

## *Response to Topical Editor*

Topical Editor Decision: Publish subject to minor revisions (review by editor) (30 Jul 2019) by Steven Sleutel

Comments to the Author:

The authors have very well accommodated referee requests. I agree with the proposed modifications to the manuscript. Only a few minor points of attention remain:

- 1) The abstract is too long: even detail is provided on specifics of the lab incubations. This part can be condensed without loss of information.

**Authors:** we have condensed the abstract from 333 to 301 words; we removed the specifics of the soil incubations, and replaced with condensed text. See track changes for the revised parts on page 1/line 14-16.

- 2) P2 2nd line of the introduction: remove . after Environment Canada

**Authors:** Done.

- 3) P2 L24 comma after ‘Baggs’

**Authors:** Done.

- 4) P3 L9-12 ‘The term “isotopomer” is used herein to indicate molecules of the same mass in which the trace isotopes are arranged differently. This differs from “isotopologue”, which is a more general term referring to molecules that differ in isotopic composition (Ostrom and Ostrom, 2012).’ Could be omitted.

**Authors:** We have deleted the text from the Introduction, but moved the reference to Ostrom and Ostrom (2012) to Page 2/Line 20–21. In addition, we have added a second reference regarding terminology (McNaught and Wilkinson, 1997). Please note that these references were added to the text in response to a comment from Reviewer No. 2 (Comment #2).

- 5) It would indeed be a good idea to provide ‘the new fig 5’ as supplementary material and refer to it in the text. While your approach is plausible, like referee 1 most readers will be at first skeptic about setting SP endmembers based on these bulk measurements of soil emitted N<sub>2</sub>O. Fig. 5 clearly demonstrates that in fact using literature based SP endmembers yields unrealistic N<sub>2</sub>O source fraction calculations. This is an important point that does deserve a bit more attention in the discussion.

**Authors:** We have added the figure to the manuscript as Supplemental Information and have included a short discussion of its importance to Section 3.4 of the Discussion:

“As a check, the soil-specific approach presented here was compared to the independent endmember/slope approach commonly used by other researchers (Deppe et al., 2017; Lewicka-Szczebak et al., 2017). Isotopomer maps were calculated using independent literature-derived values (see Fig. S1) with the endmembers set at -2.4 to 34.4 for SP<sub>D</sub> to SP<sub>N</sub>, and 11.1 to 43.0 for  $\delta^{18}\text{O}_D$  to  $\delta^{18}\text{O}_N$ ; and a reduction slope of 0.33 (Lewicka-Szczebak et al., 2017). Using the literature-derived endmembers overestimated the contribution of denitrification-derived N<sub>2</sub>O under very dry soil conditions (i.e., 20 to 40% WFPS)—indicating that up to 40% of N<sub>2</sub>O produced under these conditions was a result of denitrification (Fig. S1)—a contradiction to common knowledge (Butterbach-Bahl et al., 2013; Davidson et al. 1991). In our case, N<sub>2</sub>O source partitioning using soil-specific endmembers provided an advantage over using independent endmembers because the endmembers for the Bradwell soil were different from the literature-derived values; likely due to real soil biological processes such as microbial communities, the low rate of production, or soil heterogeneity (Decock and Six, 2013a; Lewicka-Szczebak et al., 2014). Nonetheless, we recommend that future research aims to develop more advanced models that take into account variability or more nuanced isotope effects.” **(added at Page 10/Line 19)**

- 6) Fig. 4: Please clarify in the caption: are these observations from the three soils combined? I suspect so, but this is not immediately clear yet.

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**Authors:** You are correct in your assessment. We have revised the figure and its caption to indicate this more clearly. **(Page 10 of the revised manuscript)**

- 7) The first line of the conclusions section seems a bit redundant to me – belongs rather in an abstract – but the authors can decide upon this.

**Authors:** Whereas we can see your point, we prefer to leave this in the Conclusions section. We just feel that this helps drive home why we think it is important to investigate the source partitioning of N<sub>2</sub>O emissions.

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SOIL Discuss., <https://doi.org/10.5194/soil-2019-27-RC1>, 2019 © Author(s) 2019. This work is distributed under the Creative Commons Attribution 4.0 License.

### ***Interactive comment on “Revisiting the relationship between soil moisture and N<sub>2</sub>O production pathways by measuring <sup>15</sup>N<sub>2</sub>O isotopomers”***

#### **Anonymous Referee #1**

Received and published: 11 June 2019

SOIL Discuss., <https://doi.org/10.5194/soil-2019-27>

Revisiting the relationship between soil moisture and N<sub>2</sub>O production pathways by measuring <sup>15</sup>N<sub>2</sub>O isotopomers

#### **General comments**

The authors present a very nice and high quality dataset of N<sub>2</sub>O isotopomers from soil incubated over a gradient of moisture content. The study, however, has some major shortcomings in relating the dataset to the state of the art in N<sub>2</sub>O research. I encourage the authors to elaborate in 3 areas: 1) Latest approaches to interpret N<sub>2</sub>O isotopomer data 2) Consultation of literature on the effect of soil moisture on sources of N<sub>2</sub>O based on isotope tracer work 3) Literature on factors controlling N<sub>2</sub>O reduction With a more in depth analysis of the data and discussion of the results in relation to current literature, I believe this study can become a valuable and much appreciated contribution to the discipline.

**Authors:** Thank you for the comprehensive review. Revisions have been made to bolster references to previous literature, relating our work to previous N<sub>2</sub>O research.

The reviewer has no intentions of promoting or favoring own or colleagues' work in the comments. The cited literature is intended as a resource and starting point for a more in-depth literature search.

#### **Specific comments Title:**

- 1) P 1 The word 'revisiting' in the title implies to me that our understanding was wrong, but the isotopomers confirm what we already knew.

**Authors:** Both Reviewer#1 and #2 requested that the word 'revisiting' be changed. We have given this some thought and can see the reviewers' point; thus we have changed the title to:

“A new look at an old concept: Using <sup>15</sup>N<sub>2</sub>O isotopomers to understand the relationship between soil moisture and N<sub>2</sub>O production pathways”.

#### **Abstract:**

- 2) P 1 Line 14: the authors mention 'three soils'. I suggest adding a sentence explaining the difference between the three soils

**Authors:** The following statement has been added to the revised text (page 1/line 15):

“For each of three soils—differing in nutrient levels, organic matter and texture—soil microcosms were arranged . . .”

- 3) P 1 Line 24: I assume x is soil moisture in this equation. Please specify and explain to the reader the potential relevance or importance of these equations

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**Authors:** Revised to specify the equation variables for  $x$ ,  $F_N$ , and  $F_D$ . As well, we added the following sentence to explain the potential relevance:

“The presented equations may be helpful for other researchers to estimate  $N_2O$  source partitioning when soil moisture falls within the transition from nitrification to denitrification”. (added at page 1/line 25)

### **Introduction:**

- 4) P 2 Lines 3-4 and Lines 8-13: There are several studies that investigated the effect of soil moisture on mechanisms underlying  $N_2O$  emissions using  $^{15}N$  tracers. A few examples: - Stevens et al. 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry* 29: 139-151 - Bateman and Baggs 2005. Contributions of nitrification and denitrification to  $N_2O$  emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 41: 379-388

**Authors:** We have added a sentence acknowledging the use of  $^{15}N$  tracers:

“Indeed, our understanding of the relationship between  $N_2O$  production and soil moisture has benefited greatly from the use of  $^{15}N$  tracers (Bateman and Baggs, 2005; Stevens and Laughlin, 1997; Groffman et al., 2006).” (added at page 2/line 1)

However, the point we intended to make is that despite the advancements in understanding  $N_2O$  and soil moisture, the *precise* relationships remains fairly unclear, especially during the transition. To better convey this message, the text was revised to include the following statements:

“However, there remain surprising *grey-areas* in our understanding of the underlying mechanisms, one such area being the *precise* relationship between soil moisture and  $N_2O$  production pathways, *especially during the transition from one dominant pathway to another* (Bateman and Baggs 2005). (added at page 2/line 3)

and

“While previous research has provided important steps towards better quantifying the relationship using  $^{15}N$  enrichment and acetylene inhibition techniques (Bateman and Baggs 2005), natural abundance  $^{15}N$  techniques may provide superior information by imposing fewer confounding effects on step-wise N transformations.” (added at page 2/line 14)

- 5) P 2 Line 19: Early studies on the use of isotopomers appeared in the early years 2000 by Ostrom et al., Well et al., and Toyoda et al. Please cite key early studies on the use of  $N_2O$  isotopomers to source partition  $N_2O$ .

**Authors:** We do refer to seminal papers by Toyoda, Ostrom, and Sutka further down this paragraph, but we have now revised the first sentence of the paragraph to acknowledge this body of early work up front.

