

Interactive comment on “Isotopic fractionation of N₂O to quantify N₂O reduction to N₂ – validation with Helium incubation and ¹⁵N gas flux methods” by Dominika Lewicka-Szczebak et al.

Anonymous Referee #1

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1. General comments

This paper examines the validity of isotopic analyses to quantify the degree of N₂O reduction to N₂ occurring in soils. Molecular nitrogen is the final product in denitrification, one of the major nitrogen metabolism processes in soils, but the rate of N₂ production under natural condition is usually difficult to determine due to the presence of atmospheric N₂. Isotopic fractionation of N₂O, a precursor of N₂ in denitrification, has been often used as an alternative to estimate the N₂ production rate. However, isotopic signature of N₂O is determined not only by N₂O reduction but also N₂O production by denitrification and other microbial or abiotic processes, so there remains significant uncertainty with the isotopic method for N₂ production estimates. Based on independent

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laboratory simulation experiments, the authors showed that isotopic signature of N₂O 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 N₂O 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 N₂ originating from non-labelled pools are considered similarly in the case of N₂ because evidence of such N₂ is described in section 3.2.2.

P9, eq. 6: In P8, the authors describe that three separated gas species (N₂, N₂+N₂O, and N₂O) were measured. Then why is fp_{N_2O} not used in this equation? Or did they confirm mass balance like $fp_{N_2+N_2O} = fp_{N_2} + fp_{N_2O}$?

P11, L4: "average 15N abundance in nitrate" What was averaged? Initial and final values?

P18, section 3.2.2: Although the calculation procedure for r_{N_2O} is explained in detail in section 2.5.2, results of r_{N_2O} from the 15N treatment are not shown. Does this mean the 15N gas flux method failed to give r_{N_2O} value?

P23, L6: " r_{N_2O} 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 N₂O flux

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is higher than the initial NH_4^+ pool plus the NH_4^+ 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_3^- ” In Table S1, C_NO_3 is always lower than C_NO_3 , so there seems to be no “increase in NO_3^- ”.

P27, L8: “15N-pool derived N_2O characterized by higher $\delta^{15}\text{N}_{\text{sp}}$ values” In section 2.5, the authors did not mention that they measured $\delta^{15}\text{N}_{\text{sp}}$ of N_2O 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_2O or N_2 , but I’m not convinced that it is really necessary to determine $r_{\text{N}_2\text{O}}$ (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_2O flux]”. It was [N_2O]flux in P10, eq 7.

P12, L10: “ $\delta^{15}\text{N}_{\text{bulk}}(\text{NO}_3/\text{N}_2\text{O})$ ”: positions of NO_3 and N_2O should be reversed?

P14, L11: Which do you prefer to use “SP” or “ $\delta^{15}\text{N}_{\text{sp}}$ ”? 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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