Ammonia and nitrous oxide emissions during swine slurry composting

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Abstract

NH_3 volatilization is enhanced during thermophilic composting stage due to high temperatures and NH_4^+ levels. These same factors were thought to inhibit nitrification and denitrification, since negligible NO_3^-/NO_2^- production and N_2O emissions were registered during this period. Recent studies have contradicted those assumptions, although the complete understanding of the nitrification and denitrification processes is still scarce. We evaluated N dynamics in a swine slurry composting experiment and monitored the concentration of dominant catabolic genes in nitrifying and denitrifying bacteria. NH_3-N and N_2O-N emissions represented 23.8 and 1.7% of the total N losses, respectively. Increase of N_2O-N emissions corroborated with higher *cnorB* and *qnorB* concentrations. Unaccounted nitrogen losses correlated significantly with *narG*, *nirS* and *nosZ* genes thus providing circumstantial evidence of complete denitrification and N_2 emission since the beginning of composting process.

Introduction

Up to 47% of the total N input in compost piles can be lost to atmosphere diminishing compost quality while simultaneously contributing to potential green housing emissions (e.g., NH_3 and N_2O [1]). During the earlier stages of composting the high temperature and NH_4^+ content enhance NH_3 volatilization which accounts for 9-29% of the total N losses [1,2]. Additionally, nitrification and partial denitrification can lead to N_2O emission that could represent up to 10% of the total N losses in SL composting [2,3].

It is plausible that the higher temperatures and NH_4^+ concentrations available at the earliest thermophilic stages of composting can inhibit nitrification and minimize N_2O emission [2,4]. Contradictorily to these findings, recent studies suggested that both nitrification and denitrification processes were dominant and N_2 emission could account for most of N loss even during the thermophilic composting stage [1,3]. However, the lack of empirical methods to accurately determine N_2 losses during composting is supposed to contribute to these discrepancies. Therefore, the use of catabolic genes from nitrifying and denitrifying bacteria can be used as valuable tool to improve our knowledge on those processes during swine slurry composting.

Material and Methods

Composting experiment

The composting experiment was carried out for 35 days at Embrapa Swine and Poultry (27º 18' 46" S, 54º 59' 16" W). Triplicates of composting piles were mounted inside wood boxes with the following dimensions: 2.1×1.5×0.8 m (L×W×H). Fresh swine slurry (SL) was mixed with 300 kg of wood shavings (WS) in a total of six applications as follows: 727 L at day 1, 724 L at day 8, 217 L at day 16, 215 L at day 17, 247 L at day 22, 252 L at day 30 in order to achieve a final ratio of 7.94 L of SL for 1 kg of WS (Table 1). The compost piles were manually mixed for aeration at days 4, 11, 14, 18, 21, 24, 28, and 32. Samples of WS, SL and composting material were collected and analysed for total kjeldahl nitrogen (TKN), ammonium-nitrogen (NH_4^+-N), nitrate- and nitrite-nitrogen (NO_3^--N + NO_2^--N), and total phosphorus (TP) by 4500.C, 4500.D, 4500.F, and 4500-P standard methods, respectively [5]. Total and organic nitrogen (TN and Org-N) in SL and compost samples were calculated as: TN = TKN + NO_3^--N + NO_2-N; and Org-N = TKN – NH_4^+-N. Non-volatile Pt inputs (WS+SL) and recovery on compost samples was used to check the mass balance of the compost piles. Average error on Pt mass balance was 15.6 ± 2.5 % and was considered low in comparison to similar studies [6].

Gases emissions measurement

A transparent PVC chamber measuring 12 m^3 was mounted over each of the composting piles for measurement of gaseous CO_2, CH_4, N_2O, NH_3 and water vapor emissions. A 300 mm PVC tube was attached to one extremity of the chamber. A 1/6 CV axial blower was inserted into the tube to exhaust the air from the chamber, providing a ventilation rate of 1.526 m^3 h^{-1}. Gases emissions rates were registered every 4 minutes in each chamber considering the air ventilation rate and the concentration of both elements in the inlet and outlet air samples collected through a PTFE tube by a multipoint gas sampler INNOVA 1309 and measured by an infrared photoacoustic gas analyzer INNOVA 1412 (Lumasense Technologies, Denmark). Gases emissions rates were calculated following the equation: $E = Q \times (C_o - C_i)$; where, E is the gas (CO_2-C, CH_4-C, NH_3-N or N_2O-N) emission rate (mg h^{-1}); Q is the air flow (m^3 h^{-1}); and C_o and C_i are the concentrations of CO_2-C, CH_4-C, NH_3-N or N_2O-N in the outlet and inlet air (mg m^{-3}), respectively.