“...(Van Groenigen et al., 2015). Early work focused on the intramolecular distribution of  $^{15}N$  within the linear  $N_2O$  molecule (Sutka et al., 2006; Toyoda et al., 2005), investigations of atmospheric or oceanic  $N_2O$  isotopomers (Popp et al., 2002; Toyoda and Yoshida, 1999; Yoshida and Toyoda, 2000), and soil emitted  $N_2O$  isotopomers (Perez et al., 2001; Yamulki et al., 2001).” (added at page 2/line 22)

- 6) **Materials and methods:** P 4 Line 25 – P 5 Line 20: A number of studies have been published on how to interpret  $N_2O$  isotopomer data. Lewicka-Szczeback published an elegant method for calculating  $N_2O$  from nitrification, denitrification and the fraction of  $N_2O$  reduced to  $N_2$  based on SP and  $d_{18O}$  of  $N_2O$ . Details on the calculation approach can be found here. I recommend that the authors revise their calculation of the sources of  $N_2O$  based on more recently published approaches. [https://www.researchgate.net/publication/328135133\\_Mapping\\_approach\\_model\\_after\\_Lewicka-Szczeback\\_et\\_al\\_2017\\_-\\_detailed\\_description\\_of\\_calculation\\_procedures](https://www.researchgate.net/publication/328135133_Mapping_approach_model_after_Lewicka-Szczeback_et_al_2017_-_detailed_description_of_calculation_procedures)

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**Authors:** We confirmed that the calculation for the sources of N<sub>2</sub>O we performed were indeed the same approach as described by Lewicka-Szczebak. We cited the mixing model as described by Deppe et al. 2017, which is parallel to that used by Lewicka-Szczebak et al 2017. We now make reference to both papers that employed this approach. **(added at page 5/line 29)**

The mapping approach that we employed is that used by Deppe et al. (2017) and Lewicka-Szczebak et al. (2017) has been used before – but based on  $\delta^{15}\text{N}$  SP and  $\delta^{15}\text{N}$  bulk to estimate the fraction of bacterial N<sub>2</sub>O (Zou et al, 2014). As described by both Deppe et al (2017) and Lewicka-Szczebak et al (2017), they decided to base the mixing model on the relationship between  $\delta^{15}\text{N}$  SP and  $\delta^{18}\text{O}$  values (rather than bulk  $^{15}\text{N}$ ) for more robust interpretations; accordingly, we followed their recommendations.

- 7) The approach used by the authors has some major limitations, outlined below.

The authors use soil-specific end-members in their isotope mass balance, based on data from their experiment. While it cannot be excluded that isotope values characteristic of nitrification and denitrification are to some extent soil-dependent, the authors' approach relies on the assumption that at low moisture content, nitrification was the sole source of N<sub>2</sub>O, while denitrification was assumed to be the sole source of N<sub>2</sub>O at one of the medium range moisture contents. There is no independent measurement of the contribution of nitrification, denitrification and N<sub>2</sub>O reduction to N<sub>2</sub>. Limitations and assumptions of their approach need to be clearly stated.

**Authors:** As requested, we added an explanation, and acknowledge the underlying assumptions and limitations (see page 5/lines 19–29 of the revised manuscript):

“Rather than relying on average literature-derived endmembers like previous work (Deppe et al., 2017; Lewicka-Szczebak et al., 2017), we used soil-specific endmembers derived from our data to perform the linear mixed model. This is because we measured a wide range of soil WFPS treatments with high frequency between dry and moist conditions for each soil, enabling us to determine the point at which the  $\delta^{15}\text{N}$  SP or  $\delta^{18}\text{O}$  values either dropped or increased as soil WFPS changed – as precisely as the data permitted. This approach is consistent with earlier recommendations that data is collected at high enough frequencies to capture gradual changes in isotope values as influenced by traditional proxies (i.e., gradual changes in soil WFPS) (Decock and Six, 2013a). However, it must be noted that the underlying assumption is that the soil-specific endmembers are more reflective of transition from nitrification to denitrification in each of the soils tested herein, than general literature-derived endmembers would be for any one soil. Moreover, it is assumed that the endmembers represent N<sub>2</sub>O fluxes when the sole source was either nitrification or denitrification.” **(added at page 5/line 12)**

- 8) Whether end-members are likely soil-dependent was discussed in a literature review by Decock and Six 2013. How reliable is the intramolecular distribution of  $^{15}\text{N}$  in N<sub>2</sub>O to source partition N<sub>2</sub>O emitted from soil? Soil Biology and Biochemistry 65: 114- 127. Empirical studies since have further tested the effect of soil on end members, for example, Lewicka-Szczebak et al. 2014. Experimental determinations of isotopic fractionation factors associated with N<sub>2</sub>O production and reduction during denitrification in soils. Geochimica et Cosmochimica Acta 134:55–73. The results of the presented study should be discussed in relation to other studies published on this topic.

**Authors:** Revisions have been made to discuss this study in relation to others. We reference the review by Decock and Six (2013a and b), and Lewicka et al (2014):

“Despite similarities among soils in the robust patterns of how SP values are influenced by soil moisture (Fig. 2; Table 2), SP exhibited a significant ( $P < 0.0001$ ) soil  $\times$  moisture region interaction. This finding agrees with earlier suggestions that, at finer scales, the  $^{15}\text{N}_2\text{O}$  isotopic signatures and SP values are likely regulated by the active soil microbial community, process rates, soil heterogeneity (Decock and Six, 2013a; Lewicka-Szczebak et al., 2014).” **(added at page 8/line 17)**

- 9) The authors use the relationship between  $\delta^{18}\text{O}$  and SP at higher soil moisture content as the line representative of N<sub>2</sub>O reduction. It should be noted, however, that a N<sub>2</sub>O reduction line is only applicable if N<sub>2</sub>O reduction was the

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only process affecting N<sub>2</sub>O. In the presented experiment, N<sub>2</sub>O production and reduction likely occurred simultaneously. How simultaneous production and reduction of N<sub>2</sub>O affects isotope maps is discussed in great detail in Decock and Six. 2013. On the potential of d18O and d15N to assess N<sub>2</sub>O reduction to N<sub>2</sub>. European journal of soil science 64:610-620.

**Authors:** We have revised the text to address the reviewer's concerns; transparently identify the limitations of our study; and discuss our study in context to others:

“Previously, the fractionation of SP due to N<sub>2</sub>O reduction was constrained to a variation of -2‰ to -8‰ (Jinuntuya-Nortman et al., 2008; Lewicka-Szczebak et al., 2014; Well and Flessa, 2009). Ostrom et al. (2007) showed that ... “ (added at page 8/line 26)

“Reduction slopes for our three soils averaged 0.28, which is similar to the literature-derived average of 0.35 or 0.33 used by Deppe et al. (2017) and Lewicka-Szczebak et al. (2014), respectively . . . ” (added at page 8/line 34)

“This finding echoes earlier work which suggested that during soil conditions when processes of N<sub>2</sub>O production and reduction occur simultaneously, the reduction line approach may be limited (Decock and Six, 2013b).” (added at page 9/line 17)

### **Results and discussion**

- 10) P 6 Figure 2: It would be useful to see results of a statistical analysis on the effect of soil on N<sub>2</sub>O fluxes and isotopomer values across the moisture gradient. In addition, it would be interesting to see the fraction of N<sub>2</sub>O derived from nitrification, denitrification and N<sub>2</sub>O reduction for each soil over the moisture gradient, including statistical analysis.

**Authors:** First, we apologize for a miscommunication regarding N<sub>2</sub>O fluxes; i.e., we did not measure N<sub>2</sub>O fluxes; rather, we measured the total amount of N<sub>2</sub>O produced during the 24-h incubation. This has been made more clear in the revised text (Section 3.1; see response to comment #4 by Reviewer #2).

As for the Reviewer's second point, we are not entirely sure what is being asked for here. To evaluate the influence of soil moisture on N<sub>2</sub>O production and isotopomers, we analyzed the relationship between WFPS and SP or source fraction during key moisture gradients via linear regressions – see Figure 4 and Table 2. As such, the statistical analysis was approached in two ways: 1) linear regression to characterize N<sub>2</sub>O isotopic changes as influenced by moisture (see Table 2), and 2) by developing linear models within the transition zone to model the changes in N<sub>2</sub>O source fraction (Eq 6 & 7), see Fig 4. We believe the way we approached the analyses is best suited for our objectives of this study. To evaluate the influence of soil attributes on N<sub>2</sub>O and isotopomers, a future study could be designed and carried out to best test numerous different soil types and soil attributes on N<sub>2</sub>O and isotopomers.

