Isotopically enriched ammonium shows high nitrogen transformation in the pile top zone of dairy manure compost

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Abstract

Nitrogen isotope ratios ($\delta^{15}N$) of NH_4^+ in dairy manure compost piles with and without bulking agent (10% w/w) were compared to understand the effects of the use of bulking agent on nitrogen conversion during manure composting. The $\delta^{15}N$-NH_4^+ values in each of three pile zones (top, side and core) were also compared. At the end of the process, piles with bulking agent showed significantly higher $\delta^{15}N$ values (17.7±1.3‰) than piles without bulking agent (11.8±0.9‰), reflecting the significantly higher nitrogen conversion and NH_3 loss in the former. The samples from the top zone, especially in the piles with bulking agent, showed very high NH_4^+ concentrations with significantly high ^{15}N ($\delta^{15}N$: 12.7-29.8‰) values, indicating that extremely high nitrogen conversion, nitrification-denitrification activity of the microbes and NH_3 volatilization occurred in

this zone.

1. Introduction

Nitrogen is one of the most abundant major elements in the Earth's atmosphere. There are two major anthropogenic activities affecting the global nitrogen cycle: energy production and food production (Galloway et al., 2004). Because nitrogen is one of the most important elements for plant nutrition, huge amounts of industrially fixed nitrogen are used as fertilizer to improve the productivity of agricultural crops (Tilman et al., 2002). Current anthropogenic nitrogen input to the environment (160 Tg per year) is already greater than the input from natural biological fixation (110 Tg) on land or in the ocean (140 Tg) (Gruber & Galloway, 2008), and the significance of agricultural nitrogen input on the global nitrogen cycle is expected to increase along with the nutritional needs of a growing population. In the livestock production industry, livestock intake organic nitrogen from their feed, and produce large quantities of organic nitrogen in the form of manure, a byproduct and potential resource which must be handled appropriately to protect the environment (Sharpley et al., 1998). Most of this manure is used as organic fertilizer for efficient nutrient cycling, and thus a proper understanding of nitrogen flow in the manure management system is critically important.

The nitrogen contained in dairy manure exists mostly as organic nitrogen or NH_4^+. Through the composting process, the heat production by degradation of organic matter leads to a significant loss of nitrogen into the atmosphere as gaseous ammonia (NH_3) (Dämmgen & Hutchings, 2008). Nitrifiers and other families of microorganisms in the manure also convert this nitrogen as nitrite (NO_2^-) or nitrate (NO_3^-), and both nitrifiers and denitrifiers can use them as electron acceptors. They reduce these nitrogen oxides into dinitrogen (N_2) and return them to the atmosphere in a process called denitrification (Zumft, 1997). Nitrous oxide (N_2O), a greenhouse gas, is emitted through the nitrogen conversion in the composting process (Sommer et al., 2009). Because it is known that N_2O has very strong greenhouse effects (298-fold greater than the greenhouse effects of CO_2 over a 100-year time horizon; IPCC, 2007), and N_2O is also known to contribute to ozone layer destruction (Ravishankara et al., 2009), these gas emissions must be mitigated.

With respect to this N_2O emission, our previous studies clarified that nitrification occurs in the compost surface, and compost turning (mixing by machines) and subsequent denitrification can be major sources of N_2O (Maeda et al., 2013b; Maeda et al., 2010b). Also, we have shown that the appropriate use of bulking agents can reduce the N_2O emission significantly (Maeda et al., 2013a). However, the mechanism of this N_2O mitigation is largely unknown. Because bulking agents are generally used to increase the supply of oxygen to the compost piles (Jolanun & Towprayoon, 2010), it is expected that the increase in oxygen increases nitrification and the subsequent N_2O production.

To solve this contradiction, we compared the level of δ^{15}N-NH_4^+ in these composts, because this parameter can be used to track the level of reaction involving NH_4^+ in the environment (Brooks et al., 1989; Garten Jr, 1992; Yeatman et al., 2001). Because it has already been established that the NO_x^- accumulation and the bacterial communities are different in different regions of the pile (Maeda et al., 2010a), we sampled from both the compost side and core independently, and surveyed them into the δ^{15}N-NH_4^+ analysis.

2. Materials and Methods

2.1. Composting experiment

The composting experiment was performed three times at the Hokkaido Agricultural Research Center (Sapporo City, Hokkaido): once from 27 May through 21 July in 2010 (Run 1), once from 15 September through 10 November in 2010 (Run 2) and once from 19 May through 14 July in 2011 (Run 3). The cows were fed orchard grass silage and corn silage, oat hay, alfalfa hay, beet pulp and two types of concentrate mixtures to meet their digestible energy requirements, as recommended by the Japanese Feeding Standard for Dairy Cattle. Lactating Holstein cow excrement and dried grass (Orchard grass; *Dactylis glomerata*) were used in this study to make the compost.

About 4000 kg of dairy cow excrement and 400 kg of dried grass were mixed to form the treatment pile (pile 1), while the control pile (pile 2) consisted of dairy cow excrement alone. The compost was piled up on a waterproof concrete floor, and turned once every two weeks with a front loader and manure spreader. Each pile had a volume of 7.5 m^3 with pile dimensions of 4 m in diameter and 1.8 m in height at the start of the experiment. The temperatures of the compost piles and the ambient air were measured hourly using a Thermo Recorder RTW-30S (Espec, Japan).

