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

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Abstract

δ^{15}N-NH_4^+ of dairy manure compost piles with and without bulking agent (10 % *w*/*w*) were compared to understand the significant mitigation of N_2O emission by the use of bulking agent. δ^{15}N-NH_4^+ of each locations of the pile (top, side and core) were also compared. Piles with bulking agent showed significantly higher δ^{15}N values (17.7 ± 1.3 ‰) than that of the piles without bulking agent (11.8 ± 0.9 ‰) at the end of the process, reflecting significant higher nitrogen conversion and NH_3 loss occurred in the pile with bulking agent. The pile top samples, especially in the piles with bulking agent, showed very high NH_4^+ concentrations with significant high ^{15}N (δ^{15}N: 12.7–29.8 ‰) values, indicating 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 on global nitrogen cycle; energy production and food production (Galloway et al., 2004). Because nitrogen is one of the most important elements for plant nutrition, modern agriculture uses huge amount of industrially fixed nitrogen as the fertilizer to improve the crop productivity (Tilman et al., 2002). Current anthropogenic nitrogen input to the environment (160 Tg year^{-1}) is already more than that of natural biological fixation (110 Tg) on land or in the ocean (140 Tg) (Gruber and Galloway, 2008), and the significance of agricultural nitrogen input on global nitrogen cycle is expected to be increased to feed the growing population. In livestock production industry, livestock intake organic nitrogen from their feedings, and they produce huge amount of organic nitrogen as manure, which should be treated appropriately to protect the environment (Sharpley et al., 1998). Most of them are used as the organic fertilizer for the efficient nutrient cycling, therefore the understanding on nitrogen flow in livestock manure management system is critically important issue.

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 significant loss of nitrogen into the atmosphere as gaseous ammonia (NH_3) (Dämmgen and Hutchings, 2008). Some microorganism groups such as nitrifier in the manure also convert this nitrogen as nitrite (NO_2^-) or nitrate (NO_3^-), and both nitrifier and denitrifiers can use them as electron acceptor. They reduce these nitrogen oxides into dinitrogen (N_2) and make them be back to the atmosphere, so called denitrification (Zumft, 1997). Through the nitrogen conversion in the composting process, greenhouse gas nitrous oxide (N_2O) is known to be emitted (Sommer et al., 2009). Because it is known that N_2O have 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.

For 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 the major source of N_2O (Maeda et al., 2013b, 2010b). Also, we have showed that appropriate use of bulking agent can reduce the N_2O emission significantly (Maeda et al., 2013a). However, the mechanism of this N_2O mitigation is largely unknown. Because the bulking agent is used to increase the oxygen supply into the compost piles (Jolanun and Towprayoon, 2010), the nitrification expected to be increased and subsequent N_2O production should be increased.

To solve this contradiction, we compared the abundance of $\delta^{15}N\text{-}NH_4^+$ of these composts, because this can be used to track the amount of reaction on NH_4^+ in the environment (Brooks et al., 1989; Garten Jr., 1992; Yeatman et al., 2001). So far, it is known that the NO_x^- accumulation and the bacterial communities are different between the locations of the pile (Maeda et al., 2010a), we sampled from both compost surface and core independently, and surveyed them into the $\delta^{15}N\text{-}NH_4^+$ 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 4 t of dairy cow excrement and 400 kg of dried grass were mixed to form the treatment piles (pile 1), while the control piles (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). Fresh samples were taken from each zone (the pile top, surface, and core) at the start and end of the three composting experiments and just before each turning.

2.2 Chemical analysis of the compost

About 1 kg of fresh compost samples was collected at the start and end of the three composting experiments and just before each turning. 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 min) and centrifuged (3000 g, 20 min). 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^+ and Rayleigh plot analysis

δ^{15}N-NH_4^+ abundance of the extracted samples or trapped NH_3 samples was determined with 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 for ammonium trap in the samples. Ten ml of NH_4^+-N samples (50 µg-N) were put into 15 mL tubes and 0.5 g of NaCl (ashed at 450 °C for 8 h) was added. Put one filter pack into the 15 mL bottle, and 0.03 g MgO (ashed at 450 °C for 8 h) was added to put NH_4^+ in the samples into NH_3. The 15 mL bottles were incubated at 40 °C for 2 weeks with 200 rpm. After incubation, filter pack was removed from the bottles and dried in the desiccators for 2 days. After drying, filters were recovered and put into tin cups. These tin cups with filters were 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 determine δ^{15}N-NH_4^+ in the samples. Calibration was conducted with IAEA-N1 and IAEA-N_2 (NH_4SO_4), and precision (1σ) was better than 0.2 ‰. The δ^{15}N of samples were expressed in parts per thousand deviations from the atmospheric N_2 as defined by the following equation:

