

***Interactive comment on “Early season N<sub>2</sub>O emissions under variable water management in rice systems: source-partitioning emissions using isotopocule signatures along a depth profile” by Elizabeth Verhoeven et al.***

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Authors: We appreciate this thoughtful review and have added some specific changes to the discussion to address the three main areas of concern. We have also addressed the minor comments. We hope these changes are acceptable and make the discussion more robust and valuable to the N<sub>2</sub>O isotope community. All page and line references refer to our amended manuscript with track changes all accepted.

Referee: There are three significant areas in this manuscript that need to be addressed

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and a number of minor issues that we list below. First, we appreciate the authors' use of the term "isotopocule" to more accurately describe the bulk and site dependent isotopic composition of nitrous oxide but, regrettably, their use of this term is incorrect (see Ostrom and Ostrom, 2017). Isotopocule is a contraction of "isotopic molecule" and this term refers specifically to the 12 distinct isotopic molecules that result when the two isotopes of nitrogen and 3 isotopes of oxygen are combined in every imaginable way. Thus it is incorrect to use isotopocules to describe isotope ratios. Isotopomer refers to the two isotopocules of nitrous oxide that have the same mass but differ in the location of  $^{15}\text{N}$ . Isotopologues is not a very useful term as it implies differences in both mass and isotopic composition. Given this, perhaps it would be best to simply use "isotope ratios" to describe both bulk and site specific isotopic information.

Authors: Thank you for this clarification. We have gone through the manuscript and changed all 'isotopocule' and 'isotopocule signature' terms to 'isotope ratio' as suggested.

Referee: Secondly, we are concerned with the use of constant values for the kinetic isotope effects (KIE) associated with nitrous oxide reduction in their models. The literature cited in the paper clearly demonstrates that the KIE associated with nitrous reduction is variable and yet the authors chose a single value of 6.6 per mil in their models. Further, the Jinuntuya-Nortman et al (2008) demonstrate that the KIE decreases with increasing water filled pore space.

Referee: Third, we are concerned with the use of ranges in  $\delta^{18}\text{O}$  of nitrous oxide associated with various sources of nitrous oxide to describe microbial origins. While SP is considered a conservative tracer of the origins of nitrous oxide it is widely known that bulk  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values are not conservative. Thus while ranges of values can be compiled from the literature it is uncertain how well these values represent what can be expected in the natural environment. It is known that  $\delta^{18}\text{O}$  values in nitrous oxide can be altered by exchange with water and, indeed, the authors estimate that 100% of the O in nitrous oxide has exchanged with water. Given this high degree of exchange,

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how reasonable is it to use constant isotope values to infer microbial origins? We don't believe that any of these concerns should result in rejection or major restructuring of the manuscript. Rather, we would like to see the authors acknowledge these concerns and discuss what the implications of variation in KIE's and source isotope values would have on their model results.

Authors: These are valid points and we agree are worthy of brining into the discussion. First, to clarify, we did not use a KIE value of 6.6 per mil in our models used for source partitioning N<sub>2</sub>O. Our models for source partitioning N<sub>2</sub>O relied on SP and 18O only, where we did use fixed isotope effects for 18O and SP during N<sub>2</sub>O reduction, as referenced in Table 2. The use of -6.6 per mil referred to the 15N isotope effect during N<sub>2</sub>O reduction, which was only used post source partitioning to evaluate the isotope effects for 15N. Here, we used our modeled N<sub>2</sub>O reduction fraction, rN<sub>2</sub>O (derived from 18O and SP model) to back calculate plausible  $\delta^{15}\text{N}_{\text{N}_2\text{O}/\text{NO}_3}$  values if we assumed a fixed value for 15N N<sub>2</sub>O reduction fractionation and our rN<sub>2</sub>O rates. Our intent was to determine if this type of correction could bring our measured  $\delta^{15}\text{N}_{\text{N}_2\text{O}/\text{NO}_3}$  closer to those seen in pure culture or controlled studies, thus adding support to the extent of N<sub>2</sub>O reduction measured in our model and helping to explain our measured  $\delta^{15}\text{N}_{\text{N}_2\text{O}/\text{NO}_3}$ .

