Relationship between respiratory quotient, nitrification, and nitrous oxide emissions in a forced aerated composting process

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Abstract

We assessed the relationship between respiratory quotient (RQ) and nitrification and nitrous oxide (N_2O) emission in forced aerated composting using lab-scale reactors. Relatively high RQ values from degradation of readily degradable organics initially occurred. RQ then stabilized at slightly lower values, then decreased. Continuous emission of N_2O was observed during the RQ decrease. Correlation between nitrification and N_2O emission shows that the latter was triggered by nitrification. Mass balances demonstrated that the O_2 consumption of nitrification (~ 24.8 mmol) was slightly higher than that of CO_2 emission (~ 20.0 mmol), indicating that the RQ decrease was caused by the occurrence of nitrification. Results indicate that RQ is a useful index, which not only reflects the bioavailability of organics but also predicts the occurrence of nitrification and N_2O emission in forced aerated composting.

1. Introduction

The spreading of livestock excrement can contribute to nitrate contamination of groundwater and eutrophication of surface waters. To mitigate these issues, a decentralized differentiable treatment system for livestock excreta that discharges minimum pollutants, produces minimal greenhouse gas (GHG) emissions, and more effectively recovers nutrients has been proposed (Fujiwara, 2012). Producing compost of better quality and less GHG emission is one of the key technologies in this innovative system, through preventing environmental pollution and enhancing resource recycling in agricultural areas. To achieve these outcomes, it is necessary to understand changes of the material during the composting process. In particular, the emission of nitrous oxide (N_2O), which is a GHG and has a reported global warming potential 298 times greater than that of carbon dioxide (CO_2) (IPCC, 2007), has become a problem in agricultural areas. Controls are needed to reduce N_2O emissions produced during the composting process.

Compost stability is an important component of the composting process, because it has implications for compost use as a fertilizer. Stability is related to the types of organic compounds that remain following composting and the microbial activity of composted materials (California Compost Quality Council, 2001). The primary goal of composting is to produce a stabilized end-product (Komilis and Kanellos, 2012). Some of the most common compost stability tests are based on quantification of microbial respiration (Komilis et al., 2011a). Microbial respiration activity indices based on oxygen (O_2) uptake rate (OUR) such as AT4 (static respiration index for 4 days)

and specific OUR (SOUR) (Lasaridi et al., 2006), and on CO₂ evolution (Bernal et al., 2009), are commonly used to test compost stability (Gómez et al., 2006). By coupling data of OUR and CO₂ evolution rates, the respiratory quotient (RQ) can be calculated. The RQ is the ratio of the number of moles of CO₂ generated to the number of moles of O₂ consumed. The value of RQ is known to reflect not only microbial respiration but also the biochemical composition of organic material (Gea et al., 2004), and is widely used in the study of soil environments (Dilly, 2003). Previous studies have reported that RQ is a useful index for composting processes (Atkinson et al., 1997), although others reported that RQ did not change significantly throughout those processes (Gea et al., 2004; Gómez et al., 2006). The applicability of RQ as an index of compost stability requires further study.

Stability indices are not only important measures of compost characteristics, but can also be used for process performance monitoring (Gómez et al., 2006). It is important to establish useful indices for both measuring compost stability and monitoring microbial reactions during composting, to identify appropriate conditions that will produce high-quality compost while lowering GHG emissions. To reduce N₂O emissions from the composting process, microbial activity in the nitrogen cycle during nitrogen transformation should be monitored. Previous studies of respiration indices such as RQ in composting processes have only focused on the biochemical composition of organic material (Gea et al., 2004; Komilis and Kanellos, 2012). In an earlier study, we constructed experimental devices for continuous monitoring of emitted gases from a composting reactor, indicating that N₂O emissions occurred simultaneously with an RQ decrease (Tsutsui et al., 2013).

However, we could not obtain information about potential mechanisms responsible for this relationship, because we did not conduct either microbial community or mass balance analyses.

