Response to Anonymous Referee #3

The authors' answer is in red fond.

This paper investigates the mechanism of oxygen isotope fractionation during N_2O production by denitrification. Based on the results from the static anoxic and dynamic incubation experiments, several main conclusions could be outlined in this study: (1) the $\delta^{18}O(N_2O)$ values in the static anoxic incubation experiment were influenced by the $\delta^{18}O$ of soil water, with complete exchange with soil water (x=1). (2) the $\delta^{18}O(N_2O)$ values in the dynamic incubation experiment were variable, and the isotope exchanges with soil water during N_2O production by denitrification were lower than that in the static anoxic incubation experiment (x<1). (3) the results from oxygen isotope fractionation model indicate that the majority of isotope exchange associated mainly with nitrite reduction. (4) the results of $\delta^{15}N^{SP}$ values suggest that fungal denitrification might be the contributing process leading to the different isotope exchanges patterns between static and dynamic incubation experiments, and among different soil types. This study presents many novel experiments and concepts based on the previous researches, deciphers the mechanism of oxygen isotope fractionation during N_2O production by denitrification and confirms the earlier related studies. However, the results of this study are not focused, some conclusions are not substantially demonstrated and the overall presentation is not well structured. I recommend many revisions before final consideration of this paper for publication.

Thank you very much for your positive opinion on the manuscript and for critical comments, which give us the opportunity to clarify a few points below.

General comments:

1. Title

The results of this paper mainly indicate that the isotopic signatures of $\delta^{18}O$, especially the values of $\delta^{18}O(N_2O/H_2O)$, could be used as indicators for differentiation of the N2O production processes by denitrification, hence, the title "The mechanism of oxygen isotope fractionation during N2O production by denitrification" did not reflect the main results in this paper and should be corrected accordingly.

I think this is not the main conclusion, this is important observation, but still rather an assumption which needs to be confirmed with pure culture studies, since it is not 100% sure that the observed additional N_2O source processes are due to fungal denitrification, which is, however, most probable. But I think there are many more important conclusions - like the ranges of O-exchange in various conditions and the magnitudes of O-isotope fractionation. We would only slightly change the title to: 'Oxygen isotope fractionation during N_2O production by soil denitrification'.

2. Abstract

Because the results and conclusions in this paper were not focused and well demonstrated, I recommend the authors rewrite this part. In p. 17010. Line 17-24: these sentences indicates that the results found bacterial denitrification and fungal denitrification had different oxygen isotope exchange and leaded to different values of $\delta^{18}O(N2O/H2O)$, however, as my understanding of the results, the results showed different oxygen isotope exchange between a static and a dynamic incubation experiments at first. With the results of 15N site preference, the authors demonstrated that the different oxygen isotope exchange between a static and a dynamic incubation experiments was probably due to the fungal denitrification processes.

We agree that this could be better phrased as "We found that N_2O formation in static anoxic incubation experiments was typically associated with almost complete oxygen isotope exchange and a stable difference in $\delta^{18}O$ between soil water and the produced N_2O of $\delta^{18}O(N_2O/H_2O)$ = (17.5±1.2) ‰. However, flow-through experiments yielded lower oxygen isotope exchange down to 56 % and a higher $\delta^{18}O(N_2O/H_2O)$ of up to 37 ‰."

In p. 17011. Line 4-6: the authors mentioned the branching isotope effects, however, the oxygen isotope exchange effects with soil water instead of branching isotope effects were the focus of this paper, and the conclusions demonstrated that the values of $\delta^{18}O(N2O/H2O)$ could be applied for differentiation between fungal and bacterial denitrification.

$\delta^{18}O(N_2O/H_2O)$ is indeed different for bacterial and fungal denitrification. The reason for this difference is a higher branching isotope fractionation for fungal than for bacterial denitrification (as observed in Exp. 1), based on results from pure culture studies (Rohe et al.,2014). Fungi, similarly to bacteria, showed high oxygen-isotope exchange, hence a lower extent of O-exchange cannot explain the differences observed.

3. Introduction

Many corrections should be made in this part. The authors should focus the scientific questions which need to be solved and introduce the research progresses for these questions. The hypothesis based on the previous

researches should be summarized and outlined at the last part of the introduction, furthermore, the research methods and objects should be introduced in detail for a good understanding of this research. Several scientific questions were provided and introduced in this part: (1) How the isotope oxygen exchange with soil water during denitrification responses to different abiotic factors such as temperature and soil moisture? (2) Do the different NOR mechanisms for fungi and bacteria have effects on the value of $\delta^{18}O$? The authors also made hypothesis according to these questions, however, the hypothesis was not well demonstrated in the results and discussion of this paper.