- 11) P 7 Line 16-18: I agree that a greater contribution of N<sub>2</sub>O reduction is a likely explanation for the observed results. The approach by Lewicka-Szczebak would allow the authors to calculate the fraction of N<sub>2</sub>O reduced to N<sub>2</sub> based on the isotopomer data.

**Authors:** Thanks for the suggestion. We have adopted this approach and revised the text to reflect the outcome of the analysis:

“Correspondingly, using the mapping model approach, we estimated much larger fractions of N<sub>2</sub>O were reduced to N<sub>2</sub> at 95% WFPS in the Bradwell soil (0.47), compared to the Sutherland or Asquith soils (0.13 to 0.14).” (added at page 7/line 34)

- 12) P 7 Line 22: A lot of literature has been published on factors controlling complete denitrification. See for example - Butterbach-Bahl et al. 2013. Nitrous oxide emissions from soil: How well do we understand the processes and their controls? Phil. Trans. R. Soc. B 2013 368, 20130122, - Groffman et al. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16:2091–2122 - and references

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therein.

- 13) P 7 Line 29-32: It is very likely that multiple processes underlying N<sub>2</sub>O emissions acted simultaneously to cause a higher than expected SP value. It needs to be very clear from the discussion that there was no independent measurement of nitrification, denitrification and N<sub>2</sub>O reduction to N<sub>2</sub>. To avoid this confounding factor in data interpretation, I strongly recommend the authors to use end-members from the literature for data-interpretation. Various studies have reviewed and summarized data for such end-members, for example: - Decock and Six 2013. How reliable is the intramolecular distribution of <sup>15</sup>N in N<sub>2</sub>O to source partition N<sub>2</sub>O emitted from soil? Soil Biology and Biochemistry 65: 114-127; - Ostrom and Ostrom 2017. Mining the isotopic complexity of nitrous oxide: a review of challenges and opportunities. Biogeochemistry 132:359– 372; - Denk et al. 2017. The nitrogen cycle: A review of isotope effects and isotope modeling approaches. Soil Biology and Biochemistry 105:121-137.

**Authors:** The assumptions, explanations, and limitations are now more clearly described (see authors' response to Comment #7, above).

To check if data interpretation would be influenced by using the soil-specific approach vs the independent endmember/slope approach, isotopomer maps were also calculated using independent literature-derived values (see below Fig). Literature-derived endmembers were set at -2.4 to 34.4 for SP<sub>D</sub> to SP<sub>N</sub>, and 11.1 to 43.0 for  $\delta^{18}\text{O}_D$  to  $\delta^{18}\text{O}_N$ ; a reduction slope of 0.33 (Lewicka-Szczebak et al., 2017). For our study, using literature-derived endmembers was deemed inappropriate because it overestimated the contribution of denitrification-derived N<sub>2</sub>O under very dry soil conditions (i.e., 20 to 40% WFPS), to fractions of up to 40% of N<sub>2</sub>O produced (see figure below) – a result which is in contradiction to common knowledge (Davidson et al. 1991). In our case, N<sub>2</sub>O source partitioning using soil-specific endmembers provided an advantage over using independent endmembers because certain endmembers (Bradwell soil) were different from expected/literature-derived values; likely due to real soil biological processes such as microbial communities, the low rate of production, or soil heterogeneity (Decock and Six, 2013a; Lewicka-Szczebak et al., 2014). So, it makes more sense in our case to use soil-specific endmembers (especially because we have the isotopic data over a fine-scale WFPS change, enabling us to pinpoint soil-specific endmembers as best we can). We are transparent about the approach, the assumptions, and limitations (see revisions to page 5 which clearly explains that the endmembers are assumed to represent sole nitrification or denitrification). We are open to including the below figure and explanation as a Supplemental material if you deem it necessary, but please note that our objectives were not to compare different endmember approaches. Rather, our goal was to use a reasonable endmember approach to estimate source partitions, and evaluate how soil moisture affects N<sub>2</sub>O production. See attached Figure.

- 14) P8 Line 1 – P 9 Line 9: Here and elsewhere, please edit based on previous comments.

**Authors:** This section has been revised to reflect our response to the Reviewer's previous comments. In brief, these revisions have to do with placing our results in better context with the published literature. (See tracked changes in accompanying revised manuscript.)

- 15) P 9 Line 10-27: This is an interesting analysis. I am interested to see models relating soil moisture to sources of N<sub>2</sub>O based on updated source calculations in line with the most recent literature. Based on the raw isotope data, I suspect a significant moisture by soil interaction with respect to sources of N<sub>2</sub>O. Statistical tests for such an interaction should be shown. Such an interaction may also have implications for the modeling approach in section 3.4 of this paper.

**Authors:** See response to Comment #8 above in which we added a statement showing indicating that site preference exhibited a significant ( $P < 0.0001$ ) soil  $\times$  moisture region interaction. This provides additional support for our decision to use site-specific endmembers to calculate the source fractions attributable to nitrification and denitrification in our modeling. Further, the site-specific end-member approach effectively normalizes the source partitions among soils, enabling data to be pooled for the modeling approach (Fig 4). Refer to discussion under Comment 13.

- 16) P 9 Line 27-28: Please refer to isotope tracer work, as suggested earlier.



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**Authors:** The text was revised to reference isotope tracer work in response to Comment # 4 above; however, we do not feel that it needs to be repeated here. Our purpose was to determine whether we could use  $^{15}\text{N}$  isotopomers—i.e., natural abundance, not  $^{15}\text{N}$  tracers—to better elucidate the relationship between soil moisture and  $\text{N}_2\text{O}$  production pathways. Nevertheless, we have revised the text in this section to read:

“Our results largely support the foundational studies that established the relationship between soil moisture and  $\text{N}_2\text{O}$  emissions (Davidson, 1991; Linn and Doran, 1984); however, we provide a method that moves beyond just inferring  $\text{N}_2\text{O}$  source pathways towards quantifying the pathway contributions over a range of soil moisture—and does so without having to add a  $^{15}\text{N}$  label.”

**(underlined text added at page 11/line 10)**

### 17) **Conclusion**

Please edit commensurate with previous comments.

**Authors:** The conclusions have been revised to reflect previous comments.

### 18) **Technical corrections**

None observed.

### **Anonymous Referee #2**

Received and published: 7 July 2019

Overall the data set is very interesting and will be of interest to readers.

- 1) The title uses the word 'revisiting'. While the introductory text notes the relationship between nitrification and denitrification processes with respect to soil moisture there is no prior evidence/studies introduced with respect to isotopomers and soil moisture. Thus the title may require suitable amendment or the introduction requires some additional information.

**Authors:** Text has been added to the introduction to make reference to earlier studies that have evaluated the relationship between moisture and  $\text{N}_2\text{O}$ , and which have used  $^{15}\text{N}$  tracer techniques. No previous studies that we are aware of have used isotopomers to look at this relationship. We have modified the title to better convey our meaning:

“A new look at an old concept: Using  $^{15}\text{N}_2\text{O}$  isotopomers to understand the relationship between soil moisture and  $\text{N}_2\text{O}$  production pathways”.

- 2) The introduction is nicely succinct and clear with respect to the problems associated with emissions of  $\text{N}_2\text{O}$ , the role of soil moisture as a driver of  $\text{N}_2\text{O}$  emissions, and the basics of isotopomers of  $\text{N}_2\text{O}$  as linked to nitrification and denitrification. A reference for terminology used would be good.

**Authors:** we added a citation after the terminology reference, see page 3/line 8 (Ostrom and Ostrom 2012).

- 3) In the materials and methods sections: - were the soils sieved? I assume so seeing as they were placed in Petri dishes, thus what was the mesh size? - what was the randomised block design? There are 3 soils and 4 replicates but how many water treatments (WFPS treatments) and what were they? It appears looking at Fig 2 that there are about 16 WFPS treatments

**Authors:** yes the soils were sieved using a 2-mm mesh screen; this information has been added to the revised text (page 4/line 5). The final moisture range (40 to 105% WFPS) was based on data collected during a preliminary test in which soil moisture was varied from 10% to 105%. A lack of  $\text{N}_2\text{O}$  production when WFPS was <40% led us to limit the moisture range for the final study. The number of moisture treatments within the overall range varied depending on soil texture (21 for the Sutherland soil; 17 for the Asquith soil; and 16 for the Bradwell soil). We can add this information to the text, or add a supplemental table that lists the individual moisture treatments



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for each soil (i.e., gravimetric soil water content and WFPS).

The soil microcosms were arranged using a completely randomized design (CRD) with four replicates. This information was **added at page 3/line 32**.

- 4) Note how N<sub>2</sub>O fluxes were determined. Assume it was just the one gas sample used, so there are I assume assumptions about linearity.