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

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

$$\alpha = R_{\mathrm{B}}/R_{\mathrm{A}}, \tag{2}$$

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

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

$$\varepsilon = (\alpha - 1) \times 1000 \tag{3}$$

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

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

where R and R_0 are the isotope ratio of samples just before the turning and the samples just after the previous turning. Since the piles were homogenized at every turning events, the samples just after previous turning events were used as “initial ammonium”. $\delta^{15}\mathrm{N}$ and $\delta^{15}\mathrm{N}_0$ are the respective δ values of the each NH_4^+. $[\mathrm{NH}_4^+]_0$ and $[\mathrm{NH}_4^+]$ are the ammonium concentration of the samples just after previous turning events and samples just before the each turning events. Using the approximation of $\ln(1 + x) \cong x$ with $x \ll 1$, the relationship between the difference of $\delta^{15}\mathrm{N}$ values between pile turnings and the reaction rate of the substrate is obtained from Eqs. (3) and (4) as follows,

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

where f is the reacted ammonium between the turning events, $(1 - [\mathrm{NH}_4^+]/[\mathrm{NH}_4^+]_0)$.

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,

$$c_\mathrm{b} = c_\mathrm{a} + c_\mathrm{s} \tag{6}$$

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

$$\delta^{15}\mathrm{N}_\mathrm{b} c_\mathrm{b} = \delta^{15}\mathrm{N}_\mathrm{a} c_\mathrm{a} + \delta^{15}\mathrm{N}_\mathrm{s} c_\mathrm{s} \tag{7}$$

where δ^{15}N represents the nitrogen isotope ratio of each ammonium. By combining Eqs. (6) and (7), we used

$$\delta^{15}\mathrm{N}_\mathrm{b} = c_\mathrm{a}\left(\delta^{15}\mathrm{N}_\mathrm{a} - \delta^{15}\mathrm{N}_\mathrm{s}\right)(1/c_\mathrm{b}) + \delta^{15}\mathrm{N}_\mathrm{s} \tag{8}$$

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 during whole process (Fig. S1 in the Supplement), while the piles without bulking agent showed significantly lower temperature (below 50 °C). Initial weight were 4543 ± 137 kg in the piles with bulking agent and 4136 ± 124 kg without bulking agent, and these values dropped significantly to 1413 ± 99 kg and 1960 ± 291 kg for with and without the bulking agent, respectively (Table 1). 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. C/N ratio of with and without bulking agent significantly dropped 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^{-1} TS; pile 1) and core samples (1.0–14.6 mg N g^{-1} TS; pile 1) contained high ammonium concentration compared to pile side samples (0.1–1.8 mg N g^{-1} TS; pile 1) (Fig. 1a–c). High NO_2^- accumulation were also observed in pile top samples (0.03–3.8 mg N g^{-1} TS; pile 1), but not from pile core samples. NO_3^- was also detected from pile top and side samples, but the concentrations were low (0–0.29 mg N g^{-1} TS; pile 1). Although this tendency was same in pile 2 (Fig. 1d–f), the NH_4^+ in pile top were generally lower (0.15–2.2 mg N g^{-1} TS) than that of pile 1. Accumulation of NO_2^- (0.08–2.2 mg N g^{-1} TS) and NO_3^- (0.02–0.7 mg N g^{-1} TS) were also detected from both pile top and pile 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 were shown in Fig. 2. All compost runs showed similar tendency. 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 slightly dropped from weeks 0–2 to 4.4 ± 2.8 ‰ and 6.1 ± 2.3 ‰ for both piles with and without bulking agent in all runs. This decrease between weeks 0 and 2 were not statistically significant. After week 4, these values showed significant increase, and reached 17.7 ± 1.3 ‰ and 11.8 ± 0.9 ‰ for with and without bulking agent, respectively, at the end of the experiments. Also, the piles with bulking agent showed higher value than that of the piles without the bulking agent, and this difference was statistically significant.

