Biogeosciences
Editorial Office

Dear Dr. Wang:

Thank you very much for your consideration of our manuscript entitled "**Isotopically enriched ammonium shows high nitrogen turnover in the pile top zone of dairy manure compost"** by Maeda K, Toyoda S, Yano M, Hattori S, Fukasawa M, Nakajima K and Yoshida N, for possible publication in *Biogeosciences* as an original article.

We have revised our manuscript according to your helpful comments. The corrected passages are indicated in red. You can find point-by-point response to the comments below. We believe that our manuscript has been significantly improved and would now be appropriate for publication in the journal *Biogeosciences*.

Thank you very much for your reconsideration.

Sincerely yours,

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Point-by-point responses to the comments

Associate editor:

The authors attempted to discuss nitrogen turnover with N isotopic enrichment of ammonia. First of all, what does the "nitrogen turnover" mean here? Please define in the text.

Thank you very much for your helpful comment. We decided that we replace this term into "transformation".

In the manuscript the authors reported the results in the top, side and core of the piles. In the experimental part of the manuscript, the authors should present how they obtained the top, side and core samples. And how many top, side and core samples each run? More details are needed for the experiment part.

Thank you very much for your helpful comment. We added Fig. S1 to describe the sampling location of the piles and the sampling time in the experiment period.

Statistical analysis: the authors altogether performed three runs. As you used Tukey's multiple range comparison tests to separate the means, what are "the means" here? Are they "means" of different samples each run (if yes, also how many samples?)? Or "mean" among the three runs?

Thank you very much for your helpful comment. We compared the means of the three runs between the treatments and the means of the samples from each location. Details of the sampling are described in Fig. S1. For one treatment, twelve samples were taken from pile top, side and core, respectively. These samples were taken four times (2, 4, 6, 8 week) in three runs. On the other hand, 15 samples were taken from mixed piles, five times (0, 2, 4, 6, 8 week) in three runs.

It seems that the results of the three runs were not so consistent with each other. The authors better present some comments or explanations about this.

Thank you very much for your helpful comment. We think most of the results show same tendency in three runs, but as you pointed, some (ex. Fig. 3) results are inconsistent each other. We have already stated this in the second paragraph in section 3.2.

Also the authors should be serious about the comments by one rewires. It is not a good answer to claim that "here we cannot put the ammonia volatilization in the equation because we did not measure the 15NH4 of the volatilized ammonia".

Thank you very much for your helpful comment. We have revised the section 2.4 Keeling plot analysis as you suggested. We have included the volatized ammonia into the equation. The modified equation still describes that $\delta^{15}N_b$ and $1/c_b$ have a linear relationship if a single source of ammonium is added to pre-existed ammonium. As a result, unfortunately we failed to explain the heavy ammonium in the pile top zone with this analysis, therefore we discuss other possible factors. We believe that this would not be the fatal for the manuscript to be published.

For the Keeling plot analysis or the interpretation of isotopic enrichment, since initial organic N or NH4+ may convert to NO2- and NO3-, is this kind of conversion a factor influencing the isotopic fractioning other than the NH3 volatilization?

Thank you very much for your helpful comment. Keeling plot analysis was used to determine whether we had the source of 'heavy' NH4+ or not. We fully agree with you that nitrification can contribute to isotopic fractionation on NH4+, therefore we also performed Raleigh plot analysis to assess the effect of nitrification (Fig. 4B).

The authors better have a native English speaker to check the English writing. Although I am not a native English speaker, I can find some grammatical or typo errors. Below are some examples: Page 7578 line 9: "significant high" should be "significantly higher"; Page 7581 line 13: "One cm"?

Thank you very much for your comment. We have had the revised manuscript entirely re-edited by this service, and we have attached a certification of this work.

