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

Koki Maeda1,2, Sakae Toyoda2, Midori Yano2,3, Shohei Hattori1, Makoto Fukasawa2, Keiichi Nakajima1, and Naohiro Yoshida3,4

1NARO, Hokkaido Agricultural Research Center, Dairy Research Division, 1 Hitsujigaoka, Sapporo 062-8555, Japan
2Department of Environmental Science and Technology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8502, Japan
3Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8502, Japan
4Earth-Life Science Institute, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8550, Japan

*now at: Center for Ecological Research, Kyoto University, 509-3, 2-chome, Hirano, Otsu, Shiga 520-2113, Japan

Correspondence to: Koki Maeda (k_maeda@affrc.go.jp)

Received: 13 April 2015 – Published in Biogeosciences Discuss.: 20 May 2015
Revised: 8 February 2016 – Accepted: 14 February 2016 – Published: 2 March 2016

Abstract. Nitrogen isotope ratios (δ15N) of NH4+ 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 δ15N–NH4+ 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 δ15N values (17.7 ± 1.3 ‰) than piles without bulking agent (11.8 ± 0.9 ‰), reflecting the significantly higher nitrogen conversion and NH3 loss in the former. The samples from the top zone, especially in the piles with bulking agent, showed very high NH4+ concentrations with significantly high 15N (δ15N: 12.7–29.8 ‰) values, indicating that extremely high nitrogen conversion, nitrification–denitrification activity of the microbes and NH3 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 year−1) is already greater than the input from 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 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 NH4+. 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 (NH3) (Dämmgen and Hutchings, 2008). Nitrifiers and other families of microorganisms in the manure also convert this nitrogen as nitrite (NO2−) or nitrate (NO3−), and both nitrifiers and denitrifiers can use them as electron acceptors. They reduce these nitrogen oxides into dinitrogen (N2) and return them to the atmosphere in a process called denitrifica-
tion (Zumft, 1997). Nitrous oxide (N$_2$O), a greenhouse gas, is emitted through the nitrogen conversion in the composting process (Sommmer et al., 2009). Because it is known that N$_2$O 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$_2$O is also known to contribute to ozone layer destruction (Ravishankara et al., 2009), these gas emissions must be mitigated.

With respect to this N$_2$O 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$_2$O (Maeda et al., 2010b, 2013b). Also, we have shown that the appropriate use of bulking agents can reduce the N$_2$O emission significantly (Maeda et al., 2013a). However, the mechanism of this N$_2$O mitigation is largely unknown. Because bulking agents are generally used to increase the supply of oxygen to the compost piles (Jolanun and Towprayoon, 2010), it is expected that the increase in oxygen increases nitrification and the subsequent N$_2$O production.

To solve this contradiction, we compared the level of $\delta^{15}$N–NH$_4^+$ in these composts, because this parameter can be used to track the level of reaction involving NH$_3$ in the environment (Brooks et al., 1989; Garten Jr., 1992; Yeatman et al., 2001). Because it has already been established that the NO$_3^-$ 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 $\delta^{15}$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 2 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 in the Supplement. 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$_3$ and NO$_3^-$ 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 $\delta^{15}$N–NH$_4^+$ levels and Rayleigh plot analysis

The amount of $\delta^{15}$N–NH$_4^+$ in the extracted samples or trapped NH$_3$ samples was determined by the diffusion method (Holmes et al., 1998).

One-centimeter diameter GF/D filters (Whatman, UK) were cut into four pieces, acidified with 20 µL H$_2$PO$_4$ (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 milliliters 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$_4^+$ into 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 $\delta^{15}$N–NH$_4^+$ in the samples. Calibration was conducted with IAEA-N1 and IAEA-
N₂ (NH₄SO₄), and the precision (1σ) was better than 0.2‰. The δ¹⁵N of samples was expressed in parts per thousand deviations from the atmospheric N₂ as defined by the following equation:

\[
\delta^{15}N(\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000,
\]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the \( ^{15}N / ^{14}N \) ratios of samples and the atmospheric N₂, respectively. Isotopic fractionation factor \( \alpha \) was expressed as

\[
\alpha = \frac{R_B}{R_A},
\]

