009). Via composting, the organic nitrogen contained within the initial fresh manure is converted to ammonium nitrogen by various microorganisms. Some of the initial nitrogen may be lost as NH_3 by volatilization, and some is converted to gaseous content such as nitrous oxide (N_2O) or dinitrogen (N_2) through the nitrification/denitrification process (Maeda et al. 2011). Because N_2O is a well-known strong greenhouse gas (GHG), which has about 300 times the global warming potential of CO_2 (Intergovernmental Panel on Climate Change 2001), and is also known to exacerbate the depletion of the ozone layer (Ravishankara et al. 2009), its emissions should be mitigated to avoid global warming. Recent knowledge shows that 0.2-9.9% of the initial nitrogen content can be emitted as N_2O (El Kader et al. 2007, Fukumoto et al. 2003, Hao et al. 2004, Szanto et al. 2007), although detailed knowledge of these emissions remains very limited.

It is well known that both nitrification and denitrification can be accomplished by various microorganisms such as bacteria, archaea or fungi (De Boer & Kowalchuk 2001, Laughlin et al. 2008, Leininger et al. 2006), and both nitrification and denitrification can be sources of N_2O. Because microorganisms are one of the key drivers for the nitrogen cycle on our planet, extensive attention has been paid to their ecology to date and various analytical tools were developed to understand their ecology and contribution. Some molecular tools were also developed; targeting functional genes involved in nitrification or denitrification to detect them from various environments (Dandie et al. 2007, Jones et al. 2012, Purkhold et al. 2003, Throback et al. 2004). A stable isotope can be an alternative powerful tool to understand the relative contribution and significance of these microbes in the environments (Yoshida & Toyoda 2000). Since isotopic fractionation varies among microorganism groups or N_2O production pathways, we can assume the origin by measuring its isotopic signature (Sutka et al. 2008, Sutka et al. 2006).

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Rceived 12 December 2013; accepted 1 April 2014.

In this review, recent advances in nitrogen conversion and the microorganisms involved in N_2O emissions from dairy manure compost were summarized and their implications for future mitigating strategy were discussed.

Measurement of N_2O emission

A precise and reliable measurement system is essential and important to quantify N_2O emissions during the composting process. The solution is to use a chamber with a controlled airflow combined with continuous N_2O concentration measurement (Fukumoto et al. 2003, Hassouna et al. 2008, Osada & Fukumoto 2001). In swine manure composting, significant N_2O emissions occur in the latter stage of the process, while most N_2O emissions occur from the initial stage to mid-stage dairy manure composting, maybe due to differences in the NH_4^+-N content of the manure. It is also known that significant N_2O emissions occur just after the pile-turning events during dairy manure composting (Ahn et al. 2011, Maeda et al. 2010b). We have shown that deploying bulking agent (10% weight base), which is used to enhance the degradation of organic matter, can reduce N_2O (62.8%) and CH_4 (74.3%) (Maeda et al. 2013a). It is estimated that GHG emissions from the livestock production sector can be reduced up to 1,907 Gg CO_2 eq./yr by applying this method. To establish a further mitigation strategy, we studied both the overall bacterial communities and nitrogen-related microbial groups, as the main actors are microbes within the composting pile.

Microbial community succession of the compost

During the initial stage of the composting process, active degradation of organic matter and significant heat production are possible. During this thermophilic stage, it is well known that thermophilic *Bacillus* species can be detected as the dominant species (Yamada et al. 2008). In the middle stage of the composting, temporal NO_2^--N accumulation can be achieved, which means incomplete nitrification occurs during this stage. As the process progresses, easily degradable organic matter is consumed and stable organic compounds such as humic acids are produced in the latter composting stage (Fialho et al. 2010). During this stage, microbial groups such as cellulose-degrading bacteria (e.g. *Cytophaga*) or other mesophilic bacteria can dominate (Maeda et al. 2009).

