

## ***Interactive comment on “Isotopic fractionation of N<sub>2</sub>O to quantify N<sub>2</sub>O reduction to N<sub>2</sub> – validation with Helium incubation and <sup>15</sup>N gas flux methods” by Dominika Lewicka-Szczebak et al.***

**Anonymous Referee #1**

Received and published: 20 September 2016

### 1. General comments

This paper examines the validity of isotopic analyses to quantify the degree of N<sub>2</sub>O reduction to N<sub>2</sub> occurring in soils. Molecular nitrogen is the final product in denitrification, one of the major nitrogen metabolism processes in soils, but the rate of N<sub>2</sub> production under natural condition is usually difficult to determine due to the presence of atmospheric N<sub>2</sub>. Isotopic fractionation of N<sub>2</sub>O, a precursor of N<sub>2</sub> in denitrification, has been often used as an alternative to estimate the N<sub>2</sub> production rate. However, isotopic signature of N<sub>2</sub>O is determined not only by N<sub>2</sub>O reduction but also N<sub>2</sub>O production by denitrification and other microbial or abiotic processes, so there remains significant uncertainty with the isotopic method for N<sub>2</sub> production estimates. Based on independent

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laboratory simulation experiments, the authors showed that isotopic signature of N<sub>2</sub>O before reduction is the most critical parameter and it can be experimentally determined under steady conditions and that combined analysis of two isotopic values is effective under non-steady conditions.

Although the authors' major finding is somewhat predictable and the “mapping approach” is not really new compared to previous studies, this work is the first one to confirm the validity of isotopic fractionation method experimentally and it contains some useful information on experimental approaches and on various N<sub>2</sub>O production processes occurring in soils. Therefore, I consider that this paper is suitable for publication in BG after minor revision, and I expect further researches are stimulated by this paper to apply the isotopic method to more complicated soil systems.

### 2. Specific comments

P9, L13 and eq. 5: It is not clear whether the N<sub>2</sub> originating from non-labelled pools are considered similarly in the case of N<sub>2</sub> because evidence of such N<sub>2</sub> is described in section 3.2.2.

P9, eq. 6: In P8, the authors describe that three separated gas species (N<sub>2</sub>, N<sub>2</sub>+N<sub>2</sub>O, and N<sub>2</sub>O) were measured. Then why is fp\_N<sub>2</sub>O not used in this equation? Or did they confirm mass balance like fp\_N<sub>2</sub>+N<sub>2</sub>O = fp\_N<sub>2</sub> + fp\_N<sub>2</sub>O?

P11, L4: “average <sup>15</sup>N abundance in nitrate” What was averaged? Initial and final values?

P18, section 3.2.2: Although the calculation procedure for r\_N<sub>2</sub>O is explained in detail in section 2.5.2, results of r\_N<sub>2</sub>O from the <sup>15</sup>N treatment are not shown. Does this mean the <sup>15</sup>N gas flux method failed to give r\_N<sub>2</sub>O value?

P23, L6: “r\_N<sub>2</sub>O values are always higher for Sc2” This is consistent with Figure 8, but I found the opposite statement in P16, L19. Please check the text and the figure.

P23, L23: “for both soils in the anoxic treatment the cumulative non-labelled N<sub>2</sub>O flux

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is higher than the initial NH<sub>4</sub><sup>+</sup> pool plus the NH<sub>4</sub><sup>+</sup> possibly added” I could not follow. When I compare the 8th, 12nd, and 13th columns in Table S1, this is the case only for Min Soil with anoxic, 15N treatment.

P24, L6: “it represents, respectively, 2 and 3 % of the nitrification rate (Table 1)” I cannot understand how I find this in Table 1. What does “respectively” means?

P24, L8: “observed increase in NO<sub>3</sub>-“ In Table S1, C\_NO3t is always lower than C\_NO30, so there seems to be no “increase in NO<sub>3</sub>-“.

P27, L8: “15N-pool derived N<sub>2</sub>O characterized by higher d15Nsp values” In section 2.5, the authors did not mention that they measured d15Nsp of N<sub>2</sub>O in 15N gas flux method. Did they measure it?

P32, L27: It seems that 15N gas flux method is useful to detect the processes such as producing hybrid N<sub>2</sub>O or N<sub>2</sub>, but I’m not convinced that it is really necessary to determine r\_N<sub>2</sub>O (see above comment on section 3.2.2).

### 3. Technical corrections

P9, L3: “. . . , of which the fp was derived” “from which” would be better.

P10, equations 11 and 12: To be exact, eq. 12 is not automatically derived from eq 11. I suggest to define f\_L together with f\_H.

P11, L8, L9, L13: Be consistent in expressing “[N<sub>2</sub>O flux]”. It was [N<sub>2</sub>O]flux in P10, eq 7.

P12, L10: “d15Nbulk(NO<sub>3</sub>/N<sub>2</sub>O)”: positions of NO<sub>3</sub> and N<sub>2</sub>O should be reversed?

P14, L11: Which do you prefer to use “SP” or “d15Nsp”? Consistent usage is preferable.

P14, L11: “. . . have been use to” . . . have been used to

P21, L26: “no significant correlation with the 1:1 line” Awkward phrase. I suppose the

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authors would like to say that slope was smaller than unity although calculated and measured values showed a correlation.

P23, L16: “Fig. 3”, L23 “Fig. 1(a)”, P24, L5 “Fig. 1” Are these referring to Fig. 2? Also I suggest to rearrange the figures in order of mention in the text.

P27, L20: “Sect. 4.2.3” Sect. 4.3?

P28, L20: “For them . . .” Awkward sentence.

Caption of Figure 6: “Eq. (13)” might be Eq. (17). Also, it should be clearly noted that this graph shows data obtained only with Min Soil.

Caption of Figure 7: Correct the equation number in “Eq. (13)”.

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-276, 2016.

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