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

4 Discussion

Stable isotope δ^{15}N value of NH_4^+ in dairy manure compost with and without bulking agent was studied to understand the mechanism of significant N_2O mitigation by use of bulking agent. Temporal decrease of δ^{15}N value of NH_4^+ were observed in both piles, which were not obtained in other work (Kim et al., 2008). This can be explained by the supply of newly formed "light" NH_4^+ by the ammonification of organic N, which has low value ($\alpha = \sim 1.000$) of isotopic fractionation (Högberg, 1997). The δ^{15}N value of NH_4^+ were significantly higher in the piles with bulking agent 17.7±1.3 ‰ than that of the piles without bulking agent (11.8 ± 0.9 ‰) (Fig. 2), indicating that the nitrogen transformation rate was much higher in these piles with bulking agent. Since the bulking agent is used to promote the organic matter degradation by introducing oxygen into the pile, which enhance microbial activity, significant higher organic matter degradation seems likely contributed the higher δ^{15}N value of NH_4^+ in the pile with bulking agent. Previously we demonstrated that the use of bulking agent certainly reduce the greenhouse gas N_2O emission (up to 62.8 %) with the exactly same manner and scale of the dairy manure composting (Maeda et al., 2013a). However the present study did not show the comprehensive reason why this could be achieved. Our first hypothesis on low N_2O emission by use of bulking agent, nitrogen transformation by nitrification- denitrification process is much lower, was not supported.

Because significantly different concentrations for not only NH_4^+ but also NO_2^- and NO_3^- can be achieved every two weeks (Fig. 1), independent phenomenon seems likely occurring in these different location of the piles. Therefore we collected the samples from each location (pile top, side and core), and it was confirmed that pile top samples just before turning events shows clearly higher NH_4^+ concentration than that of mixed samples of the last turning events (Fig. 1). This can be possibly explained by high temperature of pile core, especially in the piles with bulking agent ($> 60\,^{\circ}C$). The high temperature cause the internal convective airflow even if the piles were not aerated (Barrington et al., 2003; Lynch and Cherry, 1996; Yu et al., 2005), and the air flow can cause the transportation of NH_3-N from the specific zone where significant ammonification of organic-N occurs. $\delta^{15}NH_4^+$ were also determined for these samples, and we found that significantly enriched ^{15}N value of NH_4^+ were detected in pile top samples (Fig. 3). This data indicates that nitrogen turnover rate is very high in pile top zone, where significant high NH_4^+ and NO_2^- concentrations can be obtained. The high NH_4^+ concentrations in pile top can only be explained by the transformation from pile core as stated above, however the NH_4^+ in pile core generally showed depleted $\delta^{15}NH_4^+$ (Fig. 3). We therefore performed Keeling plot analysis to explain the phenomenon (Fig. 4a). If there was single “heavy” $^{15}NH_4^+$ source, we will have significant regression line between $^{15}NH_4^+$ values and inverse ammonium concentration. However, we could not see it, indicating that nitrogen turnover and isotope fractionation occurs independently in each locations. That mean in turn, the nitrogen turnover rate is extremely high in pile top samples, which shows high NH_4^+ concentration with highly enriched $\delta^{15}N$ values. Possible explanation on these highly enriched $\delta^{15}NH_4^+$ can be done by two points. One is due to extremely high nitrification-denitrification activity in these samples, and the other is high nitrogen loss as 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) have isotopic effect and it ranges from 14.2–38.2 ‰. Another ammonium oxidizer, ammonium oxidizing archaea (AOA), also shows isotopic fractionation during their activity and it ranges from 13–41 ‰ (Santoro and Casciotti, 2011). Because the pH and availability of ammonia is one of the critical driver partitioning these two ammonium oxidizer (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 amount of bacterial *amoA* gene, which required for ammonium oxidation by AOB can be detected from both pile top and side, but not from pile core (Maeda et al., 2010b), their contribution can be the possible explanation on these "heavy" $^{15}NH_4^+$ especially in pile top samples. Therefore we performed the Raleigh plot on our $^{15}NH_4^+$ data and tried to explain these enriched value with nitrification by the microbes (Fig. 4b). However, only some plot were included in the area which can be explained by nitrification, thus the nitrification alone cannot 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. It is also known that high NH_3 volatilization certainly contribute the enriched $\delta^{15}NH_4^+$ during cattle manure storage (Lee et al., 2011). Another study reported that NH_4^+ can be gaseous state easily at high pH environment, and the temperature can also influence the fractionation (Li et al., 2012). The $\delta^{15}N$ data of volatilized NH_3 from same scale of the compost piles showed very low values (−17.9∼ −13.5 ‰, unpublished data), thus NH_3 volatilization could seems likely partly contributed on these "heavy" NH_4^+ in the pile top.