When mature compost was used as a bulking agent, N_2O emissions are known to increase significantly, which might be attributable to the added NO_x^--N contained in the mature compost. Adding mature compost does not affect the overall bacterial community succession during the composting process. However, functional denitrifying gene analysis targeting *nirK* (gene-coding copper-containing nitrite reductase) and *nosZ* (gene-coding nitrous oxide reductase) by the DGGE reveals that *nosZ* diversity was significantly affected by adding mature compost, while *nirK* diversity was consistent (Maeda et al. 2010b). These findings suggested a potentially important relationship between *nosZ* diversity and N_2O emissions.

Source of N_2O just after the turning events

We performed isotopomer analysis on N_2O emitted just after turnings and revealed that the N_2O site preference (SP) values were close to bacterial denitrification (0‰) in a pure culture study (Fig. 1) (Sutka et al. 2006). Conversely, samples obtained between the turnings had higher SP values (~8‰). This elevation of SP values might be attributable to the mixing of nitrification-derived N_2O or the effect of N_2O reduction. However, we revealed that a major portion of the N_2O emissions was derived from denitrification (Maeda et al. 2010c). We also found that NO_2^- and NO_3^--N accumulation occurred in the pile-top and surface zones (<30 cm depth), and that NH_4^+-N could be detected in the core or bottom zones. Moreover, ammonia-oxidizing bacteria (AOB) proliferate in the compost surface, up to 1×10^9 copies of *amoA* (gene-coding ammonia omooxygenase), while the core and bottom zones contained much lower AOB-*amoA* copy numbers. These dominant *amoA* sequences were estimated to belong to the *Nitrosomonas europaea-eutropha* cluster. Similar sequences were reported from waste disposal sites containing high levels of nitrogen and high ammonium concentrations (Otawa et al. 2006, Zhu et al. 2007), meaning these AOB were likely adapted to the severe environment like compost. Some other studies show that ammonia-oxidizing archaea (AOA) are widely distributed in animal manure compost, but less abundant than

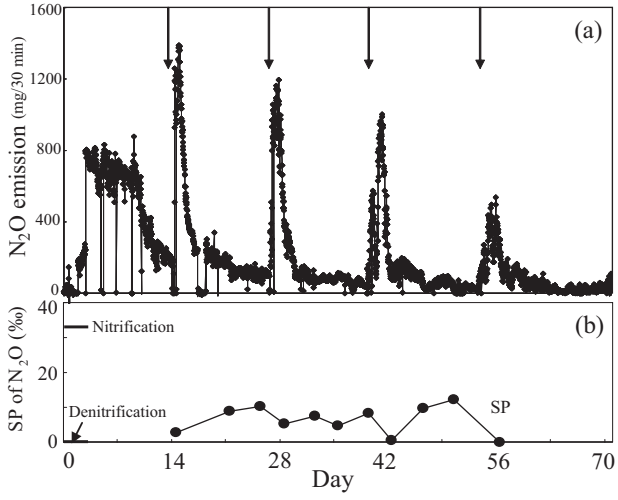

Fig. 1. Typical N_2O emission pattern from dairy manure compost. Arrows indicate the pile-turning events. SP; ^{15}N site preference value within N_2O molecule

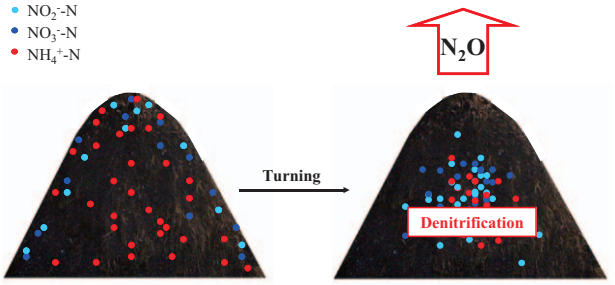

Fig. 2. Accumulation of NO_x^--N content between the turnings and N_2O emissions derived from this NO_x^--N reduction

AOB (Yamada et al. 2013, Yamamoto et al. 2012). Therefore AOB seems more important and responsible for NO_2^--N accumulation in the compost surface.