2.2. Chemical analysis of the compost

Fresh samples (About 1 kg) were taken from each of three zones (the pile top, side, and core) just before each turning. Samples were also taken just after each turning, at the start and the end of the three composting experiments. Details of the sampling are described in Fig. S1. Samples were homogenized and fresh subsamples were used to measure total solids, volatile solids, inorganic-N, pH and electrical conductivity, or stored at -20°C for total nitrogen determination. Total solids (TS) were measured after drying the samples overnight at 105°C, and dried samples were powdered and used for C/N ratio determination. Volatile solids (VS) were measured after the samples were processed at 600°C for 1 h. Total N was measured using raw samples by the Kjeldahl method. The C/N ratio was determined using a C/N analyzer (vario MAX CNS; Elementar, Germany).

To measure inorganic-N, pH and electrical conductivity, 5 g of fresh compost was placed into a 50 ml polypropylene tube with 40 ml of deionized water, then shaken (200 rpm, 30 minutes) and centrifuged (3,000 g, 20 minutes). The supernatant was collected and NH_4^+, NO_2^--N and NO_3^--N were measured using ion chromatography (ICS-1600; Dionex, USA); pH and electrical conductivity (EC) were determined with calibrated electrodes (Horiba, Japan).

2.3. Determination of δ^{15}N-NH_4^+ levels and Rayleigh plot analysis

The amount of δ^{15}N-NH_4^+ in the extracted samples or trapped NH_3 samples was determined by the diffusion method (Holmes et al., 1998).

One cm diameter GF/D filters (Whatman, UK) were cut into four pieces, acidified with 20 µl H_3PO_4 (0.02 mM) and sandwiched between 2.5 cm diameter 10 mm pore-size Teflon membranes (Millipore, USA). These filter packs were used as an ammonium trap in the samples. Ten ml of the NH_4^+-N samples (50 µg-N) was placed in 15 ml tubes and 0.5 g of NaCl (ashed at 450°C for 8 h) was added. Then a single filter pack was added to the 15 ml tube, and 0.03 g MgO (ashed at 450°C for 8 h) was added to convert NH_4^+ in the samples into NH_3. The 15 ml tubes were incubated at 40°C for 2 weeks with stirring at 200 rpm. After incubation, the filter pack was removed from the tubes and dried in a desiccator for 2 days. The dried filter was then recovered and placed in a tin cup. The tin cup containing the filter was then analyzed by an elemental analyzer (EA1110, CE Instruments, Ltd., Wigan, UK) coupled with an isotope ratio mass spectrometer (MAT252; ThermoFisher Scientific KK, Yokohama, Japan) to quantify δ^{15}N-NH_4^+ in the samples. Calibration was conducted with IAEA-N1 and IAEA-N_2 (NH_4SO_4), and the precision (1σ) was better than 0.2‰. The δ^{15}N of samples was expressed in parts per thousand deviations from the atmospheric N_2 as defined by the following equation:

$$\delta^{15}\mathrm{N}\ (‰) = ((R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1) \times 1000, \qquad (1)$$

where R_{sample} and R_{standard} are the ^{15}N/^{14}N ratios of samples and the atmospheric N_2, respectively. Isotopic fractionation factor α was expressed as

$$\alpha = R_{\mathrm{B}}/R_{\mathrm{A}}, \qquad (2)$$

where R_{A} and R_{B} are the isotopic ratio of phase A and B, respectively.

Isotopic fractionation can also be described by the enrichment factor ε, which describes the enrichment of the product relative to that of the substrate, and which is also expressed in permil (‰).

$$\varepsilon = (\alpha - 1) \times 1000 \qquad (3)$$

The evolution of the isotopic composition is described by a Rayleigh equation with a fractionation factor as follows for ^{15}N:

$$R/R_0 = (1+10^{-3}\,\delta^{15}\mathrm{N}) / (1+10^{-3}\,\delta^{15}\mathrm{N}_0) = ([\mathrm{NH}_4^+]/[\mathrm{NH}_4^+]_0)^{\,\alpha-1}, \qquad (4)$$

where R and R_0 are the isotope ratio of samples just before the turning and of the samples just after the previous turning, respectively. Since the piles were homogenized at each turning event, the amount of ammonium in a sample just after the previous turning event was taken as the “initial” ammonium.” δ^{15}N and $\delta^{15}N_0$ are the respective δ values for the each NH_4^+. $[NH_4^+]_0$ and $[NH_4^+]$ are the ammonium concentration of the samples just after the previous turning event and the samples just before the subsequent turning event, respectively. Using the approximation of $\ln(1+x) \cong x$ with $x << 1$, the relationship between the difference of δ^{15}N values between pile turnings and the reaction rate of the substrate was obtained from equations (3) and (4) as follows:

$$\delta^{15}\mathrm{N}-\delta^{15}\mathrm{N}_0 = \varepsilon \ln (1-f)\,, \tag{5}$$

where f is the amount of reacted ammonium between the turning events, defined as $f=(1 - [\mathrm{NH_4^+}] / [\mathrm{NH_4^+}]_0)$.