In this study, we took a two-step approach to examine RQ usefulness as an indicator, for both compost stability and monitoring of nitrification and N₂O emissions. In the first step, we performed a detailed gas-phase analysis to clarify the relationship between the RQ decrease and N₂O emissions. We used lab-scale forced aerated composting reactors to continuously measure gas components, toward evaluating the suitability of RQ as an indicator. In this experiment, we did not conduct sampling campaigns to avoid the variation of gas components triggered by sample mixing for clarifying the relationship between the RQ decrease and N₂O emissions. In the second step, we investigated mechanisms responsible for the simultaneous occurrence of nitrification and N₂O emissions with the RQ decrease in the forced aerated composting process. This was done by conducting gas phase, sample composition, microbial community, and mass balance analyses. In addition, temporally and spatially complex environment conditions (such as temperature, oxygen concentration, and water content) make us difficult to clear the mechanism of N₂O emission in actual composting process. To achieve the objective of our study, we conducted the forced aerated composting process under isothermal condition and maintained the water content at constant to prevent the effect of large fluctuation in environmental conditions.

2. Materials and Methods

2.1. Composting reactors and experimental devices

Composting reactors and experimental devices used were described in detail by Tsutsui et al. (2013). Briefly, we used three 1.63-L forced aerated composting reactors equipped with a porous stainless steel plate at the bottom. Inlet gas was humidified to maintain water content of the samples at ~60%. Outlet gas was bubbled into a 5 mM sulfuric acid solution to trap ammonia gas. To express the measured gas concentration in the inlet and outlet as the molar flow rate (mmol/h) at standard conditions (273.15 K; 101.325 kPa), temperature and pressure sensors (Sensez, Tokyo, Japan) and a gas flow meter RK1150PV (Kojima Instruments Inc., Kyoto, Japan) were placed upstream and downstream of the reactor, respectively. Solenoid valves were installed downstream of the sensors in the outlet gas line to guide exhaust gas into the gas analysis devices by turns.

2.2. Composting experiments

Manure from Japanese brown cattle raised at Kochi University (Kochi, Japan) and dried sawdust of Japanese cedar and Japanese cypress trees were used as composting materials. Fresh manure from four cattle was sampled and mixed well, then immediately transferred to the laboratory and processed in preparation for analysis of water content. Manure samples were used in the experiment within 12 hours of collection.

Sawdust was added to the cattle manure to adjust moisture content to 60%, and the well-mixed sawdust and manure were transferred to the composting reactors. Reactors were submerged in water baths controlled at 30 °C and inlet air was supplied to the reactor to start the

composting experiment. Gas flow rate and pressure were controlled and kept constant at 1.7 ml/min/g-TS and 161.6–167.7 kPa throughout the experiment, using a needle valve and pressure regulator, respectively.

2.2.1 Relationship between RQ and N₂O emission (Experiment I)

To ensure reproducibility of the relationship between RQ and occurrence of N₂O emission observed in the earlier study (Tsutsui et al., 2013), we conducted a forced aerated composting experiment (Experiment I) and monitored RQ and N₂O emission rates. To get clear gas component data, we did not do sampling during Experiment I, because variation of gas components was triggered by sampling of the composting mixture.

Sawdust (120.6 g) was added to the cattle manure (429.4 g) to adjust moisture content to 60%. Then, 550 g of the well-mixed sawdust and manure were transferred to the three composting reactors. Inlet air was then continuously supplied to the reactor for 30 days. During Experiment I, RQ and N₂O emission rates were monitored by following the steps in Section 2.4. Characteristics of the composting samples at the beginning and end of experiment are listed in Table 1.

2.2.2 Mechanisms of RQ decrease and N₂O emission (Experiment II)

To clarify mechanisms responsible for the simultaneous RQ decrease and occurrence of N₂O emission, a forced aerated composting experiment was executed (Experiment II). Sawdust (39.6 g) was added to the cattle manure (235.4 g) to adjust moisture content to 60%. Then, 275 g of the well-mixed sawdust and manure were transferred to the two composting reactors 1 and 2. Inlet air

was then continuously supplied to the reactor for 44 days. A portion of the composting mixture was collected on days 2, 5, 15, 20, 24, 26, 29, and 32. Before sampling, contents of each reactor were well mixed and total weight was measured. After sampling, the contents were returned to the reactor to simulate a pile mixing. The amount of sampling per sampling day depended on the measurement items and ranged from 3 g-wet to 10 g-wet (4.2 g-wet on average). Collected samples were processed for chemical and microbial community analysis, described in Sections 2.3 and 2.5, respectively. Additionally, gas analyses were done during the experiment by following the steps in Section 2.4.