We agree that the introduction needs some improvement. However, the questions named by reviewer are very detailed hypotheses and they were not the main aim of our study. Most important was the determination of isotope effects associated with oxygen exchange and with branching for different soils and different experimental conditions and the relationship between the extent of oxygen isotope exchange with soil water and the $\delta^{18}O$ values of the produced N_2O. These research questions were introduced at the end of introduction (based on the previously identified knowledge gaps) in lines 5-10 P17015, and testable hypotheses were presented in lines 13-14, 23-26, 28-01 P17014/17015. Two later hypotheses are tested in Section 3.1 lines 13-16 P17024 and first hypothesis is discussed in Section 4.5 lines 25-29 P17034.

We do not consider the detailed description of methods to be part of the introduction. However, since we use a novel approach here, the idea of the first time applied 17O tracing was explained in 5-11 P17014.

4. Methods

The experiment set-ups was not written with a clear and detailed description.

In p. 17015. Line 23-25: The two sentences were related to the results and should be put in the results part. Furthermore, which data in the results has been published in the previous paper? The authors need to highlight it with reference in the results.

This information will be added in the results section. Part of the data from Exp 1.1 ($\delta^{18}O(NO_3^-)$, $\delta^{18}O(H_2O)$, $\delta^{18}O(N_2O)$) were already published in (Lewicka-Szczebak et al., 2014). In this manuscript we expand these data with $\Delta^{17}O(N_2O)$ analyses.

In the descriptions of Experiment 1 and Experiment 2, the authors did not provide the detailed information about the treatments, the replicate number or the number of incubation jars in each treatment, and this information should be added to the method for a clear understanding of the experiment set-ups.

This is quite complex information, how we combined treatments and incubations. I have prepared an extra table showing all the experimental treatments, including soil moisture, applied nitrate and water, addition of acetylene, soil type and temperature. This table can be added to the manuscript as an extra appendix. I think the readers do not necessarily need to follow the treatments strategy to understand the paper. But this is an important additional documentation of our experimental set-up, so we decided it will be a valuable supplementary information. But additionally, we will also better clarify these issues in the revised manuscript.

In p. 17016. Line 14-15: Could the selected jars be considered as one treatment, and the non-selected jars be considered as another treatment?

'C_2H_2 treatment' will be added. The respective treatments will be also indicated in Table 1. Moreover, an appendix with detailed treatments description will be added for better clarification of treatments strategy.

In p. 17016. Line 22-25: how the N2O mole fraction f(N2O) was estimated by addition of 15N-labelled NaNO3? If this method has been described in the previous papers, it is better to add the papers as references to make a clear description of the experiment design.

Short clarification with reference can be added to the manuscript: This method allows determination of the N_2 concentration originating from the ^{15}N labelled pool and hence the N_2O mole fraction (Lewicka-Szczebak et al., 2013).

In p. 17017. Line 27-28: the sentence "f(N2O) was determined based on the direct measurement of N2O and N2 fluxes" should be followed after the sentence "The fluxes of N2O and N2 were analyzed immediately (see Sect. 2.2)" in Line 24-25.

Ok, we agree the sentence should be moved.

In p. 17020. Line 19-20: I could not understand this sentence "For both presented methods it is assumed that no further O isotope exchange between N2O and H2O occurs". Could the authors rewrite this sentence to make it be understood ?

This sentence will be rewritten: 'For both presented methods it is assumed that after N_2O is formed, no further oxygen isotope exchange with H_2O occurs.'

In p. 17021. Line 1-7: I suggest that this description of the parallel incubations for isotope exchange investigation could be inserted and fused into the contents of the experiment set-ups in p. 17015-17018. The authors should make a comprehensive introduction of the experiment design for the following analysis in the method. In addition, the authors said the parallel incubations to determine the isotope exchange were carried out in Exp 1 (p. 17021. Line 2). Did this method also carried out in Exp 2 for the isotope exchange determination? The authors did not show this content in the method.
The description of parallel incubations will be moved to description of experimental set-up.
This method ($\delta^{18}O$ method with parallel incubations) was only applied in Exp1, in Exp2 we only used ^{17}O method, where no parallel incubations with different waters are needed. This was explained in the first paragraph in Section 2.4.

In p. 17022. Line 16-17: the experiment design for the inhabitation of N2O reduction in Exp 1 were not clearly written in the part of experiment set-ups, and the sentences here could fused into the experiment set-ups. Which treatments were carried out with distinct water or nitrate isotopic signatures, and which treatments were added with acetylene for the inhabitation of N2O reduction? The authors should clarified and identified these experimental treatments in the description of the experiment design. The same corrections should be made for the contents in p. 17022. Line 2-6.
This will be clarified. We will indicate the C_2H_2 inhibited treatments in Table 1.

5. Results and Discussion
Many problems existed in the presentation of the results.
In p. 17024. Line 3-12: the paragraph had an introduction of calculation method for δ 18O(N2O/H2O) and δ 018O(N2O/H2O) , hence, this part belongs to the method and should be migrated to the method part in the paper.
The description of calculation method will be moved to method section as requested.