2.4 Keeling plot analysis

The basis of the Keeling plot method is conservation of mass. The ammonium concentration of each location of the pile before the pile turnings can be expressed as

$$c_b = c_a + c_s - c_v, \tag{6}$$

where c_b, c_a, c_s and c_v are the ammonium concentration measured in each location of the pile just before the turning, the ammonium concentration just after the previous pile turning, the additional concentration component produced by the source, and decrease in ammonium concentration caused by volatilization of NH_3, respectively. Given conservation of mass, we have

$$\delta^{15}\mathrm{N}_b c_b = \delta^{15}\mathrm{N}_a c_a + \delta^{15}\mathrm{N}_s c_s - \delta^{15}\mathrm{N}_v c_v, \tag{7}$$

where δ^{15}N represents the nitrogen isotope ratio of the ammonium in each sample or lost ammonium. Here we assume that c_v is proportional to c_b and that the difference between $\delta^{15}\mathrm{N}_v$ and $\delta^{15}\mathrm{N}_b$ is constant,

$$c_v = k c_b \tag{8}$$

$$\delta^{15}\mathrm{N}_v = \delta^{15}\mathrm{N}_b + \varepsilon_v. \tag{9}$$

Then, equations (6) and (7) are simplified as follows:

$$(1 + k)\, c_b = c_a + c_s, \tag{6'}$$

$$\{\delta^{15}\mathrm{N}_b + k(\delta^{15}\mathrm{N}_b + \varepsilon_v)\}c_b = \delta^{15}\mathrm{N}_a c_a + \delta^{15}\mathrm{N}_s c_s. \tag{7'}$$

By combining equations (6’) and (7’), we arrive at

$$\delta^{15}\mathrm{N}_b = c_a(\delta^{15}\mathrm{N}_a - \delta^{15}\mathrm{N}_s)/(1 + k)\cdot(1/c_b) + \delta^{15}\mathrm{N}_s - k\varepsilon_v/(1 + k). \tag{10}$$

Thus, $\delta^{15}\mathrm{N}_b$ and $1/c_b$ have a linear relationship if a single source of ammonium (s) is added to pre-existed ammonium (a) under the assumption described above.

2.5 Statistical analysis

The chemical component data 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.

3. Results

3.1. Composting experiments

The temperature of the piles with bulking agent (10% w/w) exceeded 60°C throughout the entire experiment (Fig. S1), while the piles without bulking agent showed significantly lower temperature (below 50°C). The initial weight was 4,543±137 kg in the piles with bulking agent and 4,136±124 kg in those without bulking agent, and the final turning these values dropped significantly to 1,413±99 kg and 1,960±291 kg, respectively (Table 1). The total solids of the piles with and without bulking agent after the composting process were 43.8±11.3% and 23.5±1.8%, respectively. The C/N ratios of the piles with and without bulking agent dropped significantly from 23.8±3.3 to 12.8±0.8 and from 22.8±1.2 to 15.6±2.6, respectively. These parameters all indicate that the organic matter degradation rate was much higher in the piles with bulking agent.

Pile top samples (2.8-7.4 mg-N/g TS; pile 1) and core samples (1.0-14.6 mg-N/g TS; pile 1) contained higher ammonium concentrations than the pile side samples (0.1-1.8 mg-N/g TS; pile 1) (Fig. 1A-C). High NO_2^- accumulation was also observed in the pile top samples (0.03-3.8 mg-N/g TS; pile 1), but not in the pile core samples. NO_3^- was also detected in the pile top and side samples, but the concentrations were low (0-0.29 mg-N/g TS; pile 1). Although similar trends were observed for pile 2 (Fig. 1D-F), the amount of NH_4^+ in the top region of pile 2 was generally lower (0.15-2.2 mg-N/g TS) than that in pile 1. Accumulations of NO_2^- (0.08-2.2 mg-N/g TS) and NO_3^- (0.02-0.7 mg-N/g TS) were also detected in both the top and side samples of pile 2.

3.2. δ^{15}N of NH_4^+ in mixed samples

δ^{15}N-NH_4^+ values of the mixed samples just after the pile turning events are shown in Fig. 2. All compost runs showed a similar tendency. The initial δ^{15}N-NH_4^+ values were 5.8±2.5‰ and 7.4±3.8‰ for the piles with and without bulking agent, respectively. These values dropped slightly between weeks 0 and 2, to 4.4±2.8‰ and 6.1±2.3‰ for piles with and without bulking agent in all runs, although these changes were not statistically significant. After week 4, these values increased significantly, and at the end of the experiments they reached 17.7±1.3‰ and 11.8±0.9‰ for the piles with and without bulking agent, respectively. Also, the piles with bulking agent showed higher values than the piles without bulking agent, and this difference was statistically significant.

δ^{15}N-NH_4^+ values were also determined for the pile top, side and core samples, and are shown in Fig. 3. The data were expressed as the difference from the mixed samples taken after the pile homogenization. The values for the pile top samples (9.6-22.5‰) were higher than those for the side samples (9.2-11.3‰) in both the piles with and without bulking agent. The core samples showed low δ^{15}N-NH_4^+ values in week 2 (1.7±1.0‰ and 4.7±2.0‰ for the piles with and without bulking agent, respectively), reflecting the newly formed “light” NH_4^+-N, which was supplied by the degradation of organic-N in the manure. On the other hand, the heaviest NH_4^+ (25.4±6.8‰) was also observed in the pile core samples at the end of the experimental period. This phenomenon was observed only from the piles with bulking agent.

4. Discussion

The stable isotope δ^{15}N value of NH_4^+ in dairy manure compost with and without bulking agent was studied to clarify the mechanism of the significant N_2O mitigation achieved using a bulking agent. A decrease in the δ^{15}N value of NH_4^+ in the first two weeks of composting was observed in both piles, although this result was not observed in the previous study (Kim et al., 2008). The discrepancy can be attributed to the supply, in the present experiments, of newly formed "light" NH_4^+ by the ammonification of organic N, which has a low value (α=~1.000) of isotopic fractionation (Högberg, 1997). The weight decrease in the piles with bulking agent (4,543±137 kg to 1,413±99 kg) was greater than that in the piles without bulking agent (4,136±124 kg to 1,960±291 kg), indicating that a relatively large amount of "light" NH_4^+ was supplied to the piles with bulking agent. The δ^{15}N value of NH_4^+ at the end of the experiments was significantly higher in the piles with bulking agent (17.7±1.3‰) than in those without bulking agent (11.8±0.9‰) (Fig. 2), indicating that the nitrogen transformation rate after the supply of newly formed ammonium was much higher in the piles with bulking agent.

