JARQ 50 (4), 353 - 359 (2016) http://www.jircas.affrc.go.jp

Regulation Trial of Nitrous Oxide Emissions Caused by Composting Swine Manure with Nitrite-Oxidizing Bacteria Added for Nitrification Promotion

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Abstract

We investigated the environmental effects of gas regulation practices using nitrite-oxidizing bacteria (NOB) for nitrification promotion in practical-scale manure composting experiments. With several tons of swine manure piled in parallel chambers, we determined the effects of adding NOB to matured swine manure by conducting two runs of composting trials. Both the control pile (without NOB) and the experimental pile (with NOB) were stored in composting chambers under continuous ventilation, and composting entailed several turnings in following the general swine farming procedure. During all periods of composting, the concentrations of greenhouse gas (GHG) and ammonia (NH_3), ventilation rate, and temperature were measured every hour. We found that the addition of NOB significantly increased both the nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) content of compost products. However, environmental gas regulations were not met in the two runs. The addition of NOB effectively reduced N_2O under oxidation facultative conditions (Run 1), but N_2O increased in the passive-type compost under cool temperature conditions (Run 2). N_2O emissions were found to largely depend on the manure composting conditions.

Discipline: Animal Industry
Additional key words: ammonia, greenhouse gas, nitrate nitrogen content, swine, composting

Introduction

In 2012, greenhouse gas (GHG) emissions from the manure management sector in Japan reached an annual total of 5,833,000 tons, accounting for 26% of the total annual emissions from the agricultural sector (National Greenhouse Gas Inventory Report of Japan 2014). Nitrous oxide (N_2O) emissions from manure management in particular are a key target of the GHG reduction policy. N_2O is a powerful GHG calculated to have 298 times the global warming potential of CO_2 over a 100-year period (IPCC Fourth Assessment Report 2007). In addition, the nitric oxide (NO) produced from N_2O destroys the ozone layer in the stratosphere through a series of reactions (Crutzen 1981). N_2O is an intermediate product generated as a by-product of nitrification and denitrification during the composting process. The emission procedure of N_2O from manure compost is not yet fully understood, and those emission patterns depend on livestock and management processes.

N_2O emissions from swine manure composting primarily occur under moderate temperature conditions after organic compounds have been destructed oxidatively in the piled manure mixture (Fukumoto and Inubushi 2009). In swine manure composting, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are involved in the nitrification process. Fukumoto et al. (2006) reported success in regulating N_2O emissions during composting at laboratory-scale facilities by harmonizing these related bacterial populations. They showed that the nitrogen dioxide (NO_2^-) accumulated and N_2O emissions generated during manure composting could be controlled by the addition of NOB, and using such as mature compost.

Based on this information, we evaluated this N_2O reduction technique during the swine manure composting process in practical-scale experiments. Ammonia (NH_3)

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Received 29 September 2015; accepted 18 January 2016.

and methane (CH_4) were also measured.

Materials and Methods

1. Experimental design

To evaluate the N_2O emission regulation abilities of the NOB additive reduction method, compost experiments were conducted using more than 1 m^3 of piled compost in Ibaraki Prefecture, Japan, in 2012 and 2014; each experiment was conducted twice. The first trial (Run 1) used an experimental chamber (W 2.0 m × D 2.0 m × H 2.5 m) at the Institute of Livestock and Grassland Science (Tsukuba, Ibaraki Prefecture) of the National Agriculture and Food Research Organization (NARO) from June to November 2012. The second trial (Run 2) used an experimental chamber (W 2.2 m × D 1.8 m × H 2.0 m) at the Ibaraki Prefectural Livestock Research Center, Swine Research Center (Inashiki, Ibaraki Prefecture), from January to May 2014. Each independent chamber system was stably ventilated, and included a control pile (nothing added) and an experimental pile (mature compost added upon turning at 6 weeks). All piles were composted for 16 weeks with weekly or biweekly turnings. The chamber system was designed to estimate the total gas emissions from compost piles, with a polyvinyl chloride (PVC) chamber equipped with a ventilation blower (TERAL-VFZ201PN: 0.86 m^3/min., Hiroshima, Japan) and a gas sampling port on the ventilation exhaust. The experimental chamber used in Run 1 had a system for forced aeration from the bottom of the pile, and that system was active at approximately 10 m^3/h aeration during the first 6 weeks of the experiment.

