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.

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 N_2O production processes by denitrification, hence, the title "The mechanism of oxygen isotope fractionation during N_2O production by denitrification" did not reflect the main results in this paper and should be corrected accordingly.

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 $\delta_0{}^{18}O(N_2O/H_2O)$, 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 ^{15}N 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.

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 $\delta_0{}^{18}O(N_2O/H_2O)$ could be applied for differentiation between fungal and bacterial denitrification.

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.

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.

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.

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?

In p. 17016. Line 22-25: how the N_2O mole fraction $f(N_2O)$ was estimated by addition of ^{15}N-labelled $NaNO_3$? 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.

In p. 17017. Line 27-28: the sentence "$f(N_2O)$ was determined based on the direct measurement of N_2O and N_2 fluxes" should be followed after the sentence "The fluxes of N_2O and N_2 were analyzed immediately (see Sect. 2.2)" in Line 24-25.

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 N_2O and H_2O occurs". Could the authors rewite this sentence to make it be understood ?

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.

In p. 17022. Line 16-17: the experiment design for the inhabitation of N_2O 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 N_2O 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.

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 $\delta^{18}O(N_2O/H_2O)$ and $\delta_0{}^{18}O(N_2O/H_2O)$, hence, this part belongs to the method and should be migrated to the method part in the paper. 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.

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 $NaNO_3$, 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. 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? In p. 17024. Line 14 and Line 22: the values of $\delta^{18}O(N_2O/H_2O)$ were not shown in the tables, should the $\delta^{18}O(N_2O/H_2O)$ here be rewritten to $\delta_0{}^{18}O(N_2O/H_2O)$? In the results, the authors presented the comparisons of x and $\delta_0{}^{18}O(N_2O/H_2O)$ 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 $\delta_0{}^{18}O(N_2O/H_2O)$. 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.

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.

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.

In p. 17028. Line 1-5: the authors said that the correlation between x and $\delta_0{}^{18}O(N_2O/H_2O)$ 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 $\delta_0{}^{18}O(N_2O/H_2O)$ for different soil types?