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 initial stage of the process, as we showed previously (Maeda et al., 2013a). The relative contribution of NH_3 volatilization and nitrification/denitrification on these $\delta^{15}NH_4^+$ increase are not clear, it is well known that nitrification occurs mainly during the latter stage of the process (Sanchez-Monedero et al., 2001), the nitrification seems to contribute this increase significantly. Interestingly, highly enriched $\delta^{15}NH_4^+$ could be observed from 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, thus it could be achieved solely by 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 *amoA* gene could be detected from the compost core even in the latter stage of the composting process, high nitrogen conversion by microbes seems likely occurred in the compost core, and this could contribute on the sharp increase of the $\delta^{15}NH_4^+$ of the mixed samples.

5 Conclusions

The $\delta^{15}NH_4^+$ measurement of the samples collected from each location of the pile enabled the explanation about what occurs between the turnings. The plausible story between the pile turnings (Fig. 5) is,

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

ii. These “light” ammonium will be transported to pile top zone by the upstream airflow generated by heat in compost core zone.

iii. Significant nitrification, denitrification and NH_3 volatilization occurs especially in the pile top zone, which leads to highly enriched $\delta^{15}NH_4^+$ in the pile top, but this seems not likely occurring in pile side significantly.

iv. Nitrification rate exceeds denitrification rate, which can lead to accumulation of NO_2^- in pile top and side, and this can contribute the 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 can be mitigated by using of bulking agent, thus further studies are needed.

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Table 1. Chemical components of compost samples.

Time	B.A.	Run	Weight kg	TS %		VS %TS		EC mS cm^{-1}		pH		NO_2^--N mg kg^{-1}TS		NO_3^--N mg kg^{-1}TS		NH_4^+-N mg kg^{-1}TS		TKN g N kg^{-1}TS		C/N	
I	–	1	4280	20.5	(0.2)	84.7	(0.3)	2.6	0.0	8.4	(0.1)	0.0	0.0	68.9	(1.8)	4646.3	(164.7)	27.6	(0.3)	24.2	(0.2)
I	–	2	4060	22.7	(0.6)	82.3	(1.1)	3.1	0.0	8.8	(0.1)	0.0	0.0	0.0	0.0	3497.5	(51.0)	24.2	(0.6)	22.2	(0.2)
I	–	3	4070	17.8	(0.4)	82.1	(0.4)	2.7	(0.1)	8.0	0.0	0.0	0.0	0.0	0.0	7347.8	(7.6)	26.2	(0.4)	22.0	(0.4)
I	+	1	4700	28.3	(0.1)	87.0	(0.3)	2.9	0.0	8.3	(0.1)	0.0	0.0	40.1	(0.9)	2929.3	(34.0)	20.8	(1.0)	23.0	(0.1)
I	+	2	4480	31.2	(0.7)	87.1	(0.8)	3.2	0.0	8.8	0.0	0.0	0.0	0.0	0.0	2288.0	(10.4)	21.6	(0.7)	27.5	(1.7)
I	+	3	4450	22.6	(0.3)	86.5	(0.6)	3.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0	5840.3	(126.1)	20.4	(0.4)	21.0	(0.3)
F	–	1	1710	24.8	(0.6)	70.0	(2.9)	2.5	0.0	9.3	0.0	0.0	0.0	0.0	0.0	1353.1	(75.1)	32.3	(1.7)	13.0	(0.1)
F	–	2	2280	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	1890	21.4	(0.4)	77.2	(0.5)	3.0	0.0	9.2	(0.1)	0.0	0.0	0.0	0.0	3817.1	(177.4)	28.9	(1.1)	17.5	(0.2)
F	+	1	1190	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	1480	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	1570	31.1	(1.0)	71.9	(1.6)	4.9	(0.1)	9.5	(0.1)	53.9	(6.7)	49.5	(12.1)	1809.8	(97.8)	29.2	(0.2)	12.7	0.0

B.A., bulking agent; I, initial; F, final; C, control; T, treatment; 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 (SD). Values followed by different letters indicate significant difference ($P < 0.05$).