2. Experimental procedure

Fresh swine manure was collected from the swine barn of each research institute from swine fed concentrated mixtures recommended by the Japanese feeding standard for swine. The collected swine manure was mixed with sawdust to adjust the moisture content to approximately 65% for the piled mixture (Table 1). Approximately one metric ton of the swine manure and sawdust mixture was mixed and then piled up using a front loader in each chamber on the waterproof floor. Upon the turning at 6 weeks, mature compost (1% of the initial piled weight) was supplied to the surface of the newly turned material of the experimental compost as an NOB inoculation.

3. Gas measurement

The temperatures of the compost piles (upper and lower sites) and of the ambient air were measured hourly using a Thermo Recorder RTW-30S (Espec Mic Corporation, Aichi, Japan). The object gas (NH_3, N_2O, CH_4 and CO_2) concentrations in the exhaust air from the chamber were continuously measured by an infrared photoacoustic detector (IPD; Innova trace gas monitor, type 1312, and multipoint sampler, type 1309; Lumasense Technol. Inc., Ballerup, Denmark). The air samples from each sampling point were automatically carried to the IPD through a Teflon tube (4 mm in diameter) at 3-min. intervals by the multipoint sampler. According to the IPD technical data, the detection limit of N_2O was 0.03 ppm at a pressure of 1 atm and a temperature of 25°C. The gas concentrations in ambient air were also measured by using the same procedure. The total amount of gas emissions was estimated as described in our previous study (Osada and Fukumoto 2001). The emission rate (E) of each substance (CH_4 and N_2O) was computed based on the amount of ventilation and differences in the concentration of each substance between the inlet and outlet air samples.

$$E\ (mg/h) = (\text{Conc. of outlet air } (mg/m^3)$$
$$- \text{Conc. of inlet air } (mg/m^3)\ x\ \text{Ventilation rate } (m^3/h)$$

4. Chemical analysis of composting materials

Approximately 1 kg of composting materials was collected at the weekly or biweekly turning, and at the beginning and end of each run. Moisture content, organic matter (OM), total nitrogen content, NH_4^+-N, NO_x^--N, pH, and biochemical oxygen demand (BOD) were measured. The moisture content and OM were determined after drying and ashing periods of more than 24 h at 65°C and 550°C, respectively. Total nitrogen was calculated as the sum total of Kjeldahl nitrogen (Bremner 1965). NH_4^+-N, NO_x^--N, and pH (glass electrodes, Yokokawa, Japan) were measured in a 1:10 (compost sample:2 N KCl, w/v) extract of the compost. The extract samples were analyzed using the method described by Bremner and Keeney (1965). BOD was determined using a BOD Trak (Hach Co., Loveland, CO, USA).

5. Enumeration of nitrifying microorganisms

The population density of nitrifying microorganisms was determined in most probable number (MPN) analyses by following the procedures for estimating the MPN of AOB and NOB as described by Schmidt and Belser (1982) and Kraamer and Conrad (1991). The population sizes of ammonia-oxidizers and nitrite-oxidizers of the compost materials were determined by using the MPN method. The basal medium and procedures were based on a previous study by Fukumoto (2006). Ten grams of raw sample with 90 ml of sterilized distilled water (DW) were shaken for 20 min., and then the shaken slurry was diluted for a tenfold series. Sixty-six microliters and 40 μL of the dilution series were inoculated into 200 μL of an ammonia- and nitrite-oxidizer medium placed into 96-well microplates in five replicates per dilution, and then incubated in the dark at 30°C for 28 days. All experiments were conducted twice. The presence or absence of nitrite was determined by the