Based on these results, the N_2O emissions just after the turnings were assumed to be derived from the reduction in NO_x^--N content accumulated in the compost surface layer (Fig. 2). This was confirmed using the acetylene block method and it was also revealed that surface samples which naturally contained NO_x^--N content produced significant N_2O just after the sampling, while core samples produced no measurable N_2O, and N_2O production correlated with the NO_x^--N content (Maeda et al. 2013b). Moreover, we compared the SP values of produced N_2O and found that NO_2^--amended core samples produced N_2O with a significantly higher SP value, which strongly indicates that the denitrifiers in the surface zone are responsible for actual denitrification.

To understand the main N_2O producer in the surface zone, we compared the bacterial communities of the surface and core zones using the PCR-DGGE method targeting the 16S rRNA gene, and found that mesophiles belonging to class *Bacteroidetes* or *Proteobacteria* always dominated in the surface zone throughout the composting process, while thermophiles or anaerobes such as *Bacillus* or *Clostridium* only dominated in the core or bottom zones (Maeda et al. 2010a). Mesophiles in the surface zones were a potential concrete target when establishing effective N_2O mitigation strategies. However, because there are numerous species in these bacteria classes, further investigation is needed to reveal which specific species are responsible for N_2O production.

Future direction

Previously it was revealed that 62% of N_2O emissions can be mitigated by mixing 10% (weight base) dried grass as the bulking agent (Maeda et al. 2013a). However, the mechanism of this mitigation is completely unknown. Using a bulking agent promotes an oxidative environment in the compost piles, which may enhance nitrification activity. Because N_2O can be produced by the nitrification-denitrification process, enhancing the oxidative environment can promote N_2O emissions. Therefore, to resolve this contradiction, the effect of using a bulking agent on both nitrifiers and denitrifiers should be investigated in future studies.

Also, our previous results clearly show that NO_x^--N accumulation in compost surface and subsequent denitrification (including nitrifier-denitrification), which occurs just after the compost turning events, are source of significant N_2O emissions. To narrow down the specific species responsible for actual N_2O production, incubation experiments under enhanced denitrification conditions can be effective. A molecular-based approach such as 16S rRNA gene or functional genes analysis can be used to analyze the enhanced denitrification conditions and obtain the genetic information of the species truly responsible for N_2O production and consumption (Ishii et al. 2011b). In addition, the lack of any relationship between phylogenetic information and functional gene information in denitrifiers (Heylen et al. 2006, Ishii et al. 2011a), means an additional approach to link these types of information is needed. Previously, Hoshino and Scramm (Hoshino & Schramm 2010) suggested that the phylogenetic and functional gene information could be linked using the RCA-FISH approach, which can stain both genes independently. Moreover, other researchers introduced the functional single cell (FSC) isolation approach, which uses antibiotics to inhibit cell division that leads to cell elongation, and manipulates active cells directly using a micromanipulator (Ashida et al. 2010, Ishii et al. 2011b). To obtain truly responsible N_2O producers or reducers, these novel approaches will be needed in future studies.

However, these approaches are insufficient to mitigate N_2O emissions and maintaining NO_x^--N in an organic matter-rich environment such as compost will be difficult. Accordingly, to mitigate N_2O emissions, there is a need to enhance N_2O reducers (which emit N as N_2 into the atmosphere), and we also need to know the actual NO_x^- reducers (N_2O producers). Philippot et al. (Philippot et al. 2011) previously reported that adding denitrifier lacking N_2O reductase to the soil enhances the N_2O/N_2 ratio. It can also be said that increasing N_2O reducers will lead to a reduction in N_2O/N_2. Therefore, a management method allowing the N_2O reducers to dominate is required, without affecting the organic matter degradation primarily required for the composting process. Although finding such a method is very difficult, it can be facilitated by further understanding N_2O production and consumption, which will help improve management methods.

In conclusion, our recent studies clearly show that N_2O is emitted from the NO_x^--N content accumulated in compost surface and denitrifiers located in the compost surface are the main source of N_2O. These findings thus shed light on

the nitrogen conversion in this extremely heterogeneous process. Further studies are needed to elucidate the details of the denitrifying microbes in the compost surface, and this knowledge will help us establish concrete and truly effective mitigation strategies in future.

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