Referee #1

1) Lack of scientific novelty. Quite a few studies have already reported that the manure nitrogen or ammonium becomes isotopically enriched during compost and this enrichment has been attributed to ammonia volatilization and nitrogen transformation. They only novel point is that the current study found that the enrichment was stronger in the top zone than in the side and core zones.
   Thank you very much for your comment. There have been only a few published studies on isotopically enriched ammonium during manure composting, and none of them have focused on the individual zones of the piles. The 15N values of the samples from different zones enabled us to interpret how nitrogen transformation occurs between pile turnings. To our knowledge this is the first report focusing on this topic. Therefore, we believe that our manuscript has significant novelty and could provide insight into the processes of manure composting and its nitrogen transformation.

   Nevertheless, the manuscript is largely based on qualitative analyses while the underlying mechanisms was not presented, i.e., the mechanisms underlying the decrease in nitrous oxide emission (this should be a major objective of this study according to the abstract) following bulking agent use or the greater enrichment in 15N in the top zone of the manure piles (this should be another major objective of this study according to the abstract and the title). For the compost piles with bulking agent, the inside temperature reached more than 60 _C. Normally under such high temperature, nitrification and denitrification or the microbial activities are much low although these processes may take place in some geothermal ecosystems. The decreased emission of nitrous oxide after bulk agent integration may due to decreased nitrification and denitrification. But this needs experiment evidence.
   Thank you very much for this helpful comment. We fully agree with you that the temperature is a possible explanation for the mitigation of N2O emission. A previous report suggested that the optimum temperature for nitrification or denitrification was that under a mesophilic condition (Willers et al., 1998), and another report showed that the N2O production rate can be higher under a thermophilic than under a mesophilic condition (Benoit et al., 2015). The high heterogeneity of temperature in different pile zones makes it very difficult to analyze such results. As we have already stated, the mitigation of N2O emission cannot be explained by the present dataset. We added only a few sentences on N2O emission because we did not provide data on N2O in this manuscript. However, we found many interesting phenomena in terms of 15NH4, and therefore we focused on the nitrogen transformation process between the pile turnings.

2) Mistake in methodology. An isotopic mass balance equation is presented as equation (7). The prerequisite to use an isotopic mass balance model is that the isotopic masses in both sides of the equation are balanced. In terms of manure compost, large nitrogen loss (e.g., ammonia volatilization) is usually taking place. For equation (7), ammonia volatilization should at least be included.
   Thank you very much for this helpful comment. We agree with you that the isotopic masses on both sides of the equation should be balanced. However, here we cannot put the ammonia volatilization in the equation because we did not measure the 15NH4 of the volatilized ammonia. However, to truly understand this phenomenon we will need to analyze 15NH4 data obtained using the current analysis methods. As a result, the large ammonia volatilization could be one of the major obstacles to a clear explanation of the phenomenon. We believe that our present data suggest some interesting hypotheses about the sequential events between the pile turnings, as stated in the conclusion section.

3) Understandability, clarity and concise. Throughout the manuscript, there are lots of grammar issues which make the paper hard to understand.
   Thank you very much for your comment. The original manuscript was already edited by a professional English editing service. We have had the revised manuscript entirely re-edited by this service, and we have attached a certification of this work.

   The experiment needs to be more clearly described. In addition, the terms need to be consistent. For example,

according to line 19 in page 7580, samples were collected “just before each turning”. However, in the following sections or the figures, it seems that samples were collected “just after the turning”.
Thank you very much for your comment. We fully agree with you that this can cause confusion for the readers. Actually, samples in each zone (pile top, side and core) should be taken BEFORE each turning because the turnings increase homogenization. Therefore we collected samples from each zone BEFORE each turning. We also collected the homogenized samples AFTER each turning, because the homogenized samples were also needed to understand the changes in the compost piles. We thus collected the samples both before and after the turning events.

For another, in line 1-2 of page 7581, “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)”. So total N was measured using two methods?
Yes, we measured total N in two different ways. A C/N analyzer can miss the ammonium nitrogen, so we considered that it would be best to cross check this parameter using two approaches. We do not believe that this constitutes a limitation of the study design.

In summary, the manuscript needs substantially improvement.
The manuscript was rewritten and, we believe, substantially improved through the help of your insightful comments.

