

We would like to thank Nathaniel Ostrom for his efforts in reviewing our manuscript and for his comments and suggestions, which have led to a valuable improvement of our manuscript. Below, the referee's comments (RC1) are displayed followed by the authors' response, which are highlighted in grey.

General comments:

The authors present an innovative and thorough assessment of the microbial origins of N₂O emissions from a grassland soil based on spectroscopic measurements. This data set is clearly an advance for the field as extensive time-series data sets for the isotopic composition of N₂O are rare but very important to constrain the dynamic nature of N₂O production from soils. While I appreciate the thoroughness of the data set and interpretations, I have a few central concerns regarding calibration and with the assessment of microbial origins of N₂O described in the paper.

RC1 Comment 1:

Characterization of sample isotope values based on two isotopically characterized standards is certainly the minimum necessary. Critical is also that the range of isotope values of the samples be encompassed by the range in isotope values of the standards. The range in isotope values for $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ is quite good (although I don't know what fraction of the samples lie outside these ranges) the range in $\delta^{18}\text{O}$ values of the standards is small and does not well encompass the range in sample values. The issue with this is not a just with the precision (which can be determined) but with the accuracy; values outside the range of the standards cannot be considered accurate even if precise. My personal opinion is to not publish isotope values outside the range of standards however, I appreciate that there may be a statistical approach for providing greater confidence in this situation.

Authors' response: one of the central concerns the referee points out is related to calibration of N₂O delta values, in particular the usage of a two-point calibration approach covering all measurement data. We acknowledge that this is of key importance to guarantee the accuracy of measurement data. In past years our laboratory has put significant efforts in the preparation of standard gases covering the range of delta values from strongly ¹⁵N depleted to enriched and gases were anchored to the international isotope ratio scales in close collaboration with Sakae Toyoda & Naohiro Yoshida / TIT and Willi A. Brand / MPI-BGC. Based on these gases we demonstrated linearity of delta measurements by QCLAS versus calibration gases already in 2008 (Waechter et al., 2008) and to our knowledge are one of few laboratories, which implemented a two point calibration approach up to now, while most labs in the N₂O isotope community report results using a one point (offset) correction. Our laboratory has organized an inter – laboratory comparison to improve the comparability of N₂O isotope results (Mohn et al., 2014) and participate in a similar very recent approach led by Nathaniel Ostrom (Ostrom et al., 2018). In both campaigns, the results reported by our laboratory were always in very good agreement with results reported by Tokyo Institute of Technology as already stated in the manuscript. In addition, the N₂O isotope community since recently has first reference materials, USGS 51 and 52 (Ostrom et al., 2018), but with limited coverage of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$.

In summary, we appreciate the comments of the referee that a two-point calibration approach covering all measurement data should be implemented, but would like to state that this is currently confined by the non-availability of suitable reference materials and the adapted approach in this manuscript therefore presents current best practice. We added the following statement to page 5 Line 18 ff:

... measured to monitor the data quality (Table 2). While S1 and S2 cover the range of $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ values of the sample gas, for $\delta^{18}\text{O}$ this is currently confined by the non-availability of suitable standard gases.

Nonetheless, the implemented calibration procedure presents current best practice in particular as the linearity of the delta scale for QCLAS measurements was demonstrated already in 2008 (Waechter et al., 2008).

RC1 Comment 2:

While the authors present an extensive data set on the concentration and isotopic composition of N₂O in air much of the data set is derived from periods of low flux when the concentration of N₂O is only slightly above atmospheric. The calculation of soil-derived N₂O is obtained from a simple isotope mixing model (soil-derived mixing with atmospheric N₂O). In this model, there is considerable error associated with the soil-derived isotope values at low N₂O concentration (the error increases as the concentration of N₂O declines). Further, the error around each calculated soil-derived isotope value increases as the difference in the isotopic composition between soil and atmospheric N₂O decreases. For this reason, in our laboratory, we generally don't publish data on samples in which the N₂O concentration is less than 30% greater than atmospheric (although this is an arbitrary value). The Keeling-plot approach does provide greater accuracy and precision but it is critical that the authors (1) report the uncertainty obtained for all soil-derived isotope values, (2) provide a clear discussion of this issue, and (3) consider not reporting isotope values for which the error is very large and a reasonable explanation for what the cut-off error should be.