**Authors:** We apologize for the miscommunication. We did not measure N<sub>2</sub>O fluxes; rather, we measured the total amount of N<sub>2</sub>O produced during the 24-h incubation. The text has been revised (Section 3.1) to reflect this fact. We have changed the y-axis title in Figure 2 from “ng g<sup>-1</sup> d<sup>-1</sup>” to “ng g<sup>-1</sup> 24-h<sup>-1</sup>”; and have changed “N<sub>2</sub>O flux” to “N<sub>2</sub>O production” throughout the manuscript.

- 5) For the Picarro CRDS is there a maximum/minimum N<sub>2</sub>O concentration? Were SP effects constant over a range of concentration?

**Authors:** The minimum detectable N<sub>2</sub>O concentration of the G5131-*i* (ca. 0.05 ppb) is well below any of the concentrations we measured during the study (the minimum concentrations being at ambient levels). The maximum concentration we can measure without difficulty is about 1000 ppb; consequently, any sample determined to exceed this concentration (based on the GC analyses) was diluted with ultra-pure zero-air to a concentration of approximately 300 ppb for <sup>15</sup>N<sub>2</sub>O isotopomer analysis. As well, during earlier testing of the instrument performance it was determined that site preference was independent of gas concentration. (Note: instrument performance is being described in a separate manuscript that is still in the works.)

- 6) The results and discussion are well considered and it is good the authors have considered N<sub>2</sub>O reduction effects on SP and interpreted results accordingly. For clarity, in the figure captions please state if the data presented are means or single points etc.

**Authors:** The data presented in Fig 2 are the means (n = 4) means and bars represent the standard error of the mean. This information has been added to the figure caption (**page 6**). Likewise, the data presented in Fig. 3 are the mean site preference values; this information has been added to the figure caption (**page 9**).

# A new look at an old concept: Using $^{15}\text{N}_2\text{O}$ isotopomers to understand the relationship between soil moisture and $\text{N}_2\text{O}$ production pathways

Kate A. Congreves<sup>1</sup>, Trang Phan<sup>1</sup>, and Richard E. Farrell<sup>2</sup>

Deleted: Revisiting the relationship between soil moisture and  $\text{N}_2\text{O}$  production pathways by measuring  $^{15}\text{N}_2\text{O}$  isotopomers

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## Abstract

Understanding the production pathways of potent greenhouse gases, such as nitrous oxide ( $\text{N}_2\text{O}$ ), is essential for accurate flux prediction and for developing effective adaptation and mitigation strategies in response to climate change. Yet, there remain surprising gaps in our understanding and precise quantification of the underlying production pathways – such as the relationship between soil moisture and  $\text{N}_2\text{O}$  production pathways. A powerful, but arguably underutilized, approach for quantifying the relative contribution of nitrification and denitrification to  $\text{N}_2\text{O}$  production involves determining  $^{15}\text{N}_2\text{O}$  isotopomers and  $^{15}\text{N}$  site preference (SP) via spectroscopic techniques. Using one such technique, we conducted a short-term incubation where  $\text{N}_2\text{O}$  production and  $^{15}\text{N}_2\text{O}$  isotopomers were measured 24-h after soil moisture treatments of 40 to 105% water-filled pore space (WFPS) were established – for each of three soils that differed in nutrient levels, organic matter and texture. Relatively low  $\text{N}_2\text{O}$  fluxes and high SP values indicated nitrification during dry soil conditions, whereas at higher soil moisture, peak  $\text{N}_2\text{O}$  emissions coincided with a sharp decline in SP indicating denitrification. This pattern supports the classic  $\text{N}_2\text{O}$  production curves from nitrification and denitrification as inferred by earlier research; however, our isotopomer data enabled the quantification of source partitioning for either pathway. At soil moisture levels < 53% WFPS, the fraction of  $\text{N}_2\text{O}$  attributed to nitrification ( $F_N$ ) predominated but thereafter decreased rapidly with increasing soil moisture ( $x$ ), according to:  $F_N = 3.19 - 0.041x$ , until a WFPS of 78% was reached. Simultaneously, from WFPS of 53 to 78%, the fraction of  $\text{N}_2\text{O}$  that was attributed to denitrification ( $F_D$ ) was modelled as:  $F_D = -2.19 + 0.041x$ ; at moisture levels of > 78%, denitrification completely dominated. Clearly, the soil moisture level during transition is a key regulator of  $\text{N}_2\text{O}$  production pathways. The presented equations may be helpful for other researchers to estimate  $\text{N}_2\text{O}$  source partitioning when soil moisture falls within the transition from nitrification to denitrification.

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## 1. Introduction

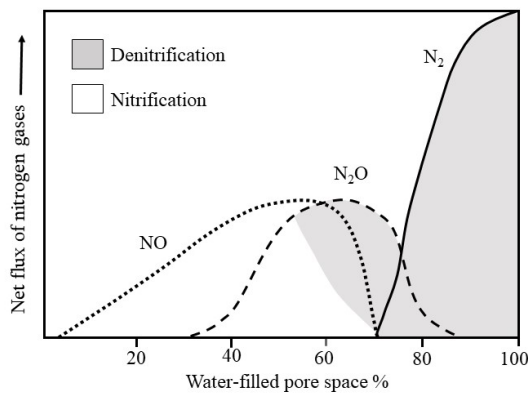
Soils are the largest source of anthropogenic  $\text{N}_2\text{O}$  emissions, accounting for up to 80% of total  $\text{N}_2\text{O}$  emissions (Environment Canada, 2015). Understanding the mechanisms leading to the emission of this potent greenhouse gas is essential for accurate flux prediction and for developing effective adaptation and mitigation strategies in response to climate change. Decades of research have strengthened our understanding of  $\text{N}_2\text{O}$  fluxes—namely, how  $\text{N}_2\text{O}$  production is regulated by soil oxygen, substrate availability, and microbial activity (Butterbach-Bahl et al., 2013; Chapuis-Lardy et al., 2007; Wagner-Riddle et al., 2017); as well as how  $\text{N}_2\text{O}$  emission is regulated by advection,

solubility and diffusion (Balaine et al., 2013; Clough et al., 2005). ~~Indeed, our understanding of the relationship~~ ~~between N<sub>2</sub>O production and soil moisture has benefited greatly from the use of <sup>15</sup>N tracers~~ (Bateman and Baggs, 2005; Stevens and Laughlin, 1997; Groffman et al., 2006). However, there remain surprising ~~grey-areas~~ in our understanding of the underlying mechanisms, one such area being the ~~precise~~ relationship between soil moisture and N<sub>2</sub>O production pathways, ~~especially during the transition from one dominant pathway to another~~ (Bateman and Baggs, 2005).

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Nitrous oxide is a product of nitrification and denitrification—microbially driven processes that depend on the aeration status of the soil (Banerjee et al., 2016; Barnard et al., 2005). As a result, the relative contributions of nitrification and denitrification are often determined based on their relationship to soil water-filled-pore space (WFPS), which acts as a proxy for aeration status. However, the widely cited relationship between soil N<sub>2</sub>O production and soil moisture (Fig. 1) is actually an educated deduction that blends work from two different studies, from which the N<sub>2</sub>O production pathways are inferred (Davidson, 1991; Linn and Doran, 1984). As such, it may be argued that the precise relationship between soil water content and N<sub>2</sub>O production mechanisms remains ~~unclear~~ and requires more complete quantification. ~~While previous research has provided important steps towards better quantifying the relationship using <sup>15</sup>N enrichment and acetylene inhibition techniques (Bateman and Baggs, 2005), natural abundance <sup>15</sup>N techniques may provide superior information by imposing fewer confounding effects on step-wise N transformations.~~

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**Figure 1** Relative contributions of nitrification and denitrification processes to N<sub>2</sub>O production as a function of water-filled pore space (adapted from Davidson 1991).

Isotopomers—i.e., isomers having the same number of each isotopic atom, but differing in their position (McNaught and Wilkinson, 1997; Ostrom and Ostrom, 2012)—provide a powerful and novel approach for quantifying the relative contribution of N<sub>2</sub>O producing processes via nitrification and denitrification (Van Groenigen et al., 2015). ~~Early work focused on the intramolecular distribution of <sup>15</sup>N within the linear N<sub>2</sub>O molecule~~ (Sutka et al., 2006; Toyoda et al.,

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2005), investigations of atmospheric or oceanic N<sub>2</sub>O isotopomers (Popp et al., 2002; Toyoda and Yoshida, 1999; Yoshida and Toyoda, 2000), and soil emitted N<sub>2</sub>O isotopomers (Perez et al., 2001; Yamulki et al., 2001). The isotopomers of N<sub>2</sub>O (i.e., <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O and <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O) can be quantified using advanced laser spectroscopic approaches—including cavity ring-down spectroscopy (CRDS)—that enable the intramolecular <sup>15</sup>N distribution of N<sub>2</sub>O to be determined (Mohn et al., 2014). The difference between the abundance of <sup>15</sup>N within the central (alpha, α) and the terminal (beta, β) N atoms of the linear N<sub>2</sub>O molecule is expressed as site-preference (SP), and high SP values of 13 to 37‰ are attributed to nitrification (hydroxylamine oxidation) while SP values of 0‰ or less indicate nitrite or nitrate reduction (denitrification and nitrifier denitrification) (Denk et al., 2017; Ostrom et al., 2010; Sutka et al., 2006; Toyoda et al., 2005). The underlying reason for the distinct differences in SP values of N<sub>2</sub>O from either microbial pathway is due to primary kinetic isotope effects when N<sub>2</sub>O is produced (Popp et al., 2002).

