

Peer-review of the manuscript ‘Attribution of N₂O sources in a grassland soil with laser spectroscopy based isotopocule analysis’

Table of contents:

a) Authors’ response to Nathaniel Ostrom’s review.....	1
5 b) Authors’ response to the review by the anonymous referee 2.....	7
c) Authors’ response to the short comment by Klaus Schäfer.....	11
d) Revised manuscript with revision-traces.....	12

Notice: For better readability, the authors’ comments are highlighted in grey throughout the document. The revised manuscript with highlighted changes can be found in section d). There, the changes resulting from Nathaniel Ostrom’s review are highlighted with green font and the changes due to the anonymous referee’s review are highlighted with red font. The reference added due to the short comment by Klaus Schäfer is highlighted with purple font. Referred page and line numbers were matched to the revised manuscript.

a) Manuscript review by Nathaniel Ostrom

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 21 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).

15 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 41 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 δ¹⁸O 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 δ¹⁸O-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, Δδ¹⁸O (N₂O/H₂O) = δ¹⁸O-N₂O – δ¹⁸O-H₂O for N₂O from bacterial denitrification in incubated soils is considered as relatively stable, given high oxygen exchange rates (Lewicka-Szczebak et al., 2017). In this respect, we agree that the sentence on page 10 Line 24 – 25 “Unfortunately, the interpretation of δ¹⁸O-N₂O is further complicated by oxygen exchange between NO₃⁻ and soil water (Well et al., 2008; Kool et al., 2011).” is misleading and was reformulated to:

“δ¹⁸O-N₂O of denitrification is affected by oxygen exchange between reaction intermediates (NO₃⁻, NO₂⁻) 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 12 Line 9 – 10 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. Δδ¹⁸O (N₂O/H₂O) 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 Δδ¹⁸O of soil-emitted N₂O according to Lewicka-Szczebak et al. (2017), where Δδ¹⁸O = δ¹⁸O-N₂O – δ¹⁸O-H₂O, the isotope effect between soil water and formed N₂O. Δδ¹⁸O 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 N₂O 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 27 ff:

... may be indicators for N₂O 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 21: 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 32: 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.

- 5 RC1 Comment 6 Page 6, line 2: 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.

- 10 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 6, Line 2: 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.

- 15

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 7, Line 11: 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.

- 20

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, but we deleted the respective statement in the legend of Figure 4.

RC1 Comment 9 Page 7, line 32: Delete "during": word not needed.

Authors' response: we agree, done.

- 25 RC1 Comment 10 Page 9, Line 30: 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 9, Line 31: Perhaps "calculated" is a better word than "extracted".

- 30 **Authors' response:** we agree, done.

RC1 Comment 12 Page 9, line 32: 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).

- 35 **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).

5 RC1 Comment 13 Page 9, line 36: 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.

10 **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.

15 RC1 Comment 14 Page 10, line 32: I am not fond of the graphical approach used by Koba et al to apportion sources of N₂O 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 N₂O, 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 N₂O.

20 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 N₂O 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-Szczebak 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.

25 RC1 Comment 15 Page 11, Line 17: Here again a single value is chosen for the KIE associated with N₂O 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 N₂O 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 frac-
30 tionation during N₂O reduction and may not reflect variation in the proportion of N₂O derived from nitrification and denitrification. The estimates of the rates of N₂O 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 N₂O/(N₂+N₂O) product ratio (Lewicka-Szczebak et al., 2015).
35 Therefore we added the following statement on page 11 Line 17 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 N₂O/(N₂+N₂O) product ratio (Lewicka-Szczebak et al., 2015).

RC1 Comment 16 Page 13, line 12: 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:

- 5 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).