In general, regarding the use of fixed isotope values and isotope effects in our model, we fully agree there is a lot of room for advancement here. Indeed, when first experimenting with our model we played around with a range of 18O values for denitrification/nitrifier-denitrification and nitrification/fungal denitrification derived N<sub>2</sub>O as we felt there was the largest range in these values in the literature (Author Response Table 1). We found the patterns between treatments to be pretty conservative but the range variable (Author Response Figure 1). An example of a previous test run is given below. In the end, we felt going in this direction was too complex for this paper and would morph it into a monster and distract from our original intent. We feel strongly though that a logical next step would be to advance the model so that isotope ratios

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and effects can be drawn from a pool of literature values using Monte Carlo simulation or a similar approach.

We have added a paragraph to P25L21, which discusses this as well as the need to account for known changes in isotope effects based on environmental conditions in more complex models.

“All modeling attempts to date rely on isotope signatures and effects determined in laboratory studies and thus changes in these values in response to environmental or microbial population dynamics in the field remains a large question. As this was an in-situ field experiment, conditions were not constant across treatments or throughout the sampling time frame, yet it has been shown that isotope effects, particularly for N<sub>2</sub>O reduction change with shifts in environmental conditions such as increasing water filled pore space (Jinuntuya-Northman et al., 2008). Therefore, the use of fixed isotope effects in our model is a simplification. Future modeling efforts may be improved by the incorporation of variable isotope effects based on soil moisture or O<sub>2</sub> for example. Careful, controlled experiments across a range of soils with different management histories are necessary to determine if consistent variation in isotope effects in relation to specific environmental parameters can be determined or if such parameters are site specific. The microbial  $\delta^{18}\text{O}$  signatures for denitrification used in our model were calculated relative to  $\delta^{18}\text{O}\text{-H}_2\text{O}$ . We therefore assumed complete exchange between N<sub>2</sub>O substrates, intermediaries and water during denitrification. We based this off of previous work showing that O exchange is high and that the isotope effect between water and N<sub>2</sub>O is relatively stable (Lewicka-Szczebak et al., 2016; Lewicka-Szczebak et al., 2017; Snider et al., 2013; Kool et al., 2007). In reality, results over time and between treatments may have been affected by varying degrees of  $^{18}\text{O}$  exchange between N<sub>2</sub>O, intermediaries and water and by variation in  $\delta^{18}\text{O}\text{-H}_2\text{O}$  values. We recommend that future studies measure the  $\delta^{18}\text{O}\text{-H}_2\text{O}$  to better constrain results. Modeling results would also be more robust if complete  $\delta^{15}\text{N}$  -N<sub>2</sub>O, -NH<sub>4</sub><sup>+</sup> and -NO<sub>3</sub><sup>-</sup> across treatments and times were available, allowing for complimentary modeling of

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SP x 15N(N<sub>2</sub>O/NO<sub>3</sub><sup>-</sup> or N<sub>2</sub>O/NH<sub>4</sub>). Employing iterative simulation techniques where a range of literature values for N<sub>2</sub>O signatures and isotope effects are used to draw from would help to highlight model sensitivity to specific isotope values and improve its accuracy. Lastly, more work needs to be done to validate results such as those generated here which rely on laboratory derived values, with complimentary measurements of microbial community dynamics, such as that by Snider et al. (2015).”

Referee: Page 4, line 1-2: Abiotic production of N<sub>2</sub>O can occur by many pathways and it seems the values cited here reflect production from hydroxylamine. We recently reported SP values of 16 per mil for N<sub>2</sub>O production from NO (Stanton et al., 2018, Geobiology (DOI :10.1111/gbi.12311)).

Authors: Clarification of hydroxylamine oxidation specifically and this additional reference have been added, P4L1.

Referee: Page 7, line 10: What are the minimum concentrations required to obtain accurate isotope values for nitrate and ammonium?

Authors: Our limit of quantification for 15N-NH<sub>4</sub> was 0.75 mgL<sup>-1</sup> or ~ 42uM NH<sub>4</sub> , this was accidently omitted, but is now added on P9L8. Our limit of quantification for 15N-NO<sub>3</sub><sup>-</sup> was 0.125 mgL<sup>-1</sup> or 2.0 uM NO<sub>3</sub><sup>-</sup> (P9L18).