In a previous work, we demonstrated that the use of bulking agent clearly reduced the greenhouse gas N_2O emission (up to 62.8%) when using the exact same scale and methods of dairy manure composting as used in the present study (Maeda et al., 2013a). Runs 2 and 3 in the previous work were identical to Runs 1 and 2 in this study. However, the present study did not provide a detailed explanation for this result. Our initial hypothesis, that the use of bulking agent reduced nitrogen transformation by nitrification-denitrification process, leading to lower N_2O emission, was not supported by the present data. One possible explanation for the difference in the mitigation of N_2O emission is the difference of temperature between the treatments, since it is known that the optimum temperature for the nitrifiers in the manure is around 35-40℃, and much lower nitrification activity can be observed above 50℃ (Willers et al., 1998). The optimum temperature for denitrification and N_2O production can be higher than these values (Benoit et al., 2015), but denitrification requires the presence of NO_2^- or NO_3^- for electron acceptor. The use of a bulking agent enabled oxygen supply into the pile, which could have enhanced the oxidation of ammonium (nitrification), but the high temperature inside the piles (>60°C) inhibited nitrification activity. Piles without a bulking agent showed lower temperature (30-40°C), which could have enhanced the nitrification, denitrification and N_2O emission in the piles without bulking agent. However, the higher nitrogen transformation achieved by other nitrogen transformations, such as NH_3 volatilization, assimilation and re-degradation of the bacterial cells, could have contributed to the higher δ^{15}N value of NH_4^+ observed in the piles with bulking agent.

Because significantly different concentrations for not only NH_4^+ but also NO_2^- and NO_3^- were observed every two weeks (Fig. 1), it was suggested that the reactions proceeded in a different manner in each of the pile regions studied. To examine this possibility, we collected samples from each location (pile top, side and core), and confirmed that the NH_4^+ concentration was clearly higher in the top region of the samples just before the first turning event than in the more homogenous samples after the last turning event (Fig. 1). This result might be attributable to the high temperature of pile core, especially in the piles with bulking agent (>60°C). The high temperature causes an internal convective airflow even if the piles are not aerated (Barrington et al., 2003; Lynch & Cherry, 1996; Yu et al., 2005), and this air flow can cause the transportation of NH_3-N from the specific zone where significant ammonification of organic-N occurs. $\delta^{15}NH_4^+$ levels were also determined for these samples, and we found that the ^{15}N value of NH_4^+ was significantly enriched in the top pile samples (Fig. 3). This finding indicated that the reaction rate was very high in the top pile zone, where significantly high NH_4^+ and NO_2^- concentrations were observed. The high NH_4^+ concentrations in the pile top could only be explained by the transport from the pile core, as stated above, but the NH_4^+ in the pile core generally showed depleted $\delta^{15}NH_4^+$ (Fig. 3). We therefore performed a Keeling plot analysis to explain the phenomenon (Fig. 4A). If there were a single “heavy” $^{15}NH_4^+$ source, we would expect to see a significant regression line between the $^{15}NH_4^+$ values and inverse ammonium concentration. However, we did not see such a line, indicating that the nitrogen transformation and isotope fractionation occurred independently in each location. In turn, this means that the nitrogen transformation rate was extremely high in the pile top samples, which showed high NH_4^+ concentration with highly enriched $\delta^{15}N$ values. We can think of two possible explanations for the highly enriched $\delta^{15}NH_4^+$.One is that the enrichment was due to extremely high nitrification-denitrification activity in these samples, and the other is that it was due to high loss of nitrogen in the gaseous NH_3 state.

Previously Casciotti et al. (2003) reported that biological ammonium oxidation by beta-proteobacterial ammonium oxidizing bacteria (AOB; four *Nitrosomonas* and one *Nitrosospira* species) has an isotopic effect that ranges from 14.2-38.2‰. Another family of ammonium oxidizers, ammonium oxidizing archaea (AOA), also show isotopic fractionation during their activity, and this fractionation ranges from 13-41‰ (Santoro & Casciotti, 2011). Because the pH and availability of ammonia is one of the critical drivers partitioning these two ammonium oxidizers (Hatzenpichler, 2012), and manure compost shows high pH values and contains very high NH_4^+ concentration in general, AOB rather than AOA seems to be the main oxidizer in the compost (Yamamoto et al., 2012). Because significant amounts of the bacterial *amoA* gene, which is required for ammonium oxidation by AOB, have been detected in both the pile top and side, but not in the pile core (Maeda et al., 2010b), the contribution of this gene is a possible explanation for the “heavy” $^{15}NH_4^+$, especially in pile top samples. Therefore we performed a Raleigh plot analysis on our $^{15}NH_4^+$ data and tried to explain these enriched values with nitrification by the microbes (Fig. 4B). However, only some plots were included in the area attributable to nitrification, and thus nitrification alone could not be the driving factor for these “heavy” $^{15}NH_4^+$-N.