References related to Nathaniel Ostrom's review

- 10 Haslun, J. A., Ostrom, N. E., Hegg, E. L., and Ostrom, P. H. (2018). Estimation of isotope variation of N₂O during denitrification by *Pseudomonas aureofaciens* and *Pseudomonas chlororaphis*: implications for N₂O source apportionment. *Biogeosciences*, *15*(12), 3873-3882. doi:10.5194/bg-15-3873-2018
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- 15 Koster, J. R., Well, R., Tuzson, B., Bol, R., Dittert, K., Giesemann, A., Emmenegger, L., Manninen, A., Cardenas, L., and Mohn, J. (2013). Novel laser spectroscopic technique for continuous analysis of N₂O isotopomers - application and intercomparison with isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, *27*(1), 216-222. doi:10.1002/rcm.6434
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- 25 Lewicka-Szczebak, D., Augustin, J., Giesemann, A., and Well, R. (2017). Quantifying N₂O reduction to N₂ based on N₂O isotopocules - validation with independent methods (helium incubation and N-15 gas flux method). *Biogeosciences*, *14*(3). doi:10.5194/bg-14-711-2017
- Mohn, J., Wolf, B., Toyoda, S., Lin, C. T., Liang, M. C., Bruggemann, N., Wissel, H., Steiker, A. E., Dyckmans, J., Szewc, L., Ostrom, N. E., Casciotti, K. L., Forbes, M., Giesemann, A., Well, R., Doucett, R. R., Yarnes, C. T., Ridley, A. R., Kaiser, J., and Yoshida, N. (2014). Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. *Rapid Communications in Mass Spectrometry*, *28*(18), 1995-2007. doi:10.1002/rcm.6982
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- 35 Waechter, H., Mohn, J., Tuzson, B., Emmenegger, L., and Sigrist, M. W. (2008). Determination of N₂O isotopomers with quantum cascade laser based absorption spectroscopy. *Optics Express*, *16*(12), 9239-9244. doi:Doi 10.1364/Oe.16.009239
- 40 Well, R., Flessa, H., Xing, L., Ju, X. T., and Romheld, V. (2008). Isotopologue ratios of N₂O emitted from microcosms with NH₄⁺ fertilized arable soils under conditions favoring nitrification. *Soil Biology & Biochemistry*, *40*(9), 2416-2426. doi:10.1016/j.soilbio.2008.06.003

b) Manuscript review by anonymous referee 2

We would like to thank the anonymous referee #2 for his detailed review of our manuscript and for his precious comments and suggestions, which have led to a clear improvement of our manuscript. Below, the referee's comments (RC2) are displayed followed by the authors' response, which are highlighted in grey.

5 General observations and detailed comments:

The paper by Ibraim and co-authors presents a novel technique to measure a suite of isotopic fingerprints of N₂O using a field-deployable device. Since N₂O-emissions and their isotopic ratios vary spatially and temporally to a large degree, such an instrument is very useful to allow extent our knowledge on the processes driving these N₂O emissions. The measurements presented in the paper demonstrate that the QCLAS instrumentation works well under field conditions and allows its imple-
10 mentation in further studies. This successful field deployment is certainly a central step after a yearlong construction and test phase in the laboratory and I congratulate the team for this effort. Likewise it is clear to the authors of this study that measuring the isotopic information of N₂O is just one step, and to interpret and exploit the data requires extensive other knowledge, ranging from meteorological boundary conditions, soil analyses, to interpreting the application of manure on the sampling site. As further outlined below, some of these non-measurement aspects of the paper should be improved to gain more clarity for
15 the readers. Since the current measurement set up was not perfect, i.e. only nighttime measurements provide robust results, a lot can be learned from this measurement campaign for future field applications. At some places, the authors already suggested how these limitations could be overcome with a better design etc. I have the impression that these "lesson learned statements" could be extended to guide future measurement campaigns in this area. In general, the paper is clearly structured, the figures are mostly instructive and the text is written nicely. I therefore welcome this paper for final publication after my and the other
20 reviewers comments are included in the final version.

RC2 Comment 1

In section 4.4.6 (page 13) you discuss the influence of the manure application (12th July) on the calculated source signatures of the emitted N₂O. A causal link between excessive nitrogen addition on subsequent N₂O emissions from the soil is to be expected and might be the case. However, looking at the flux time series in Fig. 3 it is equally clear that N₂O fluxes rise after
25 intense rainfall events and this also fulfils expectation. The highest N₂O emissions within the entire study follow the strong rainfall event end of July - here without a manure application. However, the manure application on the 12th July is almost synchronous with the rainfall event; the farmer apparently waited for rain to apply the manure. My feeling is that the discussion in section 4.4.6 focusses too strongly on the manure application, while the more likely driver behind the rising N₂O emissions (intense rainfall) is not really discussed equally. A second argument for the "heavy rain hypothesis" is also the wide footprint
30 of the isotope measurements. From Fig. 7 I get the impression that the largest fraction of the emissions stems from outside of the dashed rectangle where the site is located. Only 15 to 30% of the N₂O emissions came from the local field where the manure was applied, while the largest fraction comes from an area of a few km distance. It might be that the other fields in the surroundings were also fertilized at the same time by the farmers and thus the De-Fen site is representative, but this information is missing. I might be wrong, but you might gain additional insight to view the N₂O flux data and the isotopic signatures also
35 from this heavy rainfall point of view (likewise WFPS) and a wider, more realistic regional footprint. In this respect, you might also put less weight on the NH₄ and NO₃ soil extracted solute data because these data might be too local for the footprint of the measured N₂O isotope signatures.