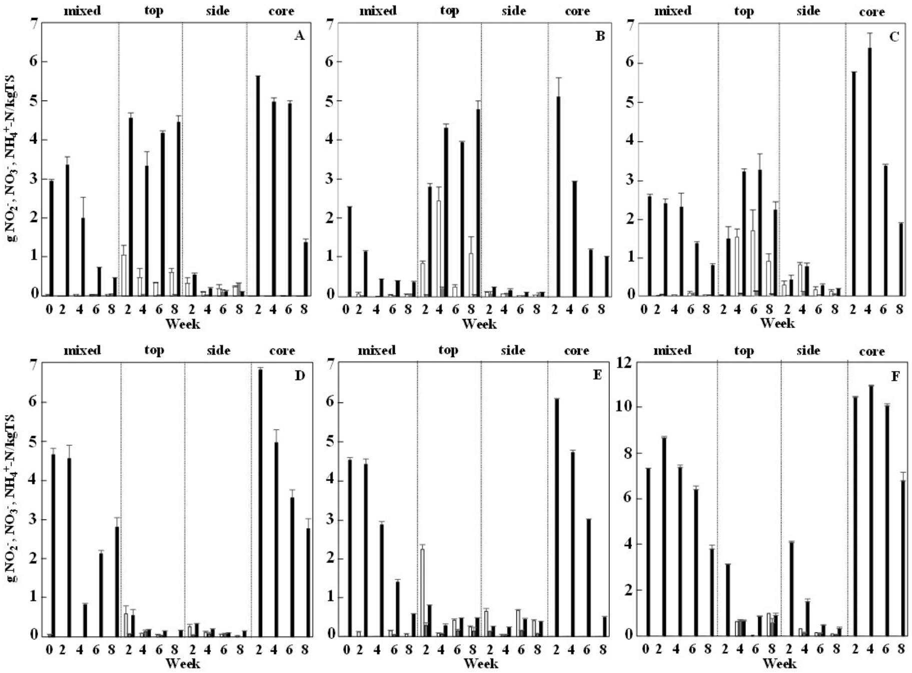

Figure 1. NO_2^- (white), NO_3^- (grey) and NH_4^+-N (black) content of the compost samples from each location (top, side and core) of the pile and the sample just after the turnings (mixed). These content were determined every two weeks, just before/after the turning events. **(a–c)** indicate the pile 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 SD ($n = 3$).

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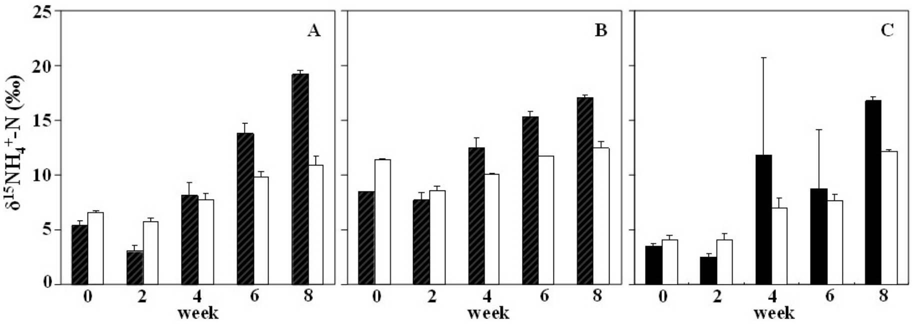

Figure 2. δ^{15}N of NH_4^+ of the mixed samples just after the turning events. Black bars indicate the compost with bulking agent (10 % *w*/*w*), and the white bars indicate the compost without bulking agent. **(a–c)** indicate the compost runs 1–3. The error bars indicate the SD ($n = 2$).

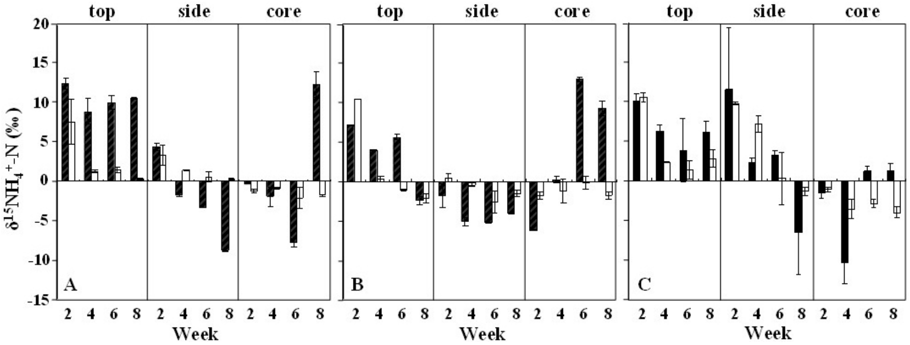

Figure 3. δ^{15}N of NH_4^+-N of the samples from each compost location (pile top, side and core). The values were expressed as the difference from the mixed samples just after the turning events. Black bars indicate the compost with bulking agent (10 % w/w), and the white bars indicate the compost without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