Referee #2

Manure compost is a major source of nitrogenous gases like ammonia (NH3) and nitrous oxide (N2O) in the atmosphere, and plays a role on global nitrogen cycle. Especially, N2O is a highly-efficient greenhouse, and also destroys ozone in the stratosphere. Therefore researches concerning manure compost, especially the emission of nitrogenous gases during manure compost, have important significance. This work is initiated from the phenomenon that the emission of N2O mitigated when bulking agent was adopted during manure compost, which was found in the authors' previous study.

From the Introduction section, the investigation on the mechanism of N2O mitigation in dairy manure compost piles with bulking agent through isotope analysis should be the major subject of this paper. However, in the Results and Discussion sections, the authors just focus on the enrichment _15N-NH4+ at the top of dairy manure compost piles, and attribute this enrichment to high nitrogen conversion, nitrification-denitrification activity and NH3 volatilization. The mechanism of N2O mitigation with bulking agent is not interrupted. It is needed a revision to make the subject clear before publication. Besides, some expressions in this paper are unclear and inconsistent, which make it difficult to understand this paper.

Thank you very much for this helpful comment. What we found in previous study was that N2O emission can be mitigated by the use of bulking agent. Therefore we tried to understand why this occurs. This was our motivation, so we need to state this. Two of the three compost runs used piles exactly the same as in the previous study. We confirmed that N2O emission was mitigated in these two runs. However these data were already published, so we did not provide the N2O emission data in this study. We stated that the same compost piles were used in the text, and added some discussion on the N2O emission.

Specific comments:

1. The authors use "pile with bulking agent", "pile with dried grass (pile 1)" to describe the dairy manure compost piles. From their previous paper (Maeda et al., 2013a), dried grass is the bulking agent, however, it is not illustrated in the present paper.

   Thank you very much for your comment. We stated this in the Materials and Methods section (P.7580, L.9-13).

   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.

2. N2O mitigation with bulking agent was found in Maeda et al. (2013a). Is similar phenomenon found in the present studies? Are experiments in the two papers the same ones?

   Thank you very much for this point. In both studies (this work and the previous one; Maeda et al., (2013a)), we used the data from three independent manure compost piles. Two of them were identical to each other.

   The manure compost piles used in the previous study (Maeda et al., (2013a)) were as follows.
   Run 1: July 21 through September 17 in 2009
   Run 2: May 27 through July 21 in 2010
   Run 3: September 15 through November 10 in 2010

   The manure compost piles in this study were as follows.
   Run 1: 27 May through 21 July in 2010
   Run 2: 15 September through 10 November in 2010
   Run 3: 19 May through 14 July in 2011

   Runs 2 and 3 in the previous work were identical to Runs 1 and 2 in this study. But we did not mention 15N of ammonium in the previous study. A mitigation effect on N2O emission was observed on at least two of three piles (we did not measure N2O emission from Run 3). We stated this in the text.

3. Line 16 of Page 7583: "Temporal decrease of _15N value of NH4+ were observed in both piles" →"The

decrease of _15N value of NH4+ in the first two weeks were observed in both piles"
Thank you very much. We have changed the expression as you suggest.

4. Line 19-21 of Page 7583: "The _15N value of NH4+ 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‰." → "The _15N value of NH4+ at the end of experiments 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 ‰.
Thank you very much. We have changed the expression as you suggest.

5. Line 22-25 of Page 7583: Why more organic matter degradation cause higher _15N value of NH4+? It seems to be inconsistent with the declaration in Line 17-18 that the ammonification of organic N supplies light NH4+.
Thank you very much for your comment. We fully agree with you that these statements are inconsistent. We changed the relevant sentences to make this more clear.

6. Table 1: The authors annotate "C, control; T, treatment; Values followed by different letters indicate significant difference ($P < 0.05$)", however, there were no "C", "T" and"letters following values" in the table. The authors should check this table carefully.
Thank you very much for this pointing. We simply deleted the unneeded descriptions from the footnote of Table 1:
~~C, control; T, treatment, Values followed by different letters indicate significant difference ($P<0.05$).~~