2.3. Chemical analysis

Moisture content and total solid (TS) content of the samples were calculated from weights obtained before and after drying at 105 °C for 12 h. The dried samples were processed at 600 °C for 2 h to calculate the content of volatile solids. We measure the chemical oxygen demand (COD_{Cr}) of composting sample suspended in ultrapure water by using COD reagent TNT822 (HACH, Loveland, CO, USA) to assess the biodegradation of organic matter contained in composting samples.

Measurements of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ concentrations in the composting mixture were made by following the methods in Tsutsui et al. (2013). Samples for pH measurement were prepared by shaking (200 rpm, 30 min) after adding distilled water in a volume equivalent to 10 times of the TS weight, and calibrated electrodes (Horiba, Kyoto, Japan) were used for pH analysis. Total carbon

and total nitrogen were determined by gas chromatography with an N-C analyzer (SUMIGRAPH NC-1000; Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

2.4. Gas analysis

Average gas molar flow rates for 60 min were used for the following OUR analysis.

Concentrations of O₂, CO₂, and N₂O in inlet and outlet gas were measured and expressed as the molar flow rate (mmol/h) at standard conditions (273.15 K; 101.325 kPa), as in the methods of Tsutsui et al. (2013). Oxygen concentration, gas pressure, and gas temperature of the input and output were measured every 1 min, and OUR was calculated from the O₂ molar flow rate in the inlet and outlet gas, using the following equation (Lopez Zavala et al., 2004).

$$\text{OUR (mmol} \cdot \text{h}^{-1}) = ([\text{O}_2]_{\text{in}} - [\text{O}_2]_{\text{out}}), \quad (1)$$

where [O₂]_{in} and [O₂]_{out} are input and output of O₂ molar flow rates (mmol·h⁻¹), respectively.

Concentrations of CO₂ and N₂O in the outlet gas were measured three times within 30 min at the outlet of each reactor, using a micro-GC GC-490 (GL Sciences, Tokyo, Japan). Additionally, CO₂ concentration in the laboratory air was monitored with a CO₂ sensor MCH-383SD (Sato Co., Tokyo, Japan) installed at the air inlet port, to correct the CO₂ concentration derived from inlet air.

The RQ was calculated using the following equation (Atkinson et al., 1997).

$$\text{RQ (-)} = \frac{\text{CO}_2 \text{ emission (mol)}}{\text{O}_2 \text{ consumption (mol)}} \quad (2)$$

Cumulative values of CO₂ emission and O₂ consumption were used for the calculation of RQ.

Duration for the calculation for cumulative values of CO₂ and O₂ were 30 min. in Experiment I and 6

h ~ 36 h in Experiment II.

2.5 Microbial community analysis

2.5.1 DNA extraction and purification

For microbial community analyses, DNA was extracted from approximately 0.2 g of wet compost samples using the ISOIL for Beads Beating kit (Nippon Gene, Tokyo, Japan) and further purified with a MagExtractor-PCR & Gel Clean up (Toyobo, Osaka, Japan) according to manufacturer's instructions.

2.5.2 Real-time PCR quantification of 16S rRNA genes of eubacteria and amoA genes of ammonia oxidizing bacteria (AOB)

Real-time PCR quantifications of 16S rRNA genes of total eubacteria and *amoA* genes of ammonia oxidizing bacteria (AOB) were carried out on a PikoReal real-time PCR system (Thermo Scientific, Waltham, MA, USA), using a GeneAce SYBR qPCR Mix α (Nippon Gene, Tokyo, Japan). Primers SRV3-1 and SRV3-2 (Lee et al., 1996) were used for quantification of 16S rRNA gene numbers, whereas primers amoA-1F and amoA-2R (Rotthauwe et al., 1997) were used for quantification of AOB *amoA* gene numbers. The reaction mixture (20 μ l) contained 1 $\times$ GeneAce SYBR qPCR Mix α , 0.2 μ M of each primer, and 2 μ l of DNA template. The thermal profile for

quantification of 16S rRNA genes consisted of initial enzyme activation at 95 °C for 10 min, then 40 cycles of denaturation at 95 °C for 30 s and annealing and extension at 60 °C for 1 min. The thermal profile for AOB *amoA* gene quantification consisted of initial enzyme activation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. After the aforementioned PCR and subsequent hold at 60 °C for 30 s, melting curve analyses were performed from 60–95 °C with a read every 0.2 °C and hold of 1 s between reads.