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 \( \varepsilon \), which describes the enrichment of the product relative to that of the substrate, and which is also expressed in per mill (‰).

\[
\varepsilon = (\alpha - 1) \times 1000
\]

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

\[
\frac{R}{R_0} = \frac{1 + 10^{-3} \delta^{15}N}{1 + 10^{-3} \delta^{15}N_0} = \left( \frac{[\text{NH}_3^+]_s}{[\text{NH}_4^+]_0} \right)^{\alpha - 1},
\]

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”. \( \delta^{15}N \) and \( \delta^{15}N_0 \) are the respective \( \delta \) values for each \( \text{NH}_3^+ \). \([\text{NH}_3^+]_s\) and \([\text{NH}_4^+]_0\) 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 \( x \ll 1 \), the relationship between the difference of \( \delta^{15}N \) values between pile turnings and the reaction rate of the substrate was obtained from Eqs. (3) and (4) as follows:

\[
\delta^{15}N - \delta^{15}N_0 = \varepsilon \ln(1 - f),
\]

where \( f \) is the amount of reacted ammonium between the turning events, defined as \( f = \left( 1 - \frac{[\text{NH}_4^+]_0}{[\text{NH}_3^+]_s} \right) \).

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

\[
c_i = c_a + c_s - c_v,
\]

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 NH₃, respectively. Given conservation of mass, we have

\[
\delta^{15}N_{cb} = \delta^{15}N_a c_a + \delta^{15}N_s c_s - \delta^{15}N_v c_v,
\]

where \( \delta^{15}N \) 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 \( \delta^{15}N_v \) and \( \delta^{15}N_b \) is constant,

\[
c_v = k c_b
\]

\[
\delta^{15}N_v = \delta^{15}N_b + \varepsilon_v.
\]

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

\[
(1 + k)c_b = c_a + c_s,
\]

\[
\{\delta^{15}N_b + k(\delta^{15}N_b + \varepsilon_v)\} c_b = \delta^{15}N_a c_a + \delta^{15}N_s c_s.
\]

By combining Eqs. (10) and (11), we arrive at

\[
\delta^{15}N_b = c_a (\delta^{15}N_a - \delta^{15}N_b)/(1 + k) \cdot (1/c_b)
\]

\[
+ \delta^{15}N_a - k \varepsilon_v/(1 + k).
\]

Thus, \( \delta^{15}N_b \) and \( 1/c_b \) have a linear relationship if a single source of ammonium (s) is added to preexisting 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 4543 ± 137 kg in the piles with bulking agent and 4136 ± 124 kg in those without bulking agent, and the final turning these values dropped significantly to 1413 ± 99 and 1960 ± 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
Table 1. Chemical components of compost samples.