Our objective was to use <sup>15</sup>N<sub>2</sub>O isotopomers to precisely quantify the relationship between soil moisture and N<sub>2</sub>O production in soils differing in soil nutrient level, organic matter, and texture.

## 2. Materials and Methods

### 2.1 Soil collection and characterization

Surface (0–10 cm) soils representing different nutrient levels and texture classes were collected from three locations in the Dark Brown soil zone in Saskatchewan, Canada. The soils—classified as Dark Brown Chernozems of the Sutherland, Asquith and Bradwell associations—were collected using a shovel, air dried and sub-samples were shipped to A&L Laboratories Inc (London, ON) for analysis (Table 1). For additional characterization, sub-samples were analyzed at the University of Saskatchewan for equilibrium soil water content, soil inorganic N levels, soil total N concentration and <sup>15</sup>N abundance (Table 1). The equilibrium soil water was determined via the long-column method based on the average of four technical replicates (Reynolds and Topp, 2007). Initial soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were determined in quadruplicate using the KCl extraction method of Maynard et al. (2007); briefly, 5 g soil was mixed with 50 mL of 2 M KCl, shaken for 30-min, filtered through Whatman 42 filter paper, and the extracts frozen at -20°C until they could be analyzed. For analysis, the extracts were thawed and allowed to equilibrate to room temperature before being analyzed using air segmented (continuous) flow analysis with a SEAL AA3 HR chemistry analyzer (SEAL Analytical; Kitchener, ON). Soil total N concentration (%) and <sup>15</sup>N content (atom%) were determined in duplicate using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia CA) coupled to a high-precision Delta V mass spectrometer (Bremen, Germany) with a precision of 0.06‰ for δ<sup>15</sup>N. Chickpea flour with an atom% <sup>15</sup>N = 0.3691 was used as a lab reference.

### 2.2 Incubation experimental design

For the incubation study, soil microcosms were established over a range of moisture treatments for each soil, and arranged in a completely randomized design with four replicates. For each microcosm, sieved (2-mm mesh screen) and air-dried soil was packed into a small (5.9 cm i.d. × 0.80 cm tall) plastic petri dish. The mass of soil needed to fill the petri dish varied with texture—ranging from 22.0 g to 29.0 g—and yielded soil bulk densities of 1.01, 1.10, and

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1.33 g cm<sup>-3</sup> for the Sutherland, Asquith, and Bradwell soils, respectively. While the quantities and bulk densities differed for each soil type, it was essential that the soil completely fill the petri dishes to avoid any differences in soil surface boundary layer or gas diffusion that would alter N<sub>2</sub>O emission.

5 Soil moisture treatments were based on gravimetric soil water content ( $\theta_g$ ) established by adding deionized water to the soil microcosms, using a fine mist of water applied from a manual spray bottle, to a predetermined weight. Gravimetric soil moisture content was varied to yield a water-filled pore space (WFPS) between 40 and 105%.

The gravimetric water, volumetric water, and WFPS were determined according to Eq. (1-3):

$$\text{Gravimetric water } \theta_g (\text{g H}_2\text{O g soil}^{-1}) = \frac{\text{water added (g)}}{\text{dry soil (g)}} \quad (1)$$

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$$\text{Volumetric water } \theta_v (\text{cm}^3 \text{H}_2\text{O cm}^3 \text{soil}^{-1}) = \theta_g \times \text{BD} \quad (2)$$

$$\% \text{ WFPS} = \left[ \frac{\theta_v}{\left(1 - \frac{\text{BD}}{\text{PD}}\right)} \right] \times 100 \quad (3)$$

15 where, BD denotes soil bulk density and PD denotes particle density (PD), which was assumed to be 2.65 g cm<sup>-3</sup>.

Immediately after moistening the soil microcosm, the petri dish was sealed inside a 1L wide-mouth mason jar fitted with a gas sampling septum, and time of sealing was recorded. Blank jars containing an empty petri dish were set up to account for background (atmospheric) gas concentrations. The microcosms were incubated at 22°C ± 1°C for 24-h.

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### 2.3 Sampling and analysis

After 24-h, a headspace gas sample was collected from each microcosm (with the time of sampling recorded) using a 20-mL plastic syringe fitted with a 22-gauge needle, injected into an evacuated 12-mL Exetainer<sup>®</sup> tube (Labco Limited, UK), and analyzed for N<sub>2</sub>O, CO<sub>2</sub>, and O<sub>2</sub> concentration using gas chromatography (Bruker 450 GC, Bruker Biosciences, Billerica, MA). Immediately thereafter, a separate 30-mL gas sample was collected from each microcosm, injected into an evacuated 12-mL Exetainer<sup>®</sup> tube, and analyzed for <sup>15</sup>N<sub>2</sub>O concentration,  $\delta^{15}\text{N}_\alpha$ ,  $\delta^{15}\text{N}_\beta$ , and  $\delta^{18}\text{O}$  using a CRDS-based Picarro G5131-*i* isotopic N<sub>2</sub>O analyzer (Picarro Inc.; Santa Clara, CA).

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### 2.4 Isotopomer approach using <sup>15</sup>N site preference and $\delta^{18}\text{O}$ for N<sub>2</sub>O source identification

Site preference was calculated by subtracting the abundance of <sup>15</sup>N from the terminal N atom (beta,  $\beta$ ) from that of the central (alpha,  $\alpha$ ) N atom. The fraction of N<sub>2</sub>O derived from hydroxylamine oxidation during nitrification (F<sub>N</sub>) or the reduction of nitrate or nitrite during denitrification (F<sub>D</sub>) was estimated for each soil by adopting the isotopomer mixing approach used by others (Deppe et al., 2017; Lewicka-Szczebak et al., 2017; Zou et al., 2014), and which use

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the  $\delta^{15}\text{N}$  SP and  $\delta^{18}\text{O}$  values of gas samples collected from the soils. As suggested by Lewicka-Szczebak et al. (2017) and by Well et al. (2012), and because SP was more closely correlated to  $\delta^{18}\text{O}$  ( $r = 0.906$ ) than  $\delta^{15}\text{N}$  ( $r = 0.849$ ), we used the relationship between  $\delta^{15}\text{N}$  SP and  $\delta^{18}\text{O}$ , instead of  $\delta^{15}\text{N}$  SP and bulk  $\delta^{15}\text{N}$ . Equations (4) and (5) show the source partitioning calculations.

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$$F_N = \frac{SP_N - SP_D}{SP_N - SP_D} \quad (4)$$

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$$F_D = 1 - F_N \quad (5)$$

10 where  $F_N$  and  $F_D$  indicate the fraction of  $\text{N}_2\text{O}$  derived from nitrification or denitrification, respectively. SP denotes the site preference for the sample ( $SP_x$ ) and the endmembers for nitrification ( $SP_N$ ) and denitrification ( $SP_D$ ).

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Rather than relying on average literature-derived endmembers like previous work (Deppe et al., 2017; Lewicka-Szczebak et al., 2017), we used soil-specific endmembers derived from our data to perform the linear mixed model.