Referee: Page 9, line 29-32. As mentioned above, this is a good representation of the literature  $\delta^{18}\text{O}$  values but given concerns about water exchange can we realistically expect these values to apply to field studies?

Authors: This is a valid point and we agree. We have tried to better acknowledge that isotope methods such as the modeling proposed here are still limited and difficult to apply and interpret in field situations. At the same time, these methods only become really useful if they can be applied in ecological or agronomic studies. No method is perfect, but we feel that given the current knowledge, the methods can be used for ecological studies as long as the uncertainty associated with data interpretation

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is acknowledged. We hope this sentiment is now better expressed in our additional discussion paragraph.

Referee: Page 10, line 5: It would seem this slope is determined from a single pair of values when a wide range of values for the KIE associated with nitrous oxide reduction can be found in the literature. What is the impact of variation in the slope on the outcomes of this model?

Authors: We did not test the sensitivity of our model to changes in this slope. We agree this, among other parameters in the model should be further tested and developed in future studies. See above.

Referee: Page 13, line 22: "In the WS treatments, high N<sub>2</sub>O emitted fluxes were also associated with lower  $\delta^{15}\text{N}$  signatures". This statement is not entirely accurate. In WS-AWD two peaks of N<sub>2</sub>O were observed (Figure 3), first on June 17, with high  $\delta^{15}\text{N}$  signatures ( $\delta^{15}\text{N}$  20‰ and the second on June 23 with lower  $\delta^{15}\text{N}$  signatures ( $\delta^{15}\text{N}$  40‰ both peaks showing similar N<sub>2</sub>O flux.

Authors: The sentence has been amended and now reads, "In the WS treatments, high N<sub>2</sub>O emitted fluxes on June 23rd, following the second fertilization, were associated with lower  $\delta^{15}\text{N}$  signatures (Fig. 3), this was not the case for a high flux in the WS-AWD on June 17th."

Referee: Page 18, lines 18-19: The use of "high" net isotope effects can be misleading because the NIE's are negative. A value of -6, for example, is higher than -16 but reflects a lower degree of isotopic discrimination. Perhaps use "greater degree of isotopic discrimination" or a similar phrase.

Authors: This is a good observation and the suggested wording has been adopted.

Referee: Page 18, line 20: The use of a single value to describe the net isotope effect for reduction of nitrous oxide is not very accurate as it is well known that this value varies. Jinuntuya-Nortman et al. (2008) demonstrated that water filled pore space is

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inversely related to the net isotope effect and at high values of water filled pore space this value approaches zero. Given that this environment is frequently characterized by high and variable water filled pore space how realistic is it to use a single value? What would be the impact on the model outcomes of allowing this value to vary over the range of literature values reported?

Authors: We feel this point is now addressed in our new discussion paragraph on P25L21. It would be interesting to assess the effect of the model outcomes if this value varied, but we feel this would be too speculative and beyond the scope of the current manuscript.

Referee: Page 19, Line 25: Authors postulates that high SP values relative to  $\delta^{18}\text{O}$  or  $\delta^{15}\text{N}$  observed in  $\text{N}_2\text{O}$  pore air from WS treatments, could be explained by greater contributions from abiotic hydroxylamine decomposition. However, in order to produce enough  $\text{N}_2\text{O}$  from abiotic hydroxylamine decomposition, to switch or enriched SP values significantly, it wouldnt require high  $\text{NH}_4^+$  concentrations (Rubasinghege et al., 2011; Heil et al., 2015)? In this study, the  $\text{NH}_4^+$  concentrations were very low during the sampling period.