Table 1. Chemical component profiles of compost piles

Compost pile			Wt (kg)	Chemical component profiles							
				pH	TS (%)	VS (%TS)	NH_4^+-N (mg/kgTS)	NO_2^--N (mg/kgTS)	NO_3^--N (mg/kgTS)	TN (%TS)	BOD (mgO_2/kgTS)
Run 1	Cont.	0w	1,875	7.1 –	34.6 –	91.7 (0.3)	3,252 (24.8)	0 (0.0)	0 (0.0)	1.4 (0.0)	106,272 –
		8w	–	7.3 (0.0)	39.6 (0.1)	86.3 (0.7)	1,120 (90.1)	284 (25.9)	1,136 (24.7)	1.8 (0.0)	732 (125)
		16w	820	6.8 (0.0)	48.2 (0.0)	84.6 (0.2)	29 (17.8)	189 (71.9)	1,299 (71.9)	2.4 (0.2)	322 (7.4)
	Exp.	0w	1,875	6.8 –	33.9 (0.0)	90.3 (0.1)	3,484 (0.0)	0 (0.0)	0 (0.0)	1.6 (0.0)	37,740 –
		8w	–	7.2 (0.0)	40.4 (0.2)	86.7 (0.2)	880 (14.4)	406 (15.8)	1,213 (28.0)	2.0 (0.1)	1,237 (210)
		16w	830	6.9 (0.0)	47.2 (0.7)	86.6 (0.1)	22 (6.4)	506 (63.4)	1,818 (62.9)	2.2 (0.2)	381 (59.8)
	mature compost				33.9 (0.0)	84.5 (1.0)	88 (35.3)	2,428 (1,276)	6,886 (1,188)	2.5 (0.0)	
Run 1	Cont.	0w	910	6.9 (0.0)	35.1 (0.2)	88.2 (0.0)	6,093 (3.9)	0 (0.0)	0 (0.0)	3.4 (0.2)	92,458 (1,694)
		8w	–	8.7 (0.1)	39.9 (0.1)	79.8 (0.1)	6,613 (7.8)	204 (33.5)	810 (46.3)	3.9 (0.0)	49,631 (1,064)
		16w	457	8.0 (0.0)	43.8 (0.1)	76.7 (0.2)	1,294 (3.2)	0 (0.0)	1,517 (43.2)	2.9 (0.1)	921 (181)
	Exp.	0w	910	7.0 (0.0)	35.5 (0.2)	88.5 (0.1)	5,606 (29.3)	0 (0.0)	0 (0.0)	3.1 (0.1)	87,717 (1,176)
		8w	–	8.8 (0.0)	38.7 (0.6)	80.6 (0.1)	7,546 (37.5)	203 (21.2)	1,123 (5.7)	4.2 (0.1)	26,075 (1,239)
		16w	471	7.6 (0.0)	42.0 (0.0)	76.3 (0.2)	1,389 (4.9)	321 (144.3)	2,192 (690.5)	3.3 (0.0)	612 (311)
	mature compost			9.0 (0.0)	75.1 (0.5)	67.5 (0.1)	438 (55.5)	187 (64.8)	708 (172.4)	4.5 (0.3)	

* Average (Standard Error)

addition of Griess-Ilosvay reagents. MPNs were estimated using the BASIC program. After incubation, the medium was analyzed in the presence or absence of NO_2^--N with the addition of Griess-Ilosvay reagents. The MPN number was determined based on the cutoff probability theory (Kohno and Fukunaga 1996, 1998).

Results

1. Physical properties and temperature changes of the pile materials

The temperature of the piled mixture of manure from fattening pigs rose within a few days to reach approximately 70-80°C in Run 1 and approximately 60°C in Run 2, and remained at that temperature range for 4-6 weeks (Fig. 1). After those temperate periods, piled manure temperatures declined to ambient temperatures in both runs. After the turning at 6 weeks in both runs, mature compost (1% of the initial piled weight) was supplied to the surface of newly turned material in the experimental pile as an NOB inoculation. Even after NOB inoculation, no obvious temperature difference was observed between the control and experimental piles in either run. In Run 2, tiny temperature differences were observed between the piles at 2-3 weeks, and after the 4th turning, both piled materials were mixed together, evenly separated, and then piled up again with the same weight in the two chambers.