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15 This is because we measured a wide range of soil WFPS treatments with high frequency between dry and moist conditions for each soil, enabling us to determine the point at which the  $\delta^{15}\text{N}$  SP or  $\delta^{18}\text{O}$  values either dropped or increased as soil WFPS changed (as precisely as the data permitted) indicating a transition from nitrification to denitrification. This approach is consistent with earlier recommendations that data be collected at high enough frequencies to capture gradual changes in isotope values as influenced by traditional proxies (i.e., gradual changes in soil WFPS) (Decock and Six, 2013a). However, it must be noted that the underlying assumption is that the soil-specific

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20 endmembers are more reflective of the transition from nitrification to denitrification in each of the soils tested herein, than general literature-derived endmembers would be for any one soil. Moreover, it is assumed that the endmembers represent  $\text{N}_2\text{O}$  produced when the sole source was either nitrification or denitrification. Endmembers for  $SP_D$  to  $SP_N$

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were set at: 2.0 to 23.7; 0.7 to 21.7; 14.4 to 23.3 for the Sutherland, Asquith, and Bradwell soils, respectively. Endmembers for  $\delta^{18}\text{O}_D$  to  $\delta^{18}\text{O}_N$  were set at: 16.0 to 35.1; 18.8 to 39.5; 25.4 to 34.2 for the Sutherland, Asquith, and Bradwell soils, respectively. The endmember ranges were based on our data where  $SP_N/\delta^{18}\text{O}_N$  represented the average values before the transition zone from nitrification to denitrification-dominated  $\text{N}_2\text{O}$  production;  $SP_D/\delta^{18}\text{O}_D$  represented the lowest values during denitrification for each soil type. For source partitioning, the influence of  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  on SP was taken into account by using the reduction and mixing line intercept approach – as described by Deppe et al. (2017) and Lewicka-Szczebak et al. (2017). However, rather than using an estimated reduction line

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30 derived from the literature, we calculated the slope and intercept for the reduction line based on our data: the  $SP/\delta^{18}\text{O}$  plot for the soil moisture range after the transition zone for each soil type. The reduction line was placed through the average SP value of gas samples derived from < 60% water-filled pore space range for each soil. The point of intersection between the endmember mixing line and the reduction line gave the estimated initial isotope values ( $SP^*$ ,  $^{18}\text{O}$ ) of produced  $\text{N}_2\text{O}$  before reduction to  $\text{N}_2$ . In the soil moisture range after the transition from nitrification to denitrification, if the  $SP^*$  value was higher than the measured SP value of the gas sample, the measured SP value was used since  $\text{N}_2\text{O}$  reduction was assumed to be negligible. The  $F_N$  and  $F_D$  were then calculated from SP values (or  $SP^*$ )

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and the SP values of the nitrification and denitrification endmembers. This calculation was done for each soil type separately.

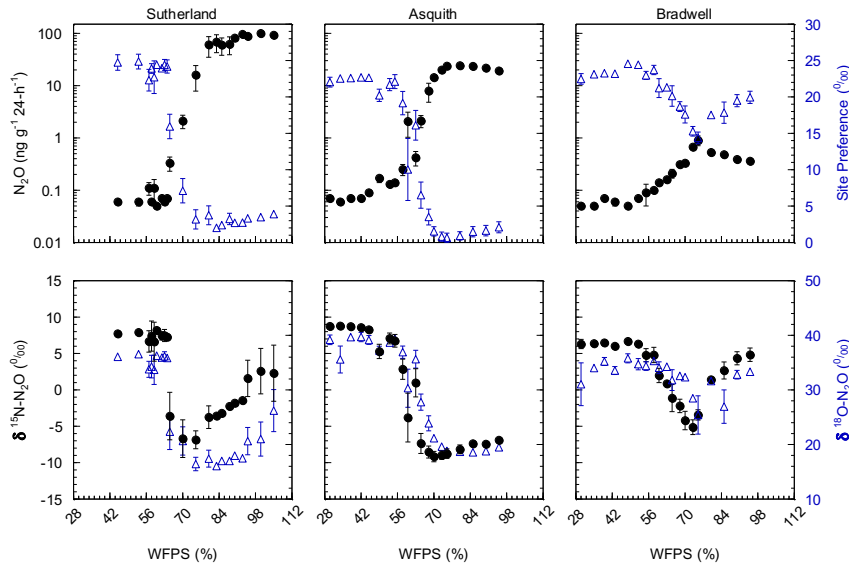
## 2.5 Statistical analysis

Correlation and linear regression analyses were conducted in CoStat (CoStat 6.451, CoHort.com) to determine associations between soil moisture and SP.

## 3. Results and Discussion

### 3.1 Nitrous oxide production

Nitrous oxide production during the 24-h incubation varied dramatically among the three soils, with peak  $N_2O$  production occurring at soil water contents equivalent to 70–80% WFPS (Fig. 2). Peak  $N_2O$  production was 100-fold greater from the Sutherland soil ( $100 \text{ ng } N_2O\text{-N } g^{-1} \text{ 24-h}^{-1}$ ) compared to the Bradwell soil ( $1 \text{ ng } N_2O\text{-N } g^{-1} \text{ 24-h}^{-1}$ ), and about 4-fold greater than that from the Asquith soil ( $24 \text{ ng } N_2O\text{-N } g^{-1} \text{ 24-h}^{-1}$ ) (Fig. 2). This differentiation follows the same trend as soil inorganic N availability and soil organic matter, which decreased in the order: Sutherland > Asquith > Bradwell (Table 1).



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15 Figure 2 Top panels:  $N_2O$  production as influenced by soil water filled pore space (WFPS), (black ink, left axis); corresponding  $^{15}N_2O$  isotopomer site preference (SP), (blue ink, right axis). Bottom panels:  $\delta^{15}N$  (black ink, left axis) and  $\delta^{18}O$  (blue ink, right axis) of emitted  $N_2O$  as influenced by soil water filled pore space (WFPS). Note:  $N_2O$  emissions were plotted on a  $\log_{10}$  scale to accommodate the large range in emissions from the different soils. Data points represent means ( $n = 4$ ) and bars represent the standard errors.



Regardless of the amount of N<sub>2</sub>O evolved, there were similarities in how soil moisture influenced relative N<sub>2</sub>O production. For all soil types, relatively low N<sub>2</sub>O fluxes were associated with drier soil treatments; N<sub>2</sub>O fluxes were incrementally magnified as soil moisture levels increased from about 55 to 80% WFPS (Fig. 2, top panels). At soil moisture levels exceeding ~80% WFPS, fluxes either remained relatively high, as was the case for the Sutherland soil, or decreased slightly, as was observed for the Asquith and Bradwell soils.

### 3.2 Nitrous oxide <sup>15</sup>N site preference, δ<sup>15</sup>N and δ<sup>18</sup>O

Not only total N<sub>2</sub>O concentration, but the <sup>15</sup>N SP, δ<sup>15</sup>N and δ<sup>18</sup>O of N<sub>2</sub>O changed with soil moisture level, and in parallel with each other (Fig. 2, bottom panels). We identified three moisture ranges—differing slightly for each soil (Table 2)—that regulated N<sub>2</sub>O production pathways based on distinct SP, δ<sup>15</sup>N and δ<sup>18</sup>O values (Fig. 2).

For each soil, the δ<sup>15</sup>N and δ<sup>18</sup>O values decreased in the same soil moisture region in which the SP values decreased (Fig. 2, bottom panels). Based on the patterns for N<sub>2</sub>O fluxes, SP, δ<sup>15</sup>N and δ<sup>18</sup>O values as related to soil moisture (Fig. 2; Table 2), our results visually indicate there was a transition between nitrification-derived and denitrification-derived N<sub>2</sub>O production at between 64 and 83; 58 and 75; 63 and 75% WFPS for the Sutherland, Asquith, and Bradwell soils, respectively.

Prior to the transition in N<sub>2</sub>O production pathway, when the soil was relatively dry, the SP values averaged 23.7, 23.3, and 21.7‰ from the Sutherland, Asquith, and Bradwell soils, respectively. These values are in line with expected SP values attributed to nitrification (Denk et al., 2017; Ostrom et al., 2010; Sutka et al., 2006; Toyoda et al., 2005). Furthermore, the observed consistency among soil types – and the negligible (near 0) slopes between WFPS and <sup>15</sup>N SP – suggests that average SPs during nitrification are relatively insensitive to the rate of production or associated N<sub>2</sub>O accumulation. It is known that isotopic fractionation governed by kinetic isotope effects occurs during the reaction sequence NH<sub>4</sub><sup>+</sup> → NH<sub>2</sub>OH → NOH → NO → N<sub>2</sub>O and NH<sub>4</sub><sup>+</sup> → NO<sub>2</sub> → NO → N<sub>2</sub>O; however, oxidation of NOH does not involve a primary kinetic isotope effect and thus should not markedly affect SP (Popp et al., 2002).

During the transition from nitrification to denitrification, SP declined rapidly in all soils (Fig. 2, Table 2). The lowest SP values were 2.0, 0.7, and 14.4‰ for the Sutherland, Asquith, and Bradwell soils, respectively. In general, sharp slopes characterized the decline in SP values with increasing soil moisture during the transition; but the Sutherland and Asquith soils had steeper slopes than the Bradwell soil (Table 2). This difference was likely related to differences in soil inorganic or mineralizable N availability (Table 1) and possibly to differences in the rates of denitrification.