The isotope fractionation for NH_3 volatilization and nitrification are similar, 1.029 and 1.015-1.035 (Högberg, 1997), respectively. In addition, it has been clearly established that high NH_3 volatilization contributes to the enriched $\delta^{15}NH_4^+$ during cattle manure storage (Lee et al., 2011). Another study reported that NH_4^+ can easily exist in a gaseous state at high pH environment, and the temperature can also influence the fractionation (Li et al., 2012).The $\delta^{15}N$ values of volatilized NH_3 from compost piles on the same scale were very low (-17.9~-13.5‰, unpublished data), and thus it would seem that NH_3 volatilization would likely have contributed to these “heavy” NH_4^+ in the pile top, at least in part.

On the other hand, the significant increase in $\delta^{15}NH_4^+$ in the latter stage of the process cannot be explained by NH_3 volatilization, because most of this occurs during their initial stage of the process, as we showed previously (Maeda et al., 2013a). Although the relative contributions of NH_3 volatilization and nitrification/denitrification to these $\delta^{15}NH_4^+$ increases are not clear, it is well known that nitrification occurs mainly during the latter stage of the process (Sanchez-Monedero et al., 2001), and the nitrification seems to contribute this increase significantly. Interestingly, highly enriched $\delta^{15}NH_4^+$ could be observed from the pile core zone at the end of the experiment in runs 1 and 2. This phenomenon cannot be explained by NH_3 volatilization because of its location in the piles, and thus it could be achieved solely by the nitrification-denitrification process. It is well known that high nitrification can occur in the latter stage of the composting process (Bernal et al., 2009; Parkinson et al., 2004), and the *amoA* gene could be detected from the compost core even in the latter stage of the composting process; therefore, high nitrogen conversion by microbes seems likely to have occurred in the compost core, and this could contribute to the sharp increase of the $\delta^{15}NH_4^+$ of the mixed samples.

5. Conclusion

The $\delta^{15}NH_4^+$ measurement of the samples collected from each location of the pile suggested an explanation for what occurred between the turnings. A plausible sequence of events between the pile turnings (Fig. 5) is as follows:

(i) Ammonification of organic N supplies a large amount of “light” ammonium in the compost core, where high organic matter degradation activity can be achieved.

(ii) This “light” ammonium is transported to the pile top zone by the upstream airflow generated by heat in the compost core zone.

(iii) Significant nitrification, denitrification and NH_3 volatilization occur in the pile top zone, leading to highly enriched $\delta^{15}NH_4^+$ in this zone, but these phenomena probably do not occur at significant levels in the pile side zone.

(ix) The nitrification rate exceeds the denitrification rate, leading to accumulation of NO_2^- in the pile top and side, which in turn contributes to significant denitrification and N_2O emission just after the turning events.

On the other hand, the $\delta^{15}NH_4^+$ measurement of piles with and without bulking agent did not explain why N_2O emission could be mitigated by the use of bulking agent, and thus further studies are needed.

Author Contribution

K.M. and S.T. designed the experiments. K.M., M.Y. and M.F. carried out the experiments. K.M., S.T. and S.H. analyzed the results. K.M., K.N. and N.Y. wrote the paper.

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Figure Captions

Fig. 1.

NO_2^- (white), NO_3^- (grey) and NH_4^+ (black) content of the compost samples from each location (top, side and core) of the pile and the sample just after the turnings (Mixed). These contents were determined every two weeks, just before/after the turning events. A-C indicate the pile 1 of the compost runs 1-3, and D-F indicate the pile 2 of the compost runs 1-3, respectively. The error bars indicate the standard deviation (n=3).

Fig. 2.

δ^{15}N of NH_4^+ of the mixed samples just after the turning events. The black bars indicate the compost with bulking agent (10% w/w), and the white bars indicate the compost without bulking agent. A-C indicate the compost runs 1-3. The error bars indicate the standard deviation (n=2).

Fig. 3.

δ^{15}N of NH_4^+ of the samples from each compost location (pile top, side and core). The values were expressed as the difference from the mixed samples just after the turning events. Black bars indicate the compost with bulking agent (10% w/w), and the white bars indicate the compost without bulking agent. A-C indicate the compost runs 1-3. The error bars indicate the standard deviation (n=3).

Fig. 4.

Keeling plot (A) and Raleigh plot (B) of the $\delta^{15}NH_4^+$. The error bars indicate the standard deviation (n=2). Black symbols indicate the compost with bulking agent (10% w/w) and white symbols indicate the compost without bulking agents. The gray zone indicates the area that can be explained by ammonium oxidation by AOB (ammonia oxidizing bacteria; ε=14.2-38.2‰) or AOA (ammonium oxidizing archaea; ε=13-41‰).