One half of the initial weight of the piled manure was lost through composting in all trials (Table 1). During composting, most of the organic matter in the piled materials was degraded through oxidation by microorganisms. Heat and carbon dioxide (CO_2) are generated as a result of the oxidative degradation of organic compounds in manure. Much of the weight loss seen in the composted manure can be attributed to the evaporation of water and the generation of CO_2. A reduction of the initial materials associated with such organic decomposition has also been observed in previous swine manure composting trials (Fukumoto et al. 2003, Osada et al. 2000). Table 1 shows the changes in the chemical characteristics of the piled materials during composting periods. We observed no significant differences between the control and experimental piles, except in terms of NO_3^--N content and NO_2^--N content.

2. Gas emissions during composting

During the experimental periods, the ventilation rates of both chambers in Runs 1 and 2 remained steady at 30 m^3/h and 40 m^3/h, respectively, except during the turning periods. This means that the air inside each chamber was continuously changed five times an hour during all experimental periods. Under these ventilation conditions, the amount of each emitted gas was calculated by following the procedure described in the *Materials and Methods* section above, and then integrated for all experimental periods. The total amounts of gas emissions from each run were calculated and are shown in Table 2. There were no differences in terms of CO_2, NH_3 and CH_4 total emissions between the control and experimental piles in either run.

During the 16-week composting periods, the predominant emission of carbon was in the form of CO_2. Total CH_4 losses, expressed as a percent of the carbon initially present in the volatile solids (VS), ranged from 0.27% to 0.41%. These data indicate that aerobic composting was present in both runs. Approximately 20% of the initial nitrogen content of the manure was lost as NH_3 in all trials, while the N loss as N_2O-N emissions was approximately 2% in Run 1 and 4-6% in Run 2.

3. The population density of nitrifying microorganisms

Figure 2 shows changes in the population density of nitrifying microorganisms in the present composting materials. A level greater than 10^2-10^6 MPN/gDM) of the AOB population was observed during all composting periods. The AOB population was not affected by the addition of mature compost, and gradually increased along with the progress of composting. After the mature compost was added, the compost materials of the experimental plot contained ap-