Authors' response: The authors agree that N₂O emissions and isotopic signatures are driven by both manure (substrate) addition and rainfalls (WFPS) and it might not be possible to disentangle both effects for the manure application on 12 July. The
40 effect of WFPS is explicitly discussed in the section 4.1 ("N₂O fluxes and WFPS") as well as in the sections 4.4.3 and 4.4.5. Nevertheless, we acknowledge the reviewer's comment on the fact that the N₂O emissions strongly raised after rainfall events since each of the three major rainfall events on 24 June, 12 July and 23 July was followed by a clear increase of the N₂O

emission rates, which is in agreement with literature (Peng et al., 2011; Liu et al., 2014). Unfortunately, for the two “rainfall – only” events no N₂O source signatures could be retrieved: around 24th June the observed data did not allow for significant Keeling plot analysis, while on 23rd July the measurement campaign was terminated and the fractional measurement data did not allow calculation of N₂O isotopic composition. We also agree that the footprint of the N₂O isotope measurements goes
5 beyond the fertilized plot. For this reason, we added a number of statements to section 4.4.6:

Page 13 line 23: After the manure application on 12 July and rainfall events in the days thereafter a strong ...

Page 13 Line 24: ... in the isotopic composition of the applied precursors, by an enhanced fractionation due to higher substrate availability or changes in process conditions (e.g. WFPS, see sections above).

Page 13 Line 31: ... during nitrification may have been used as substrate for denitrification, given the increase in WFPS due
10 to intensive rainfall events.

RC2 Comment 2

As suggested in your conclusions, it will be valuable in future measurement campaigns to also sample air from chambers that are more representative of the site. In other words, the precious isotope measurement time during the day or for meteorological situations that do not lead to sufficient N₂O accumulation in the boundary layer could be better invested.

15 **Authors' response:** In our second field study, which took place between July and December 2017 in Central Switzerland, we deployed automated flux-chambers at the day-time according to a specifically designed measurement schedule. This work is currently in preparation for publication.

RC2 Comment 3

20 Please mention the footprint shown in Fig. 7 earlier in the paper. It would be helpful for some readers (including me) to be aware that the actual footprint of the N₂O isotope data is more extended than what is visualized in Fig. 1.

Authors' response: We agree and we add the following sentence to the end of the section 2.1.1, where the De-Fen site is characterized:

Page 3 line 33: "[...] respectively (Raiffeisen Laborservice, Ormont, Germany). The average footprint area for N₂O flux and isotope measurements is given in Figure 7"

25 RC2 Comment 4

Page 1, Lines 33 and 37: (i) mentioning the sink term” while N₂O reduction acted as a major sink” may not clear to all readers. Does this refer to a consumption of N₂O produced in the soil itself or also for ambient atmospheric N₂O, i.e. a net sink to the atmosphere? (ii) “N₂O reduction to N₂ largely dictated the isotopic composition of measured N₂O. “ Does this statement refer to all measured isotope ratios; this statement seems very general.

30 **Authors' response:**

(i) Page 1 line 37: In the given context our statement referred to soil produced N₂O. We cannot exclude reduction of ambient N₂O but at least no net uptake of N₂O was observed. To emphasize this fact we added the following statement: “[...] source for N₂O, while N₂O reduction acted as a major sink for soil produced N₂O.”

35 (ii) The dual isotope mapping approach (Fig. 8) indicates that irrespective of the selected approach (SP versus $\Delta\delta^{15}\text{N}$ or SP versus $\Delta\delta^{18}\text{O}$) and scenario (1: reduction first, 2: mixing first) differences in SP to pure BD/ND were mostly controlled by N₂O reduction. We therefore would like to keep this statement.

RC2 Comment 5

Page 3, Lines 32: stick to one name for the management “cutting” vs moving (caption Fig. 4)

Authors' response: Done. "Mowing" was changed to "cutting" at the following positions: page 6 line 40, page 7 line 6 and caption of Figure 4 at page 21 line 9.

RC2 Comment 6

- 5 Page 3, Line 30: Site name: Could you use just Fendt as the site name in your paper rather than the awkward De-Fen (I know the acronym De-Fen is the more official in terms of the European Flux Database cluster).

Authors' response: We would like to keep “De-Fen” for consistency with other work related to this research site.

RC2 Comment 7

- 10 Page 3, Lines 36: (i) I am not a specialist for agricultural manures, but my understanding is that manure usually refers to animal feces (with the N mostly in form of urea) so I am confused by the ammonium N and referring to the Raiffeisen Laborservice; Do you mean inorganic fertiliser e.g. pure Ammonium sulfate? In any case, please specify this. (ii) Further, I wonder if it would have been worthwhile to obtain also the bulk N-isotopic composition of the two different kinds of fertilizers/manure. You put a lot of effort into measuring the spatial and temporal distribution of the $\delta^{15}\text{N}$ of soil-extracted nitrate while a value for the manure might be valuable as well for the input signature of $\delta^{15}\text{N}$ of soil NH_4

15 **Authors' response:**

- (i) The referee is correct, manure (and not ammonium sulphate) was applied. Raiffeisen Laborservice offers manure