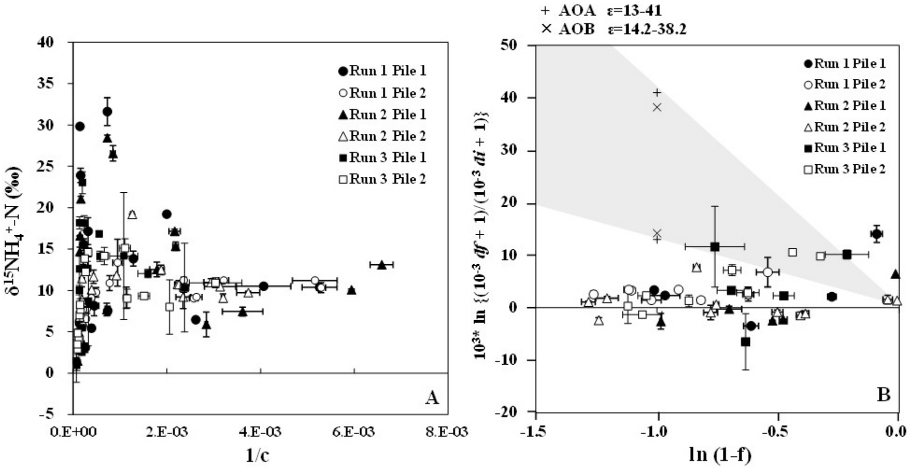

Figure 4. Keeling plot **(a)** and Raleigh plot **(b)** of the $\delta^{15}\mathrm{NH}_4^+$-N. The error bars indicate the SD ($n = 2$). Black symbols indicate the compost with bulking agent (10 % w/w) and white symbols indicate the compost without bulking agents. The gray zone indicate the area which can be explained by ammonium oxidation by AOB (ammonia oxidizing bacteria; 14.2–38.2 ‰) or AOA (ammonium oxidizing archaea; 13–41 ‰).

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Isotopically enriched ammonium shows high nitrogen turnover

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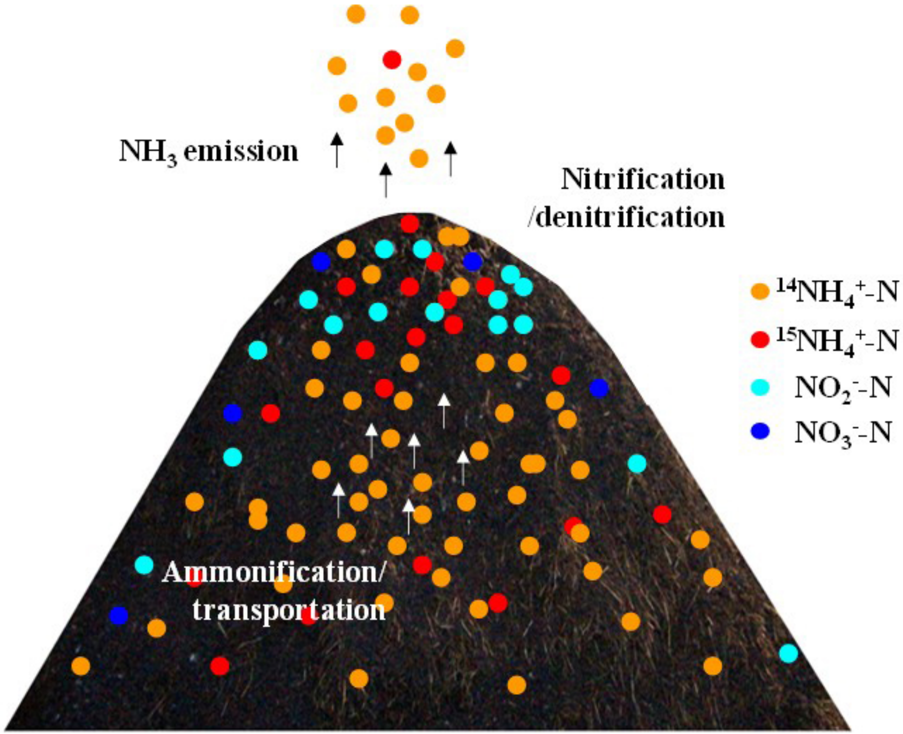

Figure 5. Summary of the events between the pile turnings.