Authors' response: the authors agree that the uncertainty of N₂O source signatures derived by the Keeling plot approach generally increases with decreasing share of source N₂O and should be given to support data interpretation. This relationship was illustrated for a similar TREX-QCLAS setup by Wolf et al. (2015), indicating that with only 12 ppb increase in N₂O an average standard error of 2.2 ‰, 1.4 ‰ and 1 ‰ for the SP, $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ isotopic source signatures can be achieved. The main reasons for the robustness of the technique applied by Wolf et al. (2015) and in the presented manuscript are the high number of gas samples (10.9 ± 0.9 measurements within each of the Keeling plot analysis) and the high sensitivity and precision of TREX-QCLAS. Furthermore, the uncertainties of the Keeling plot results were retrieved with a Monte Carlo simulation using the error seen in the target gas measurements. Thus, uncertainties for all soil-derived isotope values are contained implicitly in the given values. Nonetheless, we agree, that the criterion used in the manuscript to differentiate between valid and invalid measurements, significant correlation (p-value < 0.05) between $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$, might not be justified. Therefore, an alternative criterion was implemented and the corresponding paragraph on page 5 Line 35 ff was adapted:

"This procedure yielded 30 Keeling plot derived source signatures. The uncertainty of the source signatures was assessed based on the measured isotope delta values and N₂O concentrations using a Monte-Carlo model with 200 iterations. A benchmark value of 10 ‰ for the SP standard deviation was chosen as a criterion to distinguish valid measurements, finally leading to 12 N₂O accumulation events."

RC1 Comment 3:

There are two important assumptions in the graphical approach to tracing the microbial origins of N₂O that is used by the authors: (1) that $\delta^{18}\text{O}$ is a conservative tracer of origins and (2) that the kinetic isotope effects associated with production of N₂O as well as reduction of N₂O are constants. As I discuss below I question the validity of both of these assumptions. I will acknowledge, however, that this is a common approach in the literature but ask that the authors, at least, discuss these issues and how variation in the kinetic isotope effects might impact their model outcomes.

Authors' response: the authors agree that $\delta^{18}\text{O}$ -N₂O is not only controlled by the origin of the oxygen atom in the N₂O molecule (nitrate, nitrite, soil water or molecular O₂), but mainly driven by oxygen exchange of reaction intermediates (nitrate, nitrite) with soil water, e.g. Lewicka-Szczebak et al. (2016, 2017). Thereby,

$\Delta\delta^{18}\text{O} (\text{N}_2\text{O}/\text{H}_2\text{O}) = \delta^{18}\text{O}-\text{N}_2\text{O} - \delta^{18}\text{O}-\text{H}_2\text{O}$ for N_2O from bacterial denitrification in incubated soils is considered as relatively stable, given high oxygen exchange rates (Lewicka et al., 2017). In this respect, we agree that the sentence on page 10 Line 16–17 “Unfortunately, the interpretation of $\delta^{18}\text{O}-\text{N}_2\text{O}$ is further complicated by oxygen exchange between NO_3^- and soil water (Well et al., 2008; Kool et al., 2011).” is misleading and was reformulated to:

" $\delta^{18}\text{O}-\text{N}_2\text{O}$ of denitrification is affected by oxygen exchange between reaction intermediates (NO_3^- , NO_2^-) and soil water as a function of WFPS (Well et al., 2008; Kool et al., 2011)."

In addition, we will add the following sentence to page 11 Line 35 ff:

... using this approach reduces the uncertainty of the calculated relative contributions of the different processes as the boxes are used to span the mixing line. $\Delta\delta^{18}\text{O} (\text{N}_2\text{O}/\text{H}_2\text{O})$ for denitrification, is considered to be relatively stable (Lewicka-Szczebak et al., 2016), in particular under high WFPS associated with close to 100 % oxygen exchange between soil water and reaction intermediates (Kool et al., 2011).

In addition, we will complement the legend of Figure 8:

... (b) SP versus $\Delta\delta^{18}\text{O}$ of soil-emitted N_2O according to Lewicka-Szczebak et al. (2017), where $\Delta\delta^{18}\text{O} = d^{18}\text{O}-\text{N}_2\text{O} - d^{18}\text{O}-\text{H}_2\text{O}$, the isotope effect between soil water and formed N_2O . $\Delta\delta^{18}\text{O}$ for BD/ND is considered to be relatively stable, because of the high oxygen exchange between soil water and reaction intermediates at high WFPS.