Real-time quantitative (qPCR) analyzes

Nitrification processes were correlated with the presence of bacteria-harboring ammonium monooxygenase (*amoA*) genes. Nitrate reductase (*narG*), nitrite reductases (*nirS*), nitric oxide reductases (*cnorB* and *qnorB*) and nitrous oxide reductase (*nosZ*) genes were used to infer on denitrification. Bacterial DNA was extracted from the composting sediments using UltraClean® Microbial DNA isolation kit (MoBio Laboratories Inc., USA) according to manufacturer's instructions. PCR reactions were performed using a Rotor Gene 6000 (Corbett Research, NSW, Australia). DNA standard curves for *16S* were obtained by 10-fold dilutions of genomic DNA from *E. Coli* (ATCC 35218) and by cloning for all other genes. Serial dilutions of 10^1 to 10^9 gene copies/ mL were used to prepare calibration curves. The gene copy numbers in each sample was estimated based on the following equation: Gene copy numbers μL^{-1} = (μg DNA μL^{-1})/ (bp genome^{-1}) × bp μg DNA^{-1} × genes genome $^{-1}$. This equation assumes the size of the bacterial genome base pair (bp) used as the standard in the calibration curves (bp) (http://www.genomesonline.org) and there is approximately 9.1×10^{14} bp μg^{-1} of DNA. The detection limit of each assay was 10^2 gene copies mL^{-1}.

Results

N dynamics during swine slurry composting

Temperature remained higher than 50ºC during the first two weeks of composting, decreasing to 38-49ºC after the third manure incorporation into composting piles (Figure 1a). Moisture ranged from 68 to 76% during the same period. NH_3-N emissions were more pronounced during the first 25 days of composting, while N_2O-N emissions were negligible during the first 18 days, increasing afterwards (Figure 1b). These results are consistent with previous studies [2,3]. Low N_2O-N emissions under high temperature and very small NO_3^-+NO_2^- content in compost piles suggests inhibition of nitrification and denitrification processes as previously reported [2,4].

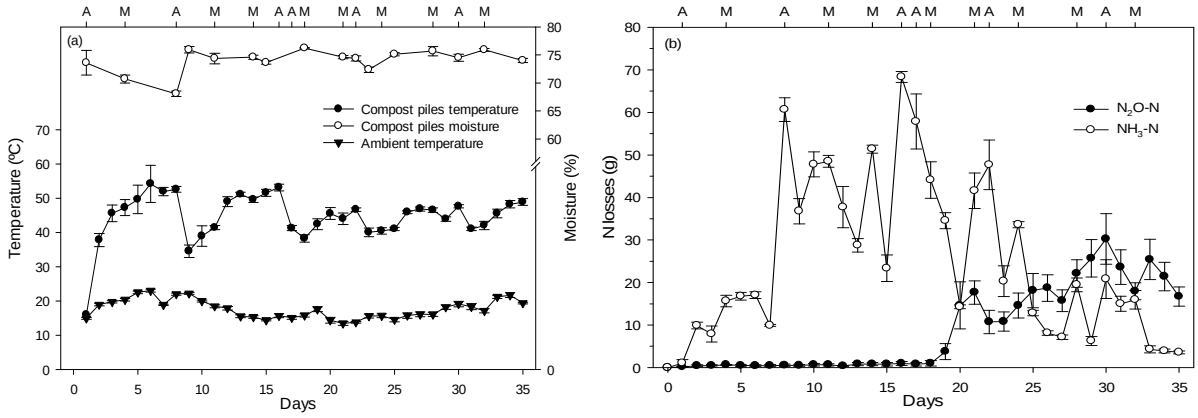

Figure 1. Temperature and moisture profiles (a) and daily NH_3-N and N_2O-N losses (b) from swine slurry composting piles. Error bars mark extent of standard errors (n=3). Letter A indicates days with slurry application and letter M indicates days when compost piles where mixed.

NH_4^+-N removal from composting piles (Figure 2a) was likely attributable to partial N immobilization as org-N (up to day 4) and NH_3-N volatilization. However, TN losses were higher than measured NH_3-N and N_2O-N losses (Figure 2b). Therefore, the unaccounted N losses could be associated with N_2 emission as result of complete denitrification [1,3].