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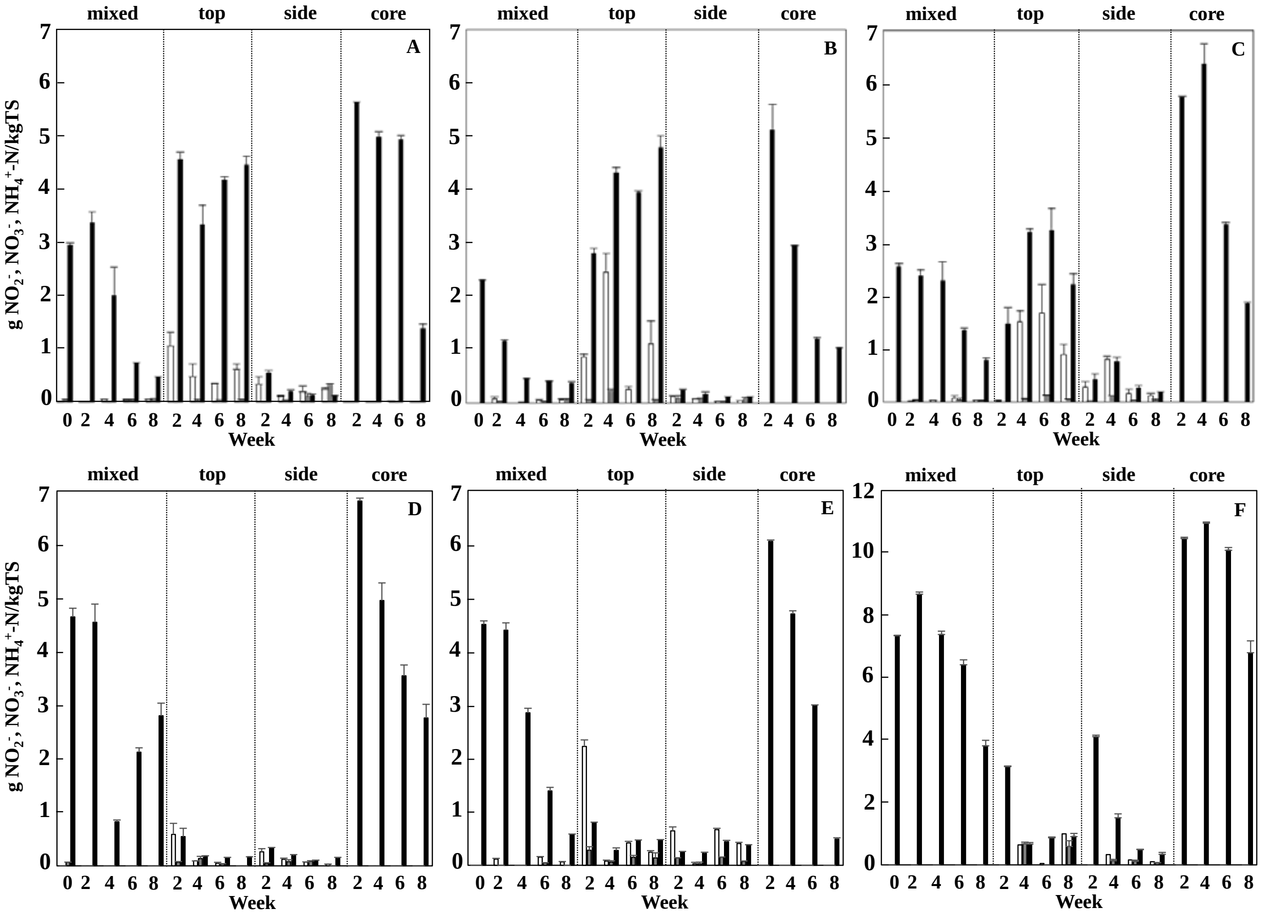
Table 1 Chemical components of compost samples

			Weight	TS		VS		EC		pH		NO_2^--N		NO_3^--N		NH_4^+-N		TKN		C/N	
Time	B.A.	Run	kg	%		%TS		$mScm^{-1}$				mg kg^{-1} TS		mg kg^{-1} TS		mg kg^{-1} TS		g-N kg^{-1}TS			
I	-	1	4,280	20.5	(0.2)	84.7	(0.3)	2.6	0.0	8.4	(0.1)	0.0	0.0	68.9	(1.8)	4,646.3	(164.7)	27.6	(0.3)	24.2	(0.2)
I	-	2	4,060	22.7	(0.6)	82.3	(1.1)	3.1	0.0	8.8	(0.1)	0.0	0.0	0.0	0.0	3,497.5	(51.0)	24.2	(0.6)	22.2	(0.2)
I	-	3	4,070	17.8	(0.4)	82.1	(0.4)	2.7	(0.1)	8.0	0.0	0.0	0.0	0.0	0.0	7,347.8	(7.6)	26.2	(0.4)	22.0	(0.4)
I	+	1	4,700	28.3	(0.1)	87.0	(0.3)	2.9	0.0	8.3	(0.1)	0.0	0.0	40.1	(0.9)	2,929.3	(34.0)	20.8	(1.0)	23.0	(0.1)
I	+	2	4,480	31.2	(0.7)	87.1	(0.8)	3.2	0.0	8.8	0.0	0.0	0.0	0.0	0.0	2,288.0	(10.4)	21.6	(0.7)	27.5	(1.7)
I	+	3	4,450	22.6	(0.3)	86.5	(0.6)	3.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0	5,840.3	(126.1)	20.4	(0.4)	21.0	(0.3)
F	-	1	1,710	24.8	(0.6)	70.0	(2.9)	2.5	0.0	9.3	0.0	0.0	0.0	0.0	0.0	1,353.1	(75.1)	32.3	(1.7)	13.0	(0.1)
F	-	2	2,280	24.3	(0.2)	75.1	(0.4)	2.6	(0.1)	9.5	(0.1)	61.2	(8.6)	0.0	0.0	451.1	(0.6)	26.7	(0.6)	16.3	0.0
F	-	3	1,890	21.4	(0.4)	77.2	(0.5)	3.0	0.0	9.2	(0.1)	0.0	0.0	0.0	0.0	3,817.1	(177.4)	28.9	(1.1)	17.5	(0.2)
F	+	1	1,190	52.7	(0.9)	69.1	(0.6)	5.0	(0.1)	9.5	0.0	44.3	(1.6)	52.6	(0.2)	460.9	(3.3)	30.0	0.0	12.0	(0.1)
F	+	2	1,480	47.6	(0.4)	73.7	(0.9)	4.3	0.0	9.0	0.0	57.4	(7.5)	60.4	(2.5)	375.5	(21.7)	29.1	(0.1)	13.6	(0.2)
F	+	3	1,570	31.1	(1.0)	71.9	(1.6)	4.9	(0.1)	9.5	(0.1)	53.9	(6.7)	49.5	(12.1)	1,809.8	(97.8)	29.2	(0.2)	12.7	0.0