In addition, the results only included the estimated values related to Table 1 and 2, without the contents related to other tables and figures. The authors should tell the results according to the tables and figures presented in the paper, and tell the story completely and fluently.
Other figures and tables belong to interpretation and discussion of the results, therefore were placed and described in discussion section. They cannot be presented in results section because of their complexity, especially the Table 4 with the calculated model is impossible to be described in results, because it is a result of our interpretations and discussion with previous studies. To avoid the problematic distinction into results and discussion, we will combine both parts into a common results & discussion section.

Table 1 and 2 showed the results of Exp 1 and 2, however, the contents in the tables were not well organized and structured. The treatments, such as reduction inhibited or non-inhibited, soil adding with heavy or light water, with natural Chile saltpeter or synthetic NaNO3, should be noted in the tables. Only one target moisture level (80% WFPS) and three target moisture level (50%, 65% and 80% WFPS) were set in the Exp 1.1 and 1.2, while one target moisture level (70% WFPS) was set in Exp 2. In the table 1 and 2, the moisture levels with small differences in the same moisture treatment could be uniformed with the target moisture levels (50%, 65%, 70%, or 80% WFPS). In p. 17015.
Treatments will be clarified in Table 1, and additional detailed outline of treatments will be added as Table A1 (appendix). I will uniform the moisture levels for exp1, as they were very consistent, however due to large differences it cannot be done for exp2.

Line 21-23, the authors said "The first part of these incubations (Exp 1.1) was performed for both soils at two different temperatures (8 and 22 ℃) but with only one moisture level of 80% WFPS (water filled pore space)", but why there was only one temperature treatment (22 ℃) for silt loam soil? Why did the results of the temperature treatment at 8 ℃ lack for silt loam soil?
We wanted to check the temperature dependence for one soil only, due to large amount of different treatments it was not possible to manage with additional temperature treatment for silt loam. The temperature dependence, if present, should occur independently of soil type.

In p. 17024. Line 14 and Line 22: the values of $\delta^{18}O(N2O/H2O)$ were not shown in the tables, should the$\delta^{18}O(N2O/H2O)$ here be rewritten to $\delta^{18}O(N2O/H2O)$?
Yes, thank you, this mistake will be corrected.

In the results, the authors presented the comparisons of x and $\delta^{18}O(N2O/H2O)$ for different temperatures, soil types, soil moisture levels, and experiment designs, however, these comparisons could not demonstrate the effects of temperature, soil types, soil moisture levels or experiment designs on the values of x and$\delta^{18}O(N2O/H2O)$. I recommend the authors make a clear and detailed description of the experiment treatment in the tables, and reanalyze the data with MANOVA to present the effects of different factors on the values.
We decided that the ANOVA analysis will be sufficient for our aims. There are not so many factors to analyse, all have been analysed with ANOVA and mostly no significant differences have been found - it has been shown that none of the analysed factors show any influence on x and $\delta^{18}O(N2O/H2O)$. The only significant difference was found between Exp1.1 and 1.2 for $\delta^{18}O(N2O/H2O)$ and this is due to differences in isotopic signature of applied nitrate as discussed later. Since the applied simple statistics indicate no significant differences for the tested hypotheses we do not see the need to apply more complicated statistics. We think this will not provide any additional information.

Many contents in Discussion were about the results in the tables and figures, and should be classified into the results part. I recommend the authors reorganize the contents in Discussion. The authors mainly discussed the results based on the analyzed data, and I recommend the authors use other previous researches to demonstrate these conclusions.
We will combine results and discussion section.
The aspects presented in the paper are quite novel, hence there are not many similar previous studies to compare with. The comparison to relevant previous studies was included. We do not know any other relevant studies which are missing in our discussion.

In p. 17027. Line 25-26: the authors said that the different values of x between the static and dynamic incubations may be due to activity of different microorganism groups, but I could not understand this conclusion based on the presented data and other information provided in the paper.
As we stated in the manuscript, we are also very surprised with this result. At this time, our suggestion is merely speculation, which was expanded on later in the section 4.5. But we will delete this sentence from here. In the discussion in Section 4.5 we present a justification for this assumption.

In p. 17028. Line 1-5: the authors said that the correlation between x and $\delta^{18}O(N2O/H2O)$ seems to differ for different soil types, and try to explain this conclusion by deciphering the theoretical model of the denitrification. However, the results of the theoretical model indicates that majority of isotope exchange associated mainly with nitrite reduction, and how did it explain the differences correlation between x and $\delta^{18}O(N2O/H2O)$ for different soil types?
This is because of the lower branching effect for sandy soils, which was also indicated by the model. By lower branching fractionation N_2O is less enriched in ^{18}O and the lower O isotope exchange results in smaller increase of $\delta^{18}O$ values, which was observed for sandy soils in Fig.4. This explanation will be added to the discussion.