Standard curves for 16S rRNA genes and AOB *amoA* genes were prepared using serial dilutions of genomic DNA from *Escherichia coli* NBRC 12713 and *Nitrosomonas europaea* NBRC 14298), respectively, concentrations of which were determined using a Qubit dsDNA HS Assay kit (Life Technologies/Molecular Probes, Eugene, OR, USA) with Qubit 2.0 fluorometer (Life Technologies/Invitrogen, Carlsbad, CA, USA). All quantifications were done in triplicate. PCR efficiencies for 16S rRNA genes and AOB *amoA* genes were 83.9% and 81.0%, respectively.

2.5.3 PCR detection of *amoA* genes of ammonia oxidizing archaea (AOA)

The presence of ammonia oxidizing archaea (AOA) *amoA* genes was examined by conventional PCR using primers Arch-*amoA*F and Arch-*amoA*R (Francis et al., 2005) in a T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The reaction mixture (50 µl) consisted of 1× SapphireAmp Fast PCR Master Mix (Takara Bio, Shiga, Japan), 0.2 µM of each primer, and 2 µl of DNA template. The thermal profiles consisted of initial enzyme activation and denaturation at 95 °C for 5 min, 40 cycles of denaturation at 94 °C for 45 s, annealing at 53–58 °C for 1 min,

extension at 72 °C for 1 min, and final extension at 72 °C for 15 min. PCR products were analyzed by electrophoresis on a 1.5% (w/v) agarose gel stained with SYBR Green I (Takara Bio, Shiga, Japan).

3. Results and discussion

3.1. Relationship between RQ value and N₂O emission in Experiment I

Profiles of the OUR and CO₂ emissions from the triplicate composting reactors are shown in Fig. 1(A) and (B), respectively. Small standard deviations of the OUR or CO₂ emission profiles throughout the experiment demonstrate good reproducibility of organic matter decomposition in this experiment. Additionally, the profile of CO₂ emission rates was similar to that of OUR in all reactors, with relatively low values. In the first hour of the experiment, OUR and CO₂ emission rates were maxima; they then decreased, with two narrow peaks at 5.5 h and 20.5 h. Relatively stable OUR values were observed from days 2 through 7. OUR values began to decrease from ~6.5 (day 7) to ~5.0 mmol/h (day 9). Simultaneously, CO₂ emission rates began to decline from about 2.3 (day 7) to about 0.80 mmol/h (day 9). Beginning on day 9, a gradual decline in OUR and CO₂ emission rates was observed, which continued until the end of the experiments (day 29.5). The thresholds of OUR and CO₂ emission rates for composting stability are proposed to be 0.4 g-O₂/g-TS/h and 2 g-CO₂/g-VS/day, respectively (California Compost Quality Council, 2001). In our study, OUR and CO₂ emission rates at the end of experiment were 0.31 g-O₂/g-TS/h and 0.22 g-CO₂/g-VS/day, respectively. These results indicate that the end product of the experiments was stabilized compost.