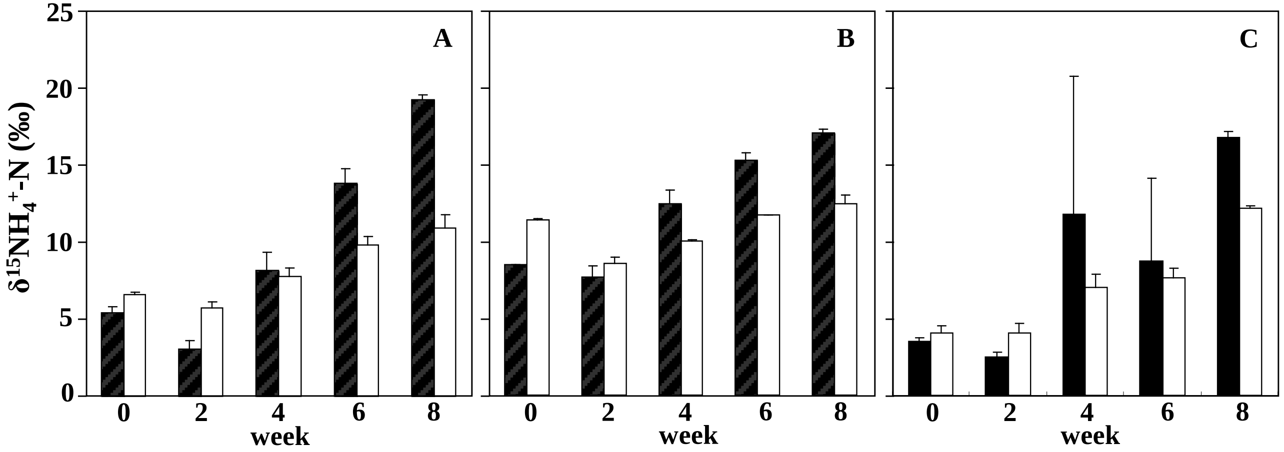
B.A., bulking agent; I, initial; F, final; TS, total solids; VS, volatile solids; EC, electrical conductivity; NO_2^--N, nitrite-nitrogen; NO_3^--N, nitrate-nitrogen; NH_4^+-N, ammonium-nitrogen; TKN, total Kjeldahl nitrogen; C/N, carbon/nitrogen ratio. The values represent the average (standard deviation).

Maeda et al., Fig. 1



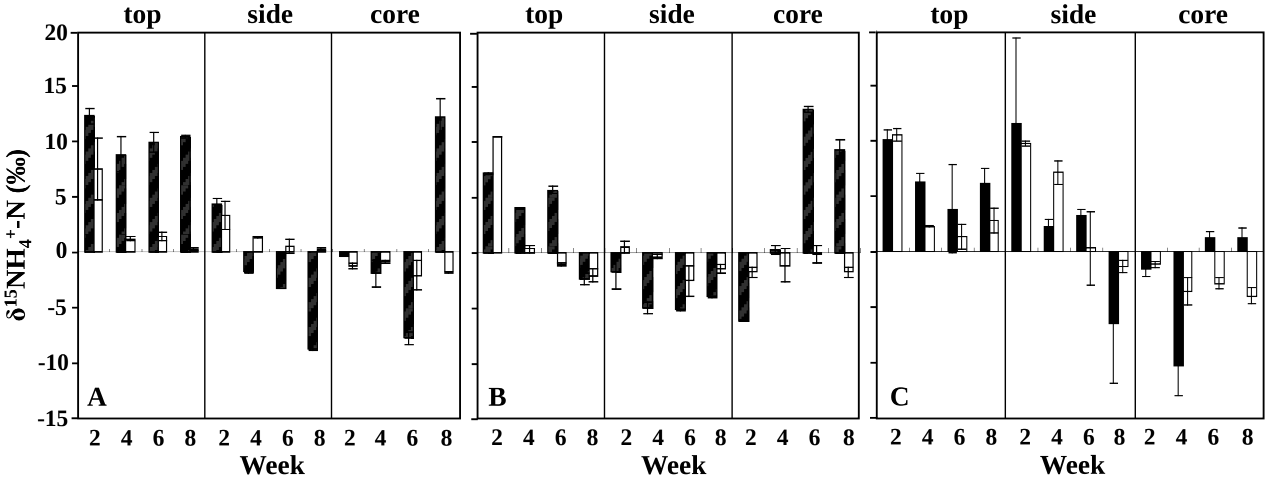
NO_2^- (white), NO_3^- (grey) and NH_4^+-N (black) content of the compost samples from each location (top, side and core) of the pile and the sample just after the turnings (Mixed). These contents were determined every two weeks, just before/after the turning events. A-C indicate the pile 1 of the compost runs 1-3, and D-F indicate the pile 2 of the compost runs 1-3, respectively. The error bars indicate the standard deviation (n=3).