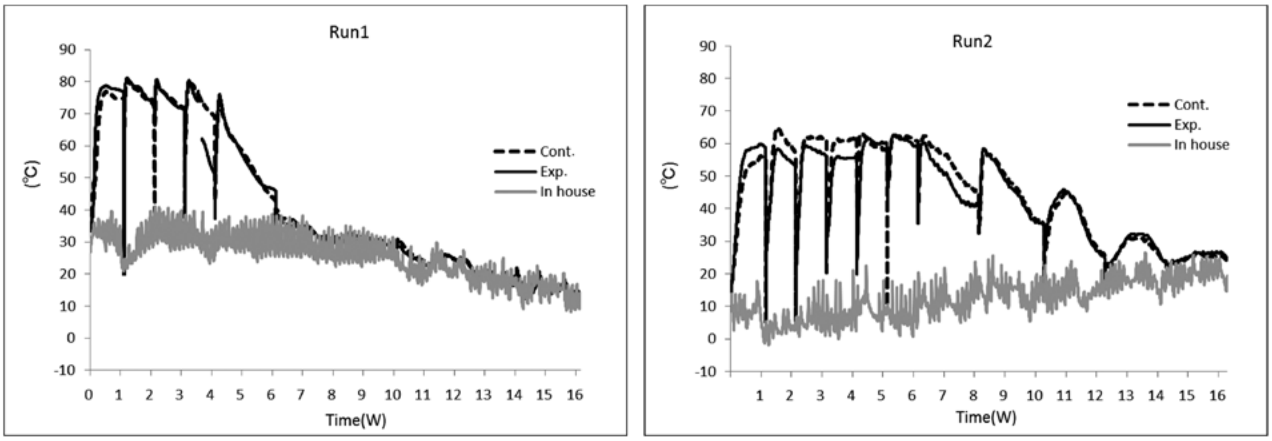

Fig. 1. Changes in piled swine manure temperature during composting periods

Table 2. Total emission and emission factor of each gas

			$NH_3^{-}N$	N_2O-N	CO_2	CH_4
			NH_3-N/TN	N_2O-N/TN		CH_4/VS
Run1	Cont.	Total (kg)	1.84	0.20	294.83	2.48
		Emission Factor (%)	21.40	2.28		0.41
	Exp.	Total (kg)	1.78	0.17	287.40	2.30
		Emission Factor (%)	20.70	2.02		0.40
Run2	Cont.	Total (kg)	1.97	0.44	192.52	0.77
		Emission Factor (%)	18.40	4.07		0.27
	Exp.	Total (kg)	1.96	0.60	240.13	1.02
		Emission Factor (%)	19.70	6.04		0.36
	*	NIR2014 EmissionFactor (%)				
	Swine					
		Piled compossing (feces and urine mixed)	24.20	2.50	–	0.16

*National GHGs Inventory Report of JAPAN (2014). Edited by Greenhouse Gas Inventory Office of Japan (GIO), Center for Global Environmental Research (CGER), National Institute for Environmental Studies (NIES) proximately 10^4-10^6 MPN/gDM of NOB, which gradually increased to 10^8 MPN/gDM in Run 1. On the other hand, in Run 2, the NOB population was detected in the initial piled materials, and gradually increased along with the progress of composting. The NOB population was not apparently affected by the addition of mature compost.

Discussion

In the present trials, the regulation of N_2O emissions during swine manure composting was not accomplished sufficiently with the addition of NOB (Table 2). At the practical scale employed in the present trials, N_2O mitigation reached 11% in Run 1, but conversely increased to 38% in Run 2. In following the procedure previously reported by Fukumoto et al. (2006), mature compost was added at the 6-week turning since N_2O generation had started in both runs. Figure 3 shows the changes in daily N_2O emissions and BOD degradation during composting. It may be useful to look more closely at some of the more important features of the N_2O generation pattern. The regulation of such N_2O emissions in the late stages of composting caused by the nitrification process is the target of the present trials (Fukumoto and Inubushi 2009). Certainly, N_2O generation in the experimental pile seemed to promptly disappear at the end of N_2O emissions (Fig. 3). However, the fact that unexpected N_2O was generated before regular nitrification activity became dominant remains problematic.

Let us consider the increased occurrence of N_2O emissions caused by denitrification. First, there was a difference in the scale of piled manure compared to that of the previous study (Fukumoto et al. 2006). In the case of piled compost, the volume of the anaerobic portions established inside the pile may increase logarithmically compared to the volume of aerobic portions as the scale of the compost pile increases. Therefore, the emission rates of N_2O and CH_4, which have high productive potentials under anaerobic conditions, increased with enlargement of the pilot piling scale in the present study. The volume of the mixture placed in the apparatus was 26 L at the start of composting (Fukumoto et al. 2006, Fukumoto and Inubushi 2009).

Secondly, the presence of a high concentration of organic matter enhanced N_2O generation at the time when oxidized nitrogen (NO_2^-, NO_3^-) dominated in the piled materials (Table 1). Generally, piled material of a scale of several cubic meters could not even be contained inside and would compress the bottom of pile; therefore, some easily degradable organic matter might remain even after

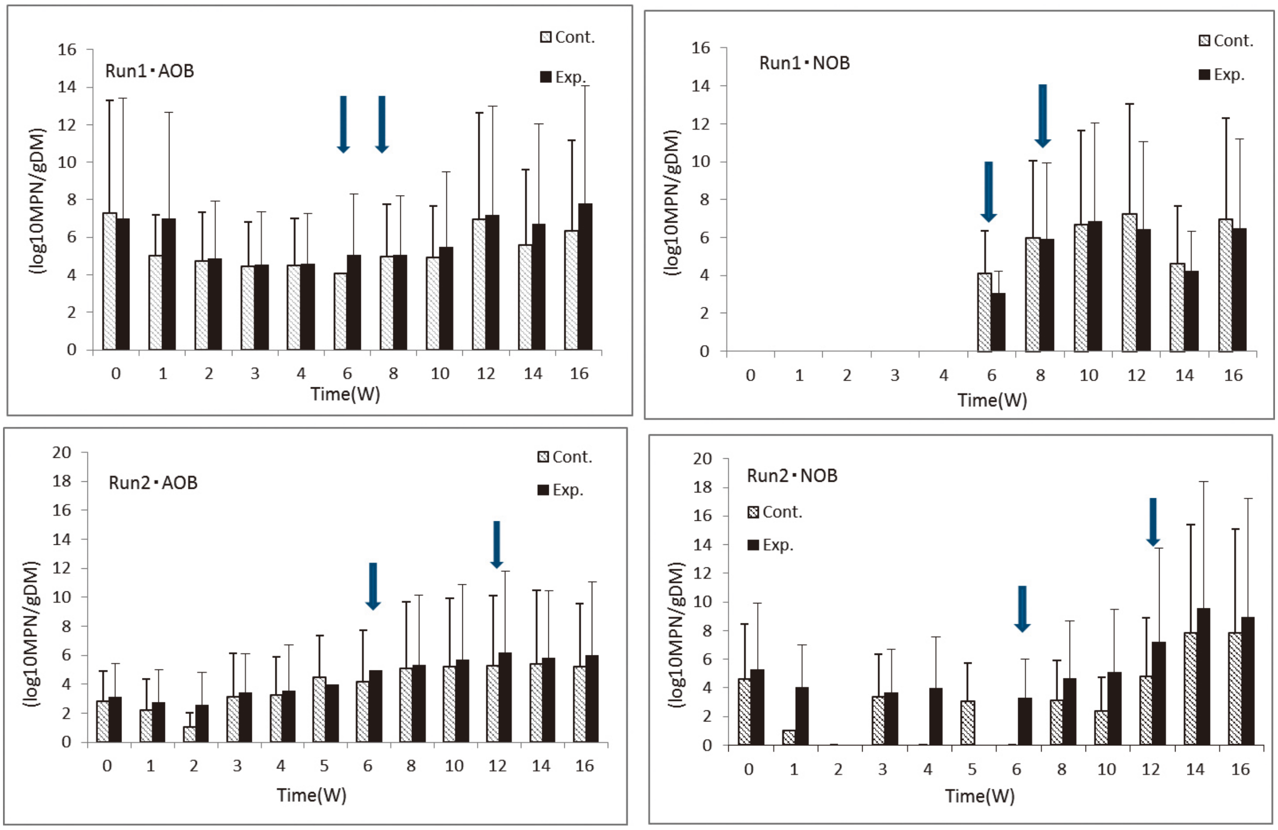

Fig. 2. Changes in the number of nitrifiers (AOB and NOB) during swine manure composting.
Arrows indicate addition of the NOB source of mature swine compost.

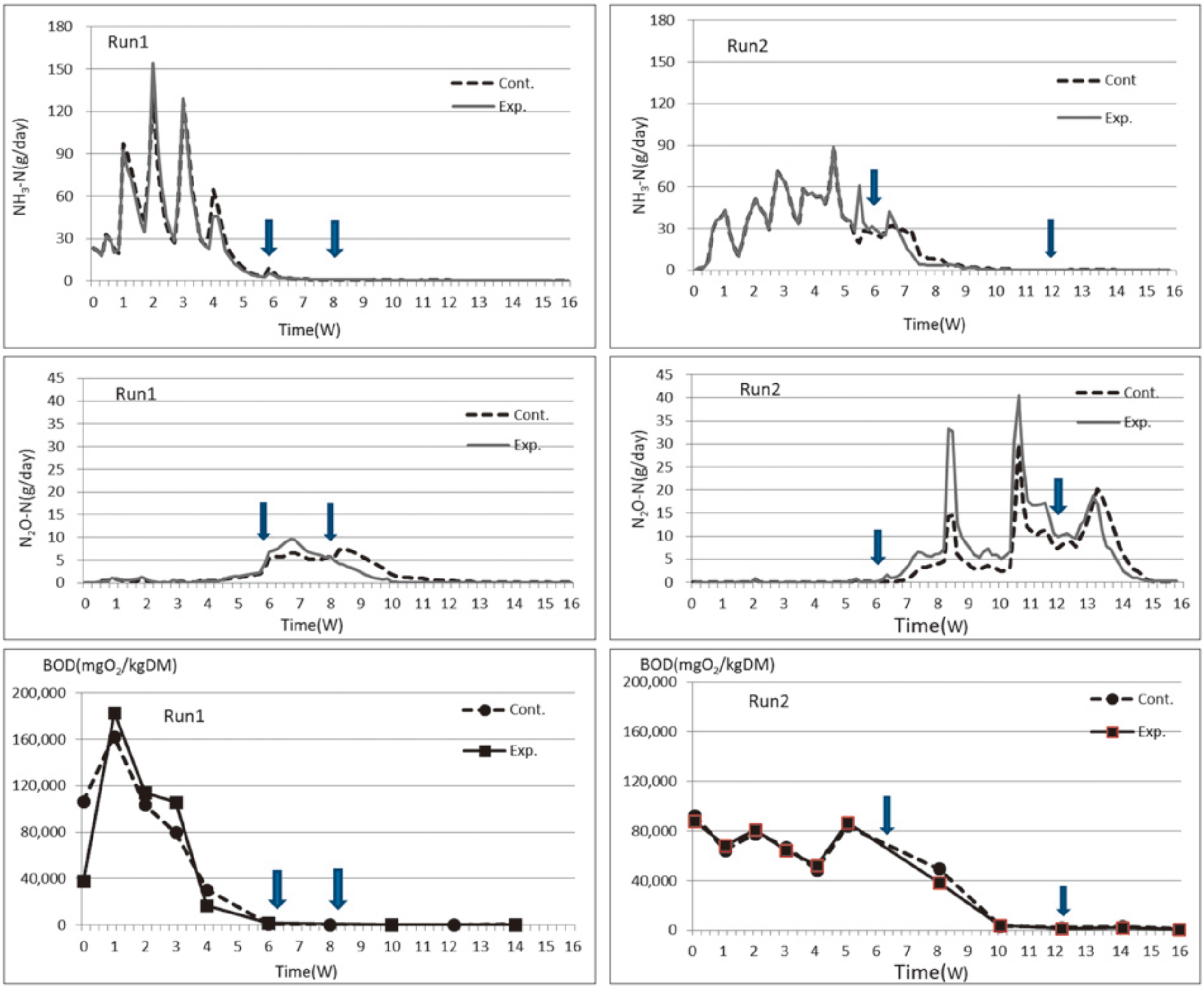

Fig. 3. Changes in nitrogenous gas emissions (NH_3-N and N_2O-N) and degradation of BOD concentration during swine manure composting. Arrows indicate addition of the NOB source of mature swine compost.

several weeks of composting (Maeda et al. 2010). BOD degradation in particular was obviously delayed under the low temperature conditions of Run 2 (Fig. 3), which may explain why the N_2O emission factor was high in Run 2 as compared to Run 1.

Finally, we must point out the negative possibilities of adding NOB for nitrification promotion. This regulation technique targets N_2O generation by the nitrification process. However, the addition of mature compost was found to enhance N_2O generation in Run 2. The addition of mature compost brings about an increase in NO_3^- by AOB and NOB inoculation, and NO_3^- in mature compost (Table 1).

In the present trials, the NOB population was not apparently affected by the addition of mature compost (Fig. 2). We also noted that N_2O generation in the denitrification process was enhanced prior to domination by regular nitrification activity. The quality of mature compost and the timing of any additions should be carefully examined and in greater detail before introducing this mitigation procedure to a pig farm.

Acknowledgements

The authors would like to thank all members of the Ibaraki Prefectural Livestock Research Center, Livestock research center swine laboratory, and NILGS-NARO, Japan, for their help with the experiments, and Mrs. K. Sumiya for her assistance. This study was supported in part by a project entitled “Development of mitigation and adaptation techniques to global warming in the sectors of agriculture, forestry, and fisheries” in the field of agro-environmental studies under Japan’s Ministry of Agriculture, Forestry and Fisheries from 2010 to 2014. This work was further supported in part by Grant-in-Aid from Japan’s

Ministry of Agriculture, Forestry and Fisheries.

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