Changes in RQ values and N₂O emission rates over the course of Experiment I are shown in Fig. 1(C) and (D), respectively. At the beginning of the experiment, a rapid decrease in RQ was observed, followed by stable values around 0.35 from day 2 through 7. An RQ decrease over time has been observed in other studies (Komilis et al., 2011b) and the initial decomposition of readily degradable material, followed by decomposition of remaining moderately and slowly degradable organic material, was expected to affect such behavior (Komilis et al., 2011b). After the stable period, RQ declined drastically from 0.35 on day 7 to 0.13 on day 10. N₂O emissions began to increase dramatically from day 7 through 10 in all three reactors, then gradually decreased until day 20. A temporal shift of correlation between RQ and N₂O emission rates is plotted in Fig. 2. The shift of the plots to the upper left side of the figure from day 7 through 11 shows that N₂O emission rates increased as RQ decreased. Stable and low RQ values in the final five days of the experiments, which were demonstrated by the stable OUR and CO₂ emission rates, indicate that there was adequate degradation of organic matter in the composting sample and low risk of O₂ depletion when the end product was applied to agricultural field soil.

3.2. RQ and N₂O emissions in Experiment II

RQ values and N₂O emission rates during Experiment II are shown in Fig. 3(A) and (B), respectively. Relatively wide variations of RQ and N₂O emission rates were observed in this experiment. This might be a result of the sampling process; decomposition of organic matter and

CO₂ emissions are enhanced by mixing of the composting sample. To identify the RQ trend more clearly, the 3-day moving average line is shown in Fig. 3(A). The RQ trend in Experiment II was generally similar to that of Experiment I, i.e., RQ decreased rapidly at the beginning of the experiment, then stabilized from day 5 through 15, and decreased again from day 15 through 23. However, relatively high N₂O emissions were observed from day 17 through 28. Although the peak N₂O emission occurred 7 days later than in Experiment I, the RQ decrease and N₂O emissions were observed in nearly the same period in Experiment II. These results and those of our previous study (Tsutsui et al., 2013) indicate that the RQ decrease and N₂O emissions occurred over the same period. Furthermore, highest N₂O emission rate in Experiment II was much lower than that in Experiment I. Amount of composting sample in Experiment I was twice as much as that in Experiment II. And the amount of NO₃⁻-N at the end of experiment I was about 1.5 times as much as that in Experiment II. In addition, we mixed the composting sample only in Experiment II. Those differences might affect the N₂O emission rate.

3.3. Mechanism of N₂O emission

Figure 4 shows the behavior of inorganic nitrogen in the composting samples during Experiment II. The decrease in NH₄⁺-N at the beginning of that experiment was induced by ammonia volatilization, and the increase in NH₄⁺-N from day 2 through 20 may have resulted from degradation of organic matter (Guo et al., 2012). From day 15, NO₂⁻-N and NO₃⁻-N began to

increase and reached 130 mg-N and 436 mg-N on day 24 and day 29, respectively, indicating nitrification between day 15 and 29. Cumulative O_2 consumption for the production of NO_2^- -N and NO_3^- -N calculated by the amount of NO_2^- -N and NO_3^- -N in composting sample from day 20 through 26 was 24.8 mmol, and was slightly higher than that for CO_2 emission. In general, the amount of O_2 uptake by nitrifier is much lower than the amount of O_2 uptake by heterotrophic microorganisms. This might be because easily degradable organics was already consumed during the beginning period (Fig. 1C), and O_2 consumption by heterotrophs for CO_2 production was significantly decreased. Combined with the coincidence in time in the decline of RQ and the increase of N_2O , the decrease of RQ was induced by both a reduction in the availability of organic material and in O_2 consumption for nitrification. Both inorganic nitrogen content and RQ finally stabilized after day 32, as shown in Figs. 3(A) and 4. The decrease in O_2 consumption by nitrification resulted in a stable RQ value in the later period of the composting process. These results indicate the usefulness of RQ as an index for monitoring the occurrence and end of nitrification. Finally, NO_3^- -N content in the composting sample (377 mg-N) was much higher than NH_4^+ -N (59.5 mg-N) and NO_2^- -N (38.6 mg-N).