Maeda et al., Fig. 2



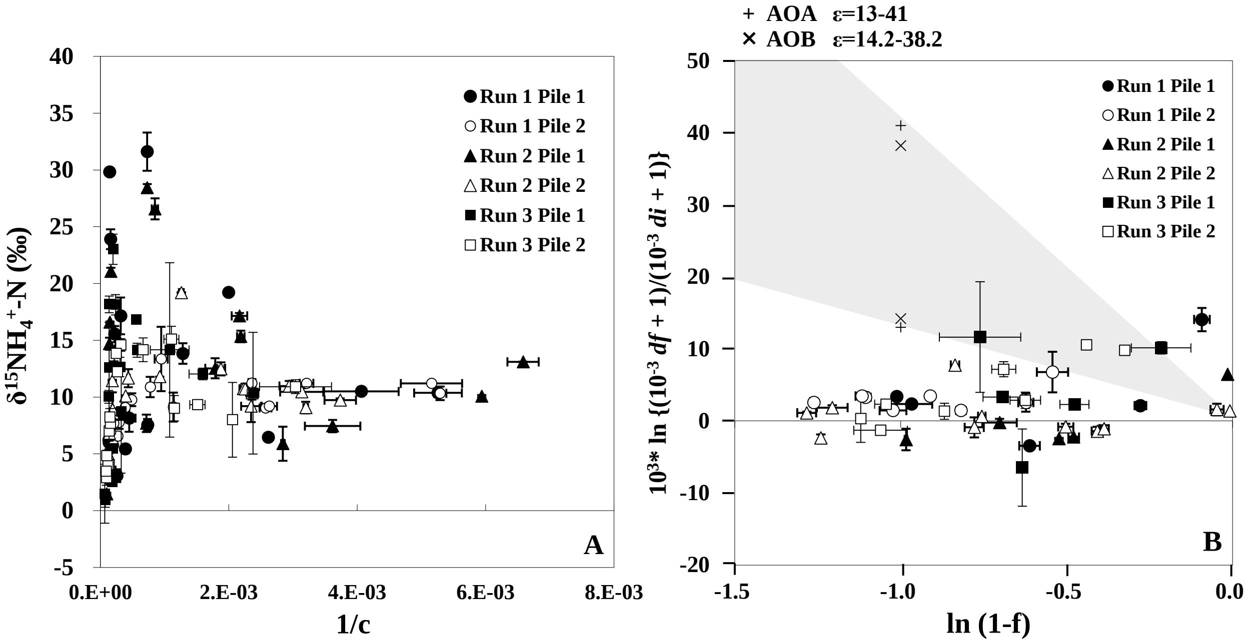
δ^{15}N of NH_4^+ of the mixed samples just after the turning events. The black bars indicate the compost with bulking agent (10% w/w), and the white bars indicate the compost without bulking agent. A-C indicate the compost runs 1-3. The error bars indicate the standard deviation (n=2).

Maeda et al., Fig. 3



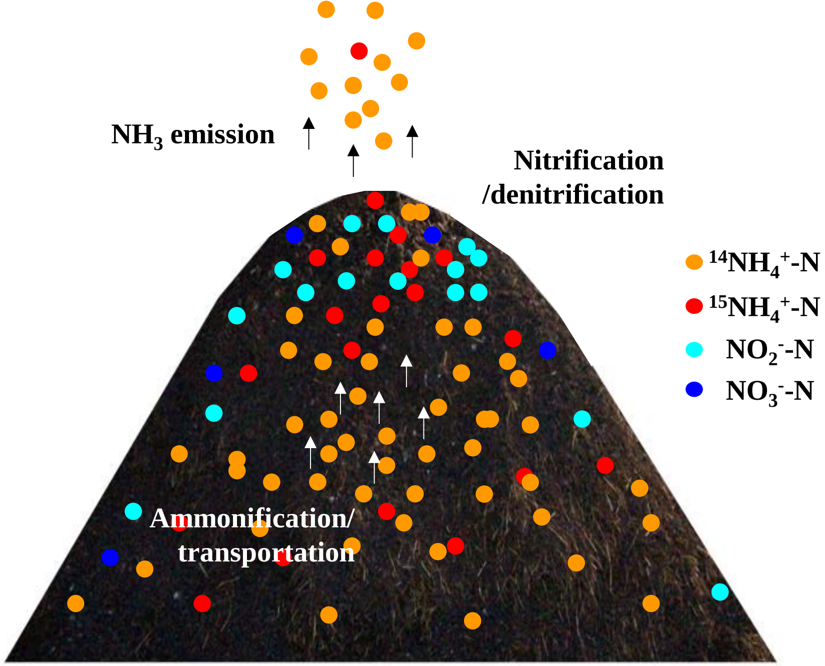
δ^{15}N of NH_4^+-N of the samples from each compost location (pile top, side and core). The values were expressed as the difference from the mixed samples just after the turning events. The black bars indicate the compost with bulking agent (10% w/w), and the white bars indicate the compost without bulking agent. A-C indicate the compost runs 1-3. The error bars indicate the standard deviation (n=3).

Maeda et al., Fig. 4



Keeling plot (A) and Raleigh plot (B) of the $\delta^{15}NH_4^+$-N. The error bars indicate the standard deviation (n=2). Black symbols indicate the compost with bulking agent (10% w/w) and white symbols indicate the compost without bulking agents. The gray zone indicate the area which can be explained by ammonium oxidation by AOB (ammonia oxidizing bacteria; 14.2-38.2‰) or AOA (ammonium oxidizing archaea; 13-41‰).

Maeda et al., Fig. 5



Summary of the events between the pile turnings