After the transition to denitrification, the SP values increased slightly as soil moisture increased (Table 2)—but more so for the Bradwell soil than the Sutherland and Asquith soils. This finding supports the sensitivity of SP values to the degree of stepwise completion of denitrification (N<sub>2</sub>O reduction to N<sub>2</sub>). We hypothesize that the ratio of N<sub>2</sub>O produced to N<sub>2</sub>O reduced was lowest for the Bradwell soil. Contrary to the large accrual of N<sub>2</sub>O from the Sutherland and Asquith soils, the low concentration of N<sub>2</sub>O produced from the Bradwell soil likely favoured complete reduction (i.e., tighter ‘holes-in-the-pipe’)—causing the Bradwell soil SP values to be the most sensitive to reduction of N<sub>2</sub>O after the transition to denitrification (Fig. 2, Table 2). Correspondingly, using the mapping model approach to calculate the fraction of denitrified-N<sub>2</sub>O reduced to N<sub>2</sub> (Lewicka-Szczepak et al., 2017) we estimated much larger fractions of N<sub>2</sub>O

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were reduced to N<sub>2</sub> at 95% WFPS in the Bradwell soil (0.47) compared to the Sutherland or Asquith soils (0.13 to 0.14). The greater amounts of N<sub>2</sub>O produced by the nutrient rich Sutherland and Asquith soils may have overwhelmed any reduction effect on the SP of N<sub>2</sub>O. Our findings attribute 'N<sub>2</sub>O leaky' soils to excess inorganic N or mineralization potential.

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### 5 3.3 The 'hole-in-the-pipe' influences site preference

As alluded to above, the Bradwell results were most dissimilar to the other soils. It is intriguing that the SP values for the Bradwell soil N<sub>2</sub>O never dropped below 14.4‰. While it is clear from the pattern of N<sub>2</sub>O fluxes, SP, δ<sup>15</sup>N and δ<sup>18</sup>O values (Fig. 2) that N<sub>2</sub>O production transitioned to denitrification as the soil water content was increased (Table 2), it is curious that the SP values were not lower (i.e., closer to 0‰) as earlier work demonstrated for denitrification (Denk et al., 2017; Ostrom et al., 2010; Sutka et al., 2006; Toyoda et al., 2005; Winther et al., 2018). Reasons for this discrepancy are as yet unclear, but we are not alone in finding SP values above 0‰ that are attributed to denitrification (Winther et al., 2018). Differences might be related to differences in microbial community structure and activity, as suggested by Decock and Six (2013a). Also, it is very likely that multiple processes underlying N<sub>2</sub>O production and consumption acted simultaneously to cause a higher than expected SP value (Decock and Six, 2013a). Otherwise, N<sub>2</sub>O reduction to N<sub>2</sub> might have played a larger than anticipated role for the Bradwell soil. Indeed, SP values within the expected range for bacterial denitrification are known to be sensitive to the reduction of N<sub>2</sub>O to N<sub>2</sub> (Deppe et al., 2017; Jinuntuya-Nortman et al., 2008; Lewicka-Szczepak et al., 2014; Ostrom et al., 2007; Well and Flessa, 2009). Despite similarities among soils in the robust patterns of how SP values are influenced by soil moisture (Fig. 2; Table 2), SP exhibited a significant ( $P < 0.0001$ ) soil × moisture region interaction. This finding agrees with earlier suggestions that, at finer scales, the <sup>15</sup>N<sub>2</sub>O isotopic signatures and SP values are likely regulated by the active soil microbial community, process rates, soil heterogeneity (Decock and Six, 2013a; Lewicka-Szczepak et al., 2014). Denitrification results in cleavage of the covalent bond between the central N and O in N<sub>2</sub>O, and based on kinetic isotope fractionation results in an increase in the <sup>15</sup>N content of the α position of the residual N<sub>2</sub>O, thereby increasing the SP (Popp et al., 2002; Ostrom et al., 2007). Thus, the increase in SP in response to N<sub>2</sub>O reduction results in a small (but important) shift away from the SP values associated with the origins of denitrification (~ 0‰) towards those of nitrification, i.e., 33‰ (Sutka et al., 2006). Previously, the fractionation of SP due to N<sub>2</sub>O reduction was constrained to a variation of -2‰ to -8‰ (Jinuntuya-Nortman et al., 2008; Lewicka-Szczepak et al., 2014; Well and Flessa, 2009). Ostrom et al. (2007) showed that the rate of reduction must be substantially greater than 10% that of production to impact the SP estimates of N<sub>2</sub>O from denitrification by more than a few percent. Because it is likely that N<sub>2</sub>O consumption was greater than production for the Bradwell soil when soil moisture exceeded 75% WFPS, our results indicate that the 'size of the hole-in-the-pipe' may influence denitrification SP to a greater extent than previously documented.

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For N<sub>2</sub>O source identification, we adopted an isotopomer mixing approach (Deppe et al., 2017; Lewicka-Szczepak et al., 2017; Zou et al., 2014) and constructed isotopomer maps (i.e., plots of SP vs. δ<sup>18</sup>O). This approach allowed us to estimate the impact of N<sub>2</sub>O reduction to N<sub>2</sub> on SP. Reduction slopes for our three soils averaged 0.28, which is similar to the literature-derived average of 0.35 or 0.33 used by Deppe et al. (2017) and Lewicka-Szczepak et al. (2017), respectively, though they varied over a wide range; i.e., from 0.16 to 0.52 (Fig. 3). A high reduction slope, such as

observed for the Bradwell soil, might be associated with the magnitude of N<sub>2</sub>O production relative to potential nitrous oxide reductase activity, or conditions that favour more complete stepwise reduction of N<sub>2</sub>O to N<sub>2</sub>. Whereas the reduction effect on SP might be stronger than previously thought, it may only be observable when conditions are favourable, as evidenced for the Bradwell soil. We echo earlier proposals made by Ostrom et al. (2007), and suggest that the current knowledge and understanding of <sup>15</sup>N<sub>2</sub>O isotopomers may have inherent biases due to methodological focus on high flux scenarios – where the rates of N<sub>2</sub>O reduction are minor and likely not of sufficient magnitude to alter isotopomer and SP data. Relatively few studies have focused on lower flux scenarios when the rates of N<sub>2</sub>O reduction relative to production may exert more of an influence on SP. Our findings support the hypothesis that N<sub>2</sub>O reduction is a minor process influencing SP during conditions of high soil N<sub>2</sub>O flux, but may be more important for conditions with low N<sub>2</sub>O flux (Ostrom et al., 2007).

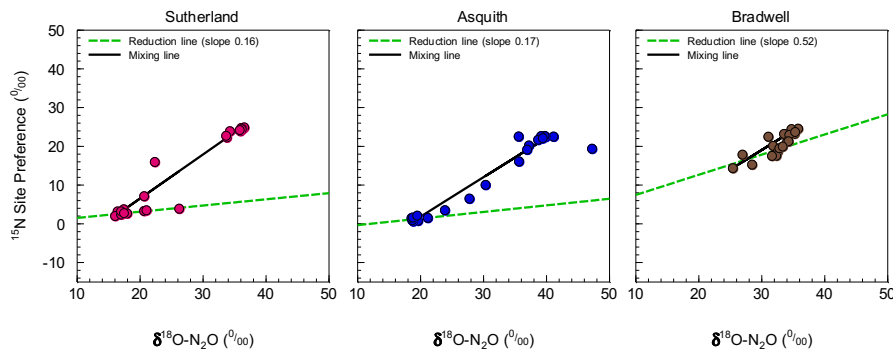


Figure 3 Isotopomer map to determine the source partitioning of N<sub>2</sub>O derived from nitrification versus denitrification using mean (n = 4) <sup>15</sup>N site preference (SP) and δ<sup>18</sup>O of N<sub>2</sub>O. The linear mixed model approach was based on Deppe et al. 2017 and Lewicka-Szczebak et al. (2017), but with mixing line end-members and reduction line slopes derived from our data.

Due to the wide range of reduction slopes observed in our study—and the differences for how SP is influenced in conditions with high flux vs low flux—we argue that using a single average reduction slope is insufficient to best predict N<sub>2</sub>O reduction. This finding echoes earlier work which suggested that during soil conditions when processes of N<sub>2</sub>O production and reduction occur simultaneously, the reduction line approach may be limited (Decock and Six, 2013b). It is recommended that further research better quantify the conditions that promote N<sub>2</sub>O reduction for improved N<sub>2</sub>O source predictions. This could be particularly important for assessing microbial source pathways of N<sub>2</sub>O production and consumption across seasonal and spatial scales, because sustained periods of low flux are not uncommon.