Nitrogen balance during Experiment II is shown in Table 2. Although the proportion of total nitrogen occurring as organic nitrogen was high (85.2%) at the beginning of that experiment, this decreased to about half the total nitrogen (54.7%) at the experiment end, because of the degradation of organic matter. That is, about half the nitrogen in the samples was inert during the composting process. A decrease of organic nitrogen was caused by transformation to inorganic nitrogen

(26.9%/initial T-N), a portion of which was emitted as NH_3 (17.1%/initial T-N) and N_2O (2.02%/initial T-N) through the end of the experiment. Emission rates of N_2O in this and earlier studies are summarized in Table 3. The N_2O emission rate in Experiment II was relatively low but within the range of reported values. The data shown in Table 2 indicate that denitrification was negligible during this experiment. Therefore, correlation of oxidized nitrogen (NO_2^- -N + NO_3^- -N) and N_2O emissions between sampling dates are plotted in Fig. 5. These plots are used to investigate the relationship between nitrification and N_2O emission rates in Experiment II. A strong linear relationship between the two implies that nitrification was the dominant pathway for N_2O emission in these experiments. The slope of the collinear approximation indicates that ~2.7% of oxidized ammonia might be converted to N_2O during composting. In Experiments I and II, we observed a clear relationship between the RQ decrease, nitrification, and N_2O emission rates under forced aeration composting. Therefore, we conclude that RQ is a useful index, reflecting not only the bioavailability of residual organics but also predicting nitrification and N_2O emissions in aerobic composting.

To obtain microbial evidence for nitrogen metabolism in Experiment II, abundance of the *amoA* gene of AOB and AOA was measured. Transition of the *amoA* gene of AOB and 16S rRNA gene of eubacteria are shown in Fig. 6. Abundance of the 16S rRNA gene of eubacteria was relatively stable; it increased slightly during the first half of the period (through day 15) and decreased slightly in the second half (Fig. 6). Growth and decrease of eubacteria might result from

degradation of readily biodegradable organic matter and endogenous respiration of heterotrophic microorganisms, respectively (Hotta and Funamizu, 2007). Abundance of the *amoA* gene of AOB was below detection limits (4.6×10^3 copies/g-TS) in both reactors at the beginning of the experiment. However, the *amoA* gene of AOB was detected from day 15, reaching 3.0×10^5 copies/g-TS in reactor 1 and 9.8×10^5 copies/g-TS in reactor 2 on day 29. These results indicate that growth of AOB with nitrification occurred in Experiment II. The abundance of the *amoA* gene of AOB was nearly the same as that reported in other studies on swine slurry composting (Angnes et al., 2013) and beef and dairy cow manure compost (Yamada et al., 2013), but much lower than that observed in dairy cow manure compost (about 10^7 – 10^9 copies/g-TS) (Maeda et al., 2010). The abundance of AOB is expected to vary between different compost types because of the difference in sample components or bedding material. The abundance of AOB in our experiment was equivalent to the average abundance found across a variety of compost types. Ammonia oxidization activity by AOA has been a focus of recent research, and studies have reported that AOA was either present in composting processes (Yamamoto et al., 2010; Zeng et al., 2011; Xie et al., 2012) or the key community for ammonia oxidization (Zhang et al., 2011). We investigated the presence of AOA *amoA* genes by conventional PCR. Although we used a widely used primer set and modified PCR conditions, non-specific amplification was observed in all DNA samples (data not shown). Although we could not determine the abundance of the *amoA* gene of AOA in the present study, a very weak band with the anticipated size for the target *amoA* gene of AOA was detected only on day 5 in reactor 1.

Therefore, the abundance of AOA in Experiment II was low, whereas AOB appears to be predominantly responsible for the observed nitrification activity. This might be because our experiment was conducted at 30 °C to encourage bacterial activity, especially that related to the nitrogen cycle. As a result, activity of AOB exceeded that of AOA.

Conclusion

We assessed the relationship between RQ, nitrification, and N₂O emission in a forced aerated composting process using lab-scale reactors. Continuous emissions of N₂O were observed during a decrease in RQ. Mass balance calculations demonstrated that nitrification occurrence caused the latter decrease. Correlation between nitrification and N₂O emission shows that nitrification triggered that emission. Our results indicate that RQ is a useful index, predicting the occurrence of nitrification and N₂O emission during forced aerated composting of cattle manure under isotherm condition. These findings may contribute to producing compost with improved quality and fewer GHG emissions.

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Tables

Table 1. Characteristics of compost samples prior to (initial) and following (final) the experimental period.