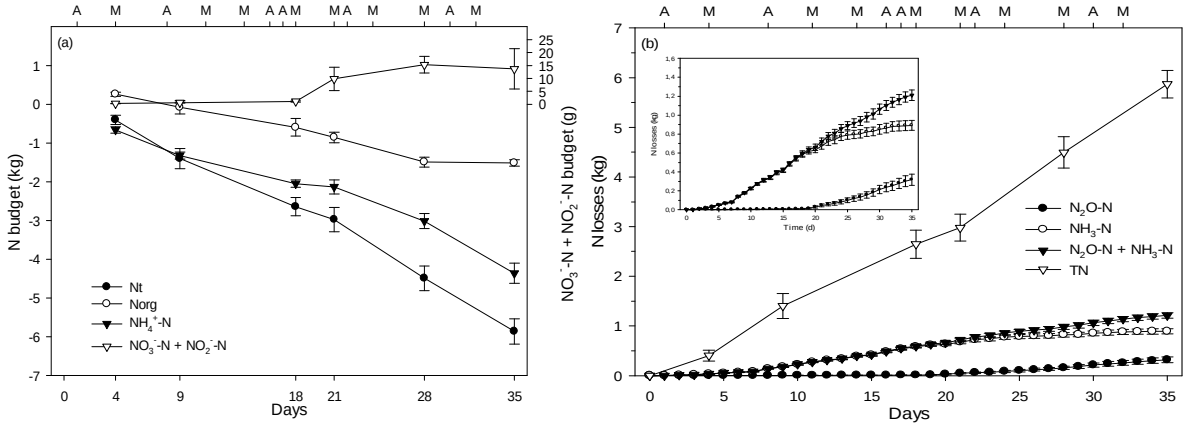

Figure 2. Budget of nitrogen species (a) and cumulative N losses (b) from swine slurry composting piles. Error bars mark extent of standard errors (n=3). Letter A indicates days with slurry application and letter M indicates days when compost piles where mixed.

Analysis of the abudance of *amoA*, *narG*, *nirS*, *cnorB*, *qnorB*, and *nosZ* genes indicated the presence of metabolic active nitrifying and denitrifying bacteria since the beginning of the composting (Figure 3). After 18 days of composting, the high concentration of c*norB* and *qnorB* relative to *nosZ* gene indicate incomplete denitrification to N_2O. The observation of incomplete denitrification to N_2O was linked to limited oxygen availability as supported by the establishment of methanogenic conditions [6].

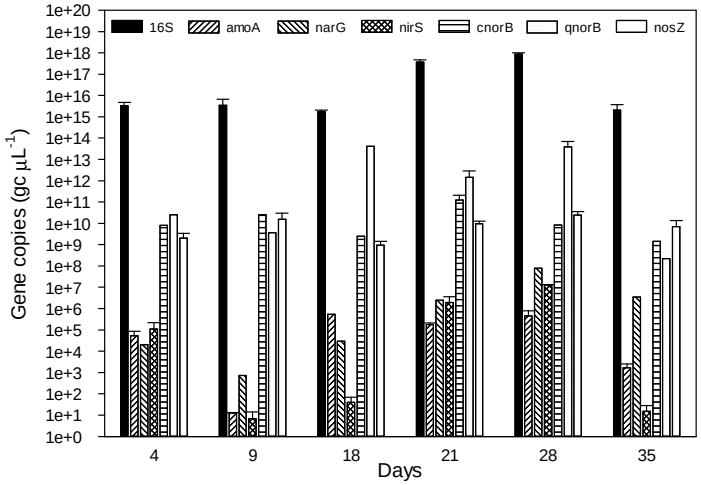

Figure 2. Abundance of 16S, amoA, narG, nirS, cnorB, qnorB, and nosZ catabolic genes from nitrifying and denitrifying bacteria during swine slurry composting.

The correlations between temperature, moisture, NO_3^-+NO_2^- content, and genes concentrations with N_2O emissions, unaccounted N (N_u) losses and N_2O+N_u is shown (Table 1). Temperature and moisture had apparent no effect on N_2O or N_u losses while NO_3^-+NO_2^- content was strongly correlated with N_2O emission. The abudance of *narG*, *nirS*, and *nosZ* genes correlated significantly with N_u and N_2O+N_u losses. The *narG*, *nirS* and *nosZ* demonstrate their usefulness as a biomarker tool to efficiently account for biological N losses that occurs during swine slurry composting, as similarly demonstrated for soils studies [7].

Table 1. Pearson's coefficients (r) obtained for correlations between nitrifying and denitrifying catabolic genes with measured N_2O emissions and/or unaccounted N losses (N_u) during swine slurry composting (n=6).

Parameter	N_2O		N_u		N_2O+N_u	
	r	p	r	p	r	p
Temperature	0.608	0.201	0.000	0.999	0.105	0.843
Moisture	0.179	0.735	0.254	0.627	0.270	0.605
$NO_3^- + NO_2^-$	**0.979**	**<0.001**	0.284	0.585	0.436	0.387
16S rDNA	0.758	0.080	0.688	0.131	0.778	0.068
amoA	0.160	0.762	0.282	0.588	0.293	0.573
narG	0.636	0.175	**0.865**	**0.026**	**0.923**	**0.008**
nirS	0.668	0.147	**0.830**	**0.041**	**0.896**	**0.015**
cnorB	0.316	0.542	-0.373	0.467	-0.296	0.568
qnorB	0.115	0.829	0.419	0.408	0.414	0.414
nosZ	0.564	0.244	**0.841**	**0.036**	**0.888**	**0.018**

Conclusion and perspectives

Unaccounted N losses correlated significantly with denitrifiers narG, nirS and nosZ genes, indicating complete denitrification to N_2 as the main source of N losses. Therefore, these biomarkers can be utilized as a tool to effectively help our current understanding of the major biological mechanism of nitrogen cycle within swine slurry composting. This is particularly important considering our empirical limitations to accurately measure N emissions at field scale.

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