We appreciate the comment by the referee that kinetic isotope effects (KIE) associated with N_2O production / consumption are not constant, which is a common (and essential) simplification using the dual isotope mapping approach in a natural, mixed culture system. We would like to stress this fact and add the sentence on page 10 Line 15 ff:

... may be indicators for N_2O reduction (Koba et al., 2009). It has to be mentioned, however, that fractionation factors may deviate depending on environmental conditions (Koster et al., 2013) or even over the course of a single experiment due to multiple reaction steps involved (Haslun et al., 2018).

RC1 Comment 4, Page 5, Line 17: Was data corrected based on measured values for the Target gas?

Authors' response: no, target gas measurements were conducted using the same analytical routine and calibration procedures as applied for the sample gas measurements. Therefore, they were not used to correct data but to assess the overall TREX-QCLAS analytical performance, e.g. repeatability.

RC1 Comment 5 Page 5, Line 26: Was the concentration correction performed daily? Did the range in concentration standards encompass the range in sample concentrations observed?

Authors' response: the concentration correction was performed based on measurements of diluted S1, and was conducted once per 24 hours. The sample gas concentrations were mostly, i.e. 511 out of 612 samples, in the concentration range covered by the concentration correction. The remaining 101 samples offered on average 5 % (maximum 14 %) higher concentrations, but were well within the linear range of the concentration correction. This linear dependency is typical for a delta correction at enhanced concentrations and a clear advantage of TREX-QCLAS versus QCLAS without preconcentration, which shows a very strong and non-linear concentration dependence.

RC1 Comment 6 Page 5, line 34: Did the Keeling plot approach include calculation of the uncertainty

surrounding the isotope values for the soil derived N₂O? Was this evaluated using the Monte Carlo model as described? At concentrations only slightly above ambient this error can be very large. We generally discard data with a N₂O concentration < 30% above atmospheric levels although the decision to discard data should probably be made on the basis of the magnitude of the uncertainty.

Authors' response: yes, a Monte Carlo model was applied to estimate the uncertainty of the N₂O isotope source signatures. In detail, the standard deviation of repeated target gas measurements, i.e. repeatability, was used as an estimate for the uncertainty of sample gas delta values and N₂O concentrations. A detailed discussion on the selected filter criterion is given in the author's response on RC1 comment 2.

RC1 Comment 7 Page 5, Line 36: Why was a correlation between $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ considered a criterion for a valid measurement? While unpublished, our data indicates that most of the variation in SP during N₂O production from the cNOR enzyme is driven by $\delta^{15}\text{N}^{\beta}$; which suggests that a lack of correlation could be a normal feature of production from denitrification.

Authors' response: The authors agree that filtering of data based on a correlation between $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ might not be justified for all source processes. Therefore, a different selection algorithm was implemented as detailed in the author's responses on RC1 comment 2.

RC1 Comment 8 Page 6, Line 5: It is not really necessary to specify the "hard disk" failure as the cause of missing data. Simply stating equipment failure is fine. This is also mentioned in the Figure 4 legend.

Authors' response: we would like to keep the statement in the text to highlight the fact that the data gap was not caused by a failure of the TREX-QCLAS.

RC1 Comment 9 Page 7, line 26: Delete "during": word not needed.

Authors' response: we agree, done.

RC1 Comment 10 Page 8, Line 22: On what basis was an r^2 of 0.2 similarly used to exclude data? This seems like a very poor degree of correlation.

Authors' response: we agree that an r^2 of 0.2 is not sufficient to filter valid source signatures. Therefore a different selection algorithm was implemented as detailed in the author's responses on RC1 comment 2.

RC1 Comment 11 Page 8, Line 23: Perhaps "calculated" is a better word than "extracted".

Authors' response: we agree, done.

RC1 Comment 12 Page 8, line 24: What are "relatively large uncertainties"? It would be better to state the uncertainty in the text as well as in the figures. Perhaps it would be best to exclude data with large uncertainties; as long as some reasonable criteria can be established to exclude data beyond a certain uncertainty (perhaps based on the range in isotope values between the sources).

Authors' response: we agree, that a quantitative indication on the uncertainties in this period should be given in the text and have changed the respective sentence to:

"[...] i.e., the extracted isotopic composition of N₂O emitted from soil ($\delta^{15}\text{N}^{\text{bulk}}$, SP, $\delta^{18}\text{O}$) derived from the Miller-Tans method showed relatively large uncertainties and amounted to 2.8 – 9.8 ‰, 2.3 – 10.6 ‰ and 4.6 – 12.9 ‰, respectively (shaded areas in Figure 5).