<table>
<thead>
<tr>
<th>Time</th>
<th>B.A.</th>
<th>Run</th>
<th>Weight (kg)</th>
<th>TS (%)</th>
<th>VS (TS%)</th>
<th>EC (mS cm⁻¹)</th>
<th>pH</th>
<th>NO₃⁻-N (mg kg⁻¹ TS)</th>
<th>NO₂⁻-N (mg kg⁻¹ TS)</th>
<th>NH₄⁺-N (mg kg⁻¹ TS)</th>
<th>TKN (g N kg⁻¹ TS)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>I –</td>
<td>1</td>
<td>4280</td>
<td>20.5 (0.2)</td>
<td>84.7 (0.3)</td>
<td>2.6 (0.0)</td>
<td>8.4 (0.1)</td>
<td>0.0</td>
<td>0.0</td>
<td>68.9 (1.8)</td>
<td>4646.3 (166.7)</td>
<td>27.6 (0.3)</td>
<td>24.2 (0.2)</td>
</tr>
<tr>
<td>I –</td>
<td>2</td>
<td>4060</td>
<td>22.7 (0.6)</td>
<td>82.3 (1.1)</td>
<td>3.1 (0.0)</td>
<td>8.8 (0.1)</td>
<td>0.0</td>
<td>0.0</td>
<td>7347.8 (7.6)</td>
<td>26.2 (0.4)</td>
<td>22.0 (0.4)</td>
<td></td>
</tr>
<tr>
<td>I –</td>
<td>3</td>
<td>4070</td>
<td>17.8 (0.4)</td>
<td>82.1 (0.4)</td>
<td>2.7 (0.1)</td>
<td>8.0 (0.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>2929.3 (34.0)</td>
<td>20.8 (1.0)</td>
<td>23.0 (0.1)</td>
<td></td>
</tr>
<tr>
<td>I +</td>
<td>1</td>
<td>4700</td>
<td>28.3 (0.1)</td>
<td>87.0 (0.3)</td>
<td>2.9 (0.0)</td>
<td>8.3 (0.1)</td>
<td>0.0</td>
<td>0.0</td>
<td>2288.0 (10.4)</td>
<td>21.6 (0.7)</td>
<td>27.5 (1.7)</td>
<td></td>
</tr>
<tr>
<td>I +</td>
<td>2</td>
<td>4480</td>
<td>31.2 (0.7)</td>
<td>87.1 (0.8)</td>
<td>3.2 (0.0)</td>
<td>8.8 (0.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>5540.3 (126.1)</td>
<td>20.4 (0.4)</td>
<td>21.0 (0.3)</td>
<td></td>
</tr>
<tr>
<td>I +</td>
<td>3</td>
<td>4450</td>
<td>22.6 (0.3)</td>
<td>86.5 (0.6)</td>
<td>3.0 (0.0)</td>
<td>7.7 (0.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>2837.1 (177.4)</td>
<td>28.9 (1.1)</td>
<td>17.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>F –</td>
<td>1</td>
<td>1710</td>
<td>24.8 (0.6)</td>
<td>70.0 (2.9)</td>
<td>2.5 (0.0)</td>
<td>9.3 (0.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>1353.1 (75.1)</td>
<td>22.3 (1.7)</td>
<td>13.0 (0.1)</td>
<td></td>
</tr>
<tr>
<td>F –</td>
<td>2</td>
<td>2280</td>
<td>24.3 (0.2)</td>
<td>75.1 (0.4)</td>
<td>2.6 (0.1)</td>
<td>9.5 (0.1)</td>
<td>61.2 (8.6)</td>
<td>0.0</td>
<td>0.0</td>
<td>451.1 (0.6)</td>
<td>26.7 (0.6)</td>
<td>16.3 (0.0)</td>
</tr>
<tr>
<td>F –</td>
<td>3</td>
<td>1890</td>
<td>21.4 (0.4)</td>
<td>77.2 (0.5)</td>
<td>3.0 (0.0)</td>
<td>9.2 (0.1)</td>
<td>0.0</td>
<td>0.0</td>
<td>3817.1 (177.4)</td>
<td>28.9 (1.1)</td>
<td>17.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>F +</td>
<td>1</td>
<td>1190</td>
<td>52.7 (0.9)</td>
<td>69.1 (0.6)</td>
<td>5.0 (0.1)</td>
<td>9.5 (0.0)</td>
<td>44.3 (1.6)</td>
<td>52.6 (0.2)</td>
<td>460.9 (3.3)</td>
<td>30.0 (0.0)</td>
<td>12.0 (0.1)</td>
<td></td>
</tr>
<tr>
<td>F +</td>
<td>2</td>
<td>1480</td>
<td>47.6 (0.4)</td>
<td>73.7 (0.9)</td>
<td>4.3 (0.0)</td>
<td>9.0 (0.0)</td>
<td>57.4 (7.5)</td>
<td>60.4 (2.5)</td>
<td>375.5 (21.7)</td>
<td>29.1 (0.1)</td>
<td>13.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>F +</td>
<td>3</td>
<td>1570</td>
<td>31.1 (1.0)</td>
<td>71.9 (1.6)</td>
<td>4.9 (0.1)</td>
<td>9.5 (0.1)</td>
<td>53.9 (6.7)</td>
<td>49.5 (12.1)</td>
<td>1809.8 (97.8)</td>
<td>29.2 (0.2)</td>
<td>12.7 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

B.A.: bulking agent; I: initial; F: final; TS: total solids; VS: volatile solids; EC: electrical conductivity; NO₃⁻-N: nitrate-nitrogen; NO₂⁻-N: nitrite-nitrogen; NH₄⁺-N: ammonium-nitrogen; TKN: total Kjeldahl nitrogen; C/N: carbon-nitrogen ratio. The values represent the average (standard deviation).

Figure 1. NO₃⁻ (white), NO₂⁻ (grey) and NH₄⁺–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 2 weeks, just before/after the turning events. (a)–(e) 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).
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⁻¹ TS; pile 1) and core samples (1.0–14.6 mg N g⁻¹ TS; pile 1) contained higher ammonium concentrations than the pile side samples (0.1–1.8 mg N g⁻¹ TS; pile 1) (Fig. 1a–c). High NO₃⁻ accumulation was also observed in the pile top samples (0.03–3.8 mg N g⁻¹ TS; pile 1), but not in the pile core samples. NO₃⁻ was also detected in the pile top and side samples, but the concentrations were low (0–0.29 mg N g⁻¹ TS; pile 1). Although similar trends were observed for pile 2 (Fig. 1d–f), the amount of NH₄⁺ in the top region of pile 2 was generally lower (0.15–2.2 mg N g⁻¹ TS) than that in pile 1. Accumulations of NO₂⁻ (0.08–2.2 mg N g⁻¹ TS) and NO₃⁻ (0.02–0.7 mg N g⁻¹ TS) were also detected in both the top and side samples of pile 2.

### 3.2 δ¹⁵N of NH₄⁺ in mixed samples

δ¹⁵N–NH₄⁺ 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 δ¹⁵N–NH₄⁺ 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.

δ¹⁵N–NH₄⁺ 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 δ¹⁵N–NH₄⁺ 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” NH₄⁺–N, which was supplied by the degradation of organic N in the manure. On the other hand, the heaviest NH₄⁺ (25.4 ± 6.8 ‰) was also observed in the pile core sam-

---

**Figure 2.** δ¹⁵N of NH₄⁺ 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).

**Figure 3.** δ¹⁵N of NH₄⁺–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).
piles at the end of the experimental period. This phenomenon was observed only from the piles with bulking agent.

4 Discussion

The stable isotope $\delta^{15}N$ 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 $\delta^{15}N$ value of $NH_4^+$ in the first 2 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 ($\alpha = \sim 1000$) of isotopic fractionation (Högberg, 1997). The weight decrease in the piles with bulking agent (4543 ± 137–1413 ± 99 kg) was greater than that in the piles without bulking agent (4136 ± 124–1960 ± 291 kg), indicating that a relatively large amount of “light” $NH_4^+$ was supplied to the piles with bulking agent. The $\delta^{15}N$ 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 nitritification–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°C, and much lower nitrogen activity can be observed above 50°C (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 acceptors. 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 $\delta^{15}N$ 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 2 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 and 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 $^15N$ 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 $NO_2^-$ $N$ 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 top pile 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 and 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 bac-
terial 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 Rayleigh 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). 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 ($-17.9$ to $-13.5\%e$, 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.

Figure 4. Keeling plot (a) and Rayleigh 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‰).

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

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.

iv. The nitrification rate exceeds the denitrification rate, leading to accumulation of NO$_3^-$ in the pile top and side, which in turn contributes to significant denitrification and N$_2$O 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$_2$O emission could be mitigated by the use of bulking agent, and thus further studies are needed.

The Supplement related to this article is available online at doi:10.5194/bg-13-1341-2016-supplement.

Author contributions. K. Maeda and S. Toyoda designed the experiments. K. Maeda, M. Yano and M. Fukasawa carried out the experiments. K. Maeda, S. Toyoda and S. Hattori analyzed the results. K. Maeda, K. Nakajima and N. Yoshida wrote the paper.

Acknowledgements. We would like to thank Atsuko Kobayashi and Kauza Azumaya for providing the laboratory-based technical assistance. This work was supported by a grant for the “Development of Mitigation and Adaptation Techniques to Global Warming in the Sectors of Agriculture, Forestry, and Fisheries” from the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan. This work was also supported by a Grant-in-Aid for Young Scientists (B) to K. Maeda and a grant from the Global Environment Research Fund (B-094) of the Ministry of the Environment, Japan to N. Yoshida.

Edited by: X. Wang

References