	Moisture %	TS %	VS %-TS	NH ₄ -N mg-N/kg-TS	NO ₂ -N mg-N/kg-TS	NO ₃ -N mg-N/kg-TS	pH -	COD _{Cr} g/kg-TS
Initial	60.0 ± 0.14	40.0 ± 0.14	94.5 ± 0.85	652 ± 0	0.01 ± 0.007	2.7 ± 0.21	7.71	1198 ± 19.8
Final	60.5 ± 0.25	39.5 ± 0.25	91.8 ± 0.42	197 ± 56.9	127 ± 10.6	1573 ± 80.6	7.99	973 ± 10.6

Table 2. Mass balance analysis of nitrogen in Experiment II.

Time (day)	0	15	29	44
Org-N (mg-N)	1.50×10^3	1.20×10^3	546	575
NH ₄ ⁺ -N (mg-N)	253	268	91.4	59.5
NO ₂ ⁻ -N (mg-N)	0.32	0.94	61.0	38.6
NO ₃ ⁻ -N (mg-N)	6.78	2.58	436	377
T-N (mg-N)	1.76×10^3	1.47×10^3	1.13×10^3	1.05×10^3
NH ₃ (mg-N)	0	184	301	301
N ₂ O (mg-N)	0	8.89	32.5	35.7
Sampling (mg-N)	0	137	287	398
Sum (mg-N)	1.76×10^3	1.80×10^3	1.75×10^3	1.78×10^3

Table 3. Nitrous oxide emission rates during composting process.

Substrate	Aeration	Initial volume (wet-)	Duration (days)	N ₂ O emission rate (%T-N)	Reference
Cattle manure	Forced aeration	275 g	44	2.02	This study
Cattle manure	Forced aeration	4.92 kg	31	0.9–6.5	Chowdhury et al., 2014
Hen manure	Forced aeration	4.92 kg	31	17.7–26.5	
Cattle and Hen manure	Forced aeration	4.92 kg	31	16.5–21.7	
Organic household waste	Forced aeration	200 L	59	<1	Jarvis et al., 2009
Pig manure	Headspace ventilation	13 kg	112	8.85	Fukumoto et al., 2006
Swine manure	Headspace ventilation	16 kg	110	9.27	Fukumoto and Inubushi, 2009
Swine manure	Headspace ventilation	16 kg	76	6.53	Fukumoto et al., 2011
	Headspace ventilation	50 kg	91	5.62	
Dairy cow manure	-	1,200 kg	80	0.37	Ahn et al., 2011
Swine manure	-	320 kg	About 80	3.70	Fukumoto et al., 2003
	-	780 kg	About 80	4.60	
Pig manure	-	1,132 kg	118	2.50	Szanto et al., 2007
	-	1,045 kg	118	9.90	

Figure captions

Fig. 1. Oxygen uptake rate (A), CO₂ emission rate (B), respiratory quotient (C), and N₂O emission rate (D) in Experiment I. Values and error bars indicate average and standard deviation in triplicate experiments.

Fig. 2. Temporal shift of correlation plots between respiratory quotient and nitrous oxide emission rates. Symbols represent following time periods: triangle, day 0 through 7; diamond, day 7 through 11; circle, day 11 through 19; square, day 19 onward.

Fig. 3. Change of respiratory quotient values (A) and N₂O emission rates (B) in Experiment II. Values and error bars indicate average and standard deviation in duplicate experiments. Dotted line in (A) represents moving average (n=3) and line in (B) represents cumulative N₂O emissions.

Fig. 4. Behavior of inorganic nitrogen in composting samples. Symbols represent the following compounds: diamond, NH₄⁺-N; square, NO₂⁻-N; triangle, NO₃⁻-N. Values and error bars indicate average and standard deviation in duplicate experiments.

Fig. 5. Correlation plots of total concentrations of NO₂⁻-N and NO₃⁻-N, plus N₂O emission rates.

Fig. 6. Behavior of 16S rRNA genes of eubacteria and *amoA* genes of AOB in reactors 1 and 2 during Experiment II. Values and error bars indicate average and standard deviation in duplicate measurements. Squares represent 16S rRNA genes of eubacteria, and circles represent *amoA* genes in AOB. N.D indicates not detected.

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