Authors:  $\text{NH}_4$  concentrations in the WS-AWD prior to the second fertilization were between 5-10 mg N/L and around 5 mg/L N in the WS-FLD and were thus higher than the DS-AWD for much of the sampling period. However, you are correct that the times of higher  $\text{NH}_4$  in the WS treatments don't necessarily correspond to the scattered high SP values and no correlation between these variables was observed for any treatment (Table 3). The plausibility of abiotic hydroxylamine oxidation during coupled nitrification-denitrification is discussed later in this same paragraph. We have amended the wording some. It now reads as follows. If this whole piece remains too speculative, we can omit. "Abiotic hydroxylamine decomposition requires nitrification for the production of  $\text{NH}_2\text{OH}$ , and iron or manganese (hydr)oxides as electron acceptors to proceed (Bremner et al., 1980). Given the moist conditions, nitrification rates were likely low in the WS treatments. Feasible co-occurrence of these species could really

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only occur directly in the rhizosphere of a flooded rice soil, where  $O_2$  is transported to the immediate root zone by the aerenchyma. Tightly coupled nitrification-denitrification in the rhizosphere of rice plants has been shown before (Arth and Frenzel, 2000) as has coupling of nitrogen – iron transformations (Ratering and Schnell, 2000) but we cannot say the extent to which this may have occurred in our system. “ P21L2

Referee: Page 21, line 13: The finding that oxygen exchange is 100% is very concerning. Doesn't 100% exchange compromise the use of  $\delta^{18}O$  to partition sources of nitrous oxide?

Authors: We politely disagree. Our modeling used isotope signatures calculated relative  $d^{18}O$  of water for denitrification based on results of (Lewicka-Szczebak et al., 2016; Lewicka-Szczebak et al., 2017). We have added the aforementioned discussion paragraph which we hope adequately addresses this issue.

Referee: Figure 4: Is there a reason why the reduction and mixing lines are plotted in A but not on the figures in B?

Authors: Yes, we did not derive a reduction and mixing line for  $SP \times 15N-N_2O$  relationship. To accurately draw such lines we need to use fixed values for the  $15N-N_2O$  signature produced from denitrification and nitrification. We have not reviewed the literature for a consensus value for these processes. Because we had limited data for  $d^{15}N$  in  $NH_4^+$  and  $NO_3^-$ , we could not use these values in modeling.

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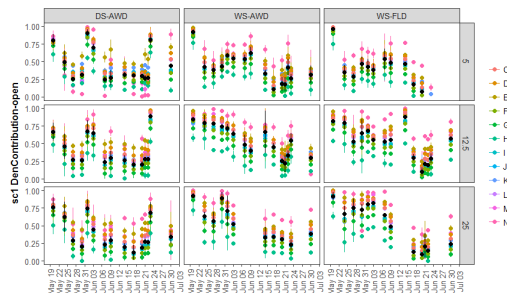
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Author Response Figure 1. Denitrification contribution results for Scenario 1, open system modeling across a range of  $\delta^{15}\text{O}-\text{N}_2\text{O}_{\text{at}}$  and  $\delta^{18}\text{O}-\text{N}_2\text{O}_{\text{den}}$  values. The range of values used in this testing are given in Table 1. The values actually used in the manuscript results are from "A" (black dots). From this analysis we chose to stick with the values derived from Lewicka-Szczekab et al. 2017 for consistency and because they represented the mean. The ranges changed with varying  $\delta^{18}\text{O}$  values but the relative patterns were conserved.



Author response Table 1.  $\delta^{18}\text{O}$  values used in model testing.

Identification	Description	$\delta^{15}\text{O}-\text{N}_2\text{O}_{\text{at}}$	$\delta^{18}\text{O}-\text{N}_2\text{O}_{\text{den}}$
A (black dots)	Default values (DF) derived from Lewicka-Szczekab et al. (2017)	36.5	12.7
C	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}+5$	36.5	17.7
D	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}+10$	36.5	22.7
E	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}+20$	36.5	32.7
F	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}-5$	36.5	7.7
G	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}-10$	36.5	2.7
H	$\text{N}_2\text{O}_{\text{at}}$ fixed, $\text{N}_2\text{O}_{\text{den}}-20$	36.5	-7.3
I	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}+5$	41.5	12.7
J	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}+10$	46.5	12.7
K	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}+20$	56.5	12.7
L	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}-5$	31.5	12.7
M	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}-10$	26.5	12.7
N	$\text{N}_2\text{O}_{\text{den}}$ fixed, $\text{N}_2\text{O}_{\text{at}}-20$	16.5	12.7

Fig. 1.