### 3.4 Source pathway partitioning and modelling

Using the pooled data from the isotopomer maps to predict source partitions, linear models were developed that fit the transitions for nitrification-derived N<sub>2</sub>O (R<sup>2</sup> = 0.65, p < 0.001) and denitrification-derived N<sub>2</sub>O (R<sup>2</sup> = 0.65, p < 0.001)

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(Fig. 4) with coefficients of variation and root-mean-square errors of 0.10 and 0.20, respectively. The models predict that over a soil moisture range of 53 to 78% WFPS, the source partitioning rapidly changed from nitrification- to denitrification-dominated N<sub>2</sub>O production. At soil moisture levels < 53% WFPS, N<sub>2</sub>O was predominately attributed to nitrification (F<sub>N</sub> = 1) but thereafter decreased rapidly, according to Eq. (6):

$$F_N = 3.19 - 0.041x \quad (6)$$

until a WFPS of 78%. This result was mirrored by the increase in N<sub>2</sub>O attributed to denitrification at a WFPS of 53% according to Eq. (7):

$$F_D = -2.19 + 0.041x \quad (7)$$

until F<sub>D</sub> = 1 at 78% and higher WFPS. These relationships exemplify the sensitivity of N<sub>2</sub>O production pathways to soil moisture changes. For instance, during the transition, a change in soil moisture as little as 10% (i.e., from 55 to 65% WFPS) is predicted to lower nitrification-derived N<sub>2</sub>O production by 56% but increase denitrification-derived N<sub>2</sub>O by >7-fold (Fig. 4). Consequently, the linear models presented here may help other researchers estimate N<sub>2</sub>O source partitioning when soil moisture falls within the transition from nitrification to denitrification.

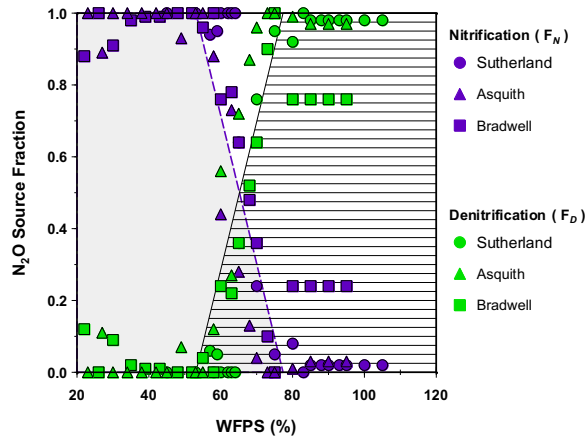


Figure 4 Fraction of emitted N<sub>2</sub>O that was attributed to nitrification (F<sub>N</sub>; shaded grey area) or denitrification (F<sub>D</sub>; lined area) based on the isotopomer mixing model (data points). Note: the dashed purple line denotes the predicted F<sub>N</sub> (Eq. 6); the solid black line denotes the predicted F<sub>D</sub> (Eq. 7).

As a check, the soil-specific approach presented here was compared to the independent endmember/slope approach commonly used by other researchers (Deppe et al., 2017; Lewicka-Szczepak et al., 2017). Isotopomer maps were calculated using independent literature-derived values (see Fig. S1) with the endmembers set at -2.4 to 34.4 for SP<sub>D</sub> to SP<sub>N</sub>, and 11.1 to 43.0 for δ<sup>18</sup>O<sub>D</sub> to δ<sup>18</sup>O<sub>N</sub>; and a reduction slope of 0.33 (Lewicka-Szczepak et al., 2017). Using the

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literature-derived endmembers overestimated the contribution of denitrification-derived N<sub>2</sub>O under very dry soil conditions (i.e., 20 to 40% WFPS)—indicating that up to 40% of N<sub>2</sub>O produced under these conditions was a result of denitrification (Fig. S1)—a contradiction to common knowledge (Butterbach-Bahl et al., 2013; Davidson et al. 1991). In our case, N<sub>2</sub>O source partitioning using soil-specific endmembers provided an advantage over using independent endmembers because the endmembers for the Bradwell soil were different from the literature-derived values; likely due to real soil biological processes such as microbial communities, the low rate of production, or soil heterogeneity (Decock and Six, 2013a; Lewicka-Szczebak et al., 2014). Nonetheless, we recommend that future research aims to develop more advanced models that take into account variability or more nuanced isotope effects.

Clearly, soil moisture change during the transition is a key regulator of which pathway dominantly produces N<sub>2</sub>O—be it nitrification or denitrification, or a mixture of both. Our results largely support the foundational studies that established the relationship between soil moisture and N<sub>2</sub>O emissions (Davidson, 1991; Linn and Doran, 1984); however, we provide a method that moves beyond just inferring N<sub>2</sub>O source pathways towards quantifying the pathway contributions over a range of soil moisture—and does so without having to add a <sup>15</sup>N label.

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#### 4 Conclusions

Determining the production pathways of soil-derived N<sub>2</sub>O is a worthwhile goal as there is potential to manage soils in ways that lead to reduced nitrification or denitrification during periods of risk for N<sub>2</sub>O loss—thereby mitigating emissions of a potent greenhouse gas. We show that isotopomer data have the potential to provide progress towards this goal. Measuring <sup>15</sup>N<sub>2</sub>O isotopomers enabled a more precise evaluation of the relationship between soil moisture and N<sub>2</sub>O production and we present a source fraction model for key soil moisture ranges. In general, our results support earlier assumptions about the relationships between moisture and N<sub>2</sub>O production pathways but can help move beyond inferring towards quantifying relative source pathways. Clearly, soil moisture level during ‘the transition zone’ is a key regulator of which pathway predominates—be it nitrification, denitrification, or a mixture of both. Hence, the models presented herein should be useful for other researchers to estimate contributions of nitrification versus denitrification when soil WFPS ranges from 53-78%.

One known caveat when using the isotopomer method for source pathway quantification is the isotope effect of N<sub>2</sub>O reduction. Previous researchers have attempted to address this limitation by using an average reduction slope and linear mixed model approach, but due to the wide range of reduction slopes observed in our study—and the differences for how denitrification SP is influenced in conditions with high N<sub>2</sub>O flux vs low flux—we argue that using a single average reduction slope is insufficient to best predict N<sub>2</sub>O reduction. It is recommended that further research better quantify the conditions which influence N<sub>2</sub>O reduction and its sensitivity on denitrification SP values for improved N<sub>2</sub>O source predictions. The creation of larger isotope databases would contribute to the development of more advanced models that take into account variability or more nuanced isotope effects.

#### 5 Author Contributions

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KAC and REF designed the experiment and TP carried it out. KAC prepared the manuscript with contributions from REF and TP.

## 6 Competing Interests

The authors declare that they have no conflict of interest.

## 5 7 Acknowledgements

Financial support was provided by the University of Saskatchewan College of Agriculture and Bioresources via a Martin Agricultural Trust Fund award to KAC and REF and by the Natural Sciences and Engineering Research Council of Canada via a Discovery Grant award to KAC. The authors are grateful to Frank Krijnen and Darin Richman for technical help in the lab.

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**Table 1 Soil physical and chemical characteristics.**

	<b>Sutherland</b>	<b>Asquith</b>	<b>Bradwell</b>
Previous cropping history	Vegetable crops	Fodder crops	Field crops
Texture class	Silty clay loam	Sandy loam	Loam
Organic matter (%)	5.9	3.9	2.7
Equilibrium soil water ( $\theta_g$ )	0.46	0.40	0.33
pH	7.6	7.5	7.9
CEC $\text{cmolc kg}^{-1}$	34.8	18.6	16.9
Total N (%)	0.42	0.21	0.16
Total $^{15}\text{N}$ (atom %)	0.371	0.370	0.368
Nitrate ( $\mu\text{g g}^{-1}$ )	194	35	10
Ammonium ( $\mu\text{g g}^{-1}$ )	3.8	1.7	5.2
Bray-Phosphorus (ppm)	542	190	23
Potassium (ppm)	1415	544	329
Sulfur (ppm)	49	28	13
Magnesium (ppm)	925	448	432
Calcium (ppm)	4650	2670	2490

**Table 2. Linear regressions between <sup>15</sup>N site preference and soil water-filled pore space (%) during three soil moisture regions for each soil type: i) before the transition from nitrification, ii) during the transition from nitrification to denitrification, and iii) after the transition to denitrification.**

Soil type	WFPS (%)	Slope	Intercept	Pearson r	<i>p</i> -value
<b>Before transition</b>					
Sutherland	< 64	-0.049	26.69	-0.30	0.4660 <sup>ns</sup>
Asquith	< 58	-0.004	22.04	-0.04	0.8973 <sup>ns</sup>
Bradwell	< 63	0.010	22.69	0.14	0.6781 <sup>ns</sup>
<b>During transition</b>					
Sutherland	64 – 83	-0.99	81.62	0.88	0.0214*
Asquith	58 – 73	-1.19	85.75	-0.89	0.0067**
Bradwell	63 – 75	-0.59	58.29	-0.99	0.0004*
<b>After transition</b>					
Sutherland	> 83	0.065	-3.01	0.86	0.0126*
Asquith	> 73	0.072	-4.77	0.99	0.0064**
Bradwell	> 75	0.262	-4.47	0.94	0.0154*

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