RC1 Comment 13 Page 8, line 30: Yes, the poorer data quality for $\delta^{18}\text{O}$ is likely a consequence of the fact that the standards do not encompass the data. Generally, standards must encompass the range in data to assure accuracy in any calibration relationship. The issue is that this does not only result in poorer precision but, more importantly, results in poorer accuracy. The authors are presenting data for which the accuracy is questionable. If the $\delta^{18}\text{O}$ values obtained are substantially outside the range of the standards the authors should give considerable thought as to whether they should be excluded.

Authors' response: we agree to the referee comments, that a two-point calibration approach covering all measurement data should be implemented, but would like to state that this is currently confined by the non-availability of suitable reference materials. Therefore, the adapted approach presents current best practice. A detailed discussion of this aspect and text added to the manuscript is given in the author's responses on RC1 comment 1.

RC1 Comment 14 Page 9, line 19: I am not fond of the graphical approach used by Koba et al to apportion sources of N_2O largely because $\delta^{18}\text{O}$ is a poor tracer of microbial origins. It is well known that oxygen exchanges with water during the microbial production of N_2O , which can alter $\delta^{18}\text{O}$ values (e.g. Kool references). Further, this approach relies on limited data sets for $\delta^{18}\text{O}$ generated by cultures of fungal denitrification, bacterial denitrification and nitrification. Given this, I suggest that the authors, at least, acknowledge the limitations of the graphical approach in assessment of the origins of N_2O .

For $\delta^{15}\text{N}$, this approach also depends not only on the isotopic composition of the nutrient source but also the kinetic isotope effect (KIE) associated with N_2O production. The KIE is more accurately described as a net isotope effect and can be highly variable depending upon conditions in the soil. The authors need to consider and discuss the effect of variation in the KIE on their model outcomes.

Authors' response: the authors agree that the dual isotope mapping approach by Koba et al. (2009) and Lewicka et al. (2017) relies on a number of assumptions, which should be stated in the text. A detailed discussion of this aspect and text added to the manuscript is given in the author's responses on RC1 comment 3.

RC1 Comment 15 Page 10, Line 5: Here again a single value is chosen for the KIE associated with N_2O reduction whereas there are many papers that show variation. In Jinuntuya-Nortman et al (2010) we demonstrate that the SP net isotope effect associated with N_2O reduction can approach zero per mil and is inversely correlated with water filled pore space. The authors demonstrate that water filled pore space varies. What is the affect of a varying NIE on the model outcomes? Indeed their inference that low SP values occur at high WFPS is entirely consistent with the role of WFPS in masking expression of fractionation during N_2O reduction and may not reflect variation in the proportion of N_2O derived from nitrification and denitrification. The estimates of the rates of N_2O reduction may not be meaningful if the net isotope effect for reduction varies (as it likely does).

Authors' response: we agree that a single value selected for the $\epsilon(\text{SP})$ is a simplification and $\epsilon(\text{SP})$ values might vary, e.g. depending on WFPS (Jinuntuya-Nortman et al, 2010) and $\text{N}_2\text{O}/(\text{N}_2+\text{N}_2\text{O})$ product ratio (Lewicka-Szczebak et al., 2015). Therefore we added the following statement on page 11 Line 6 ff:

... in accordance with Ostrom et al. (2007). Using a single $\epsilon(\text{SP})$ value is a simplification, however, as fractionation factors might vary, e.g. depending on WFPS (Jinuntuya-Nortman et al, 2010) and $\text{N}_2\text{O}/(\text{N}_2+\text{N}_2\text{O})$ product ratio (Lewicka-Szczebak et al., 2015).

RC1 Comment 16 Page 12, line 36: Given the issues raised above, I am not convinced by the author's

statement that natural abundance isotope studies are “an effective way to disentangle N₂O production pathways”. A frank assessment of the strengths and weaknesses of the approach is needed.

Authors' response: we agree and reformulated the mentioned statement to:

Our findings confirm that natural abundance isotope studies of N₂O provide a way to trace N₂O production / destruction pathways, in particular when combined with supportive parameters or isotope modelling approaches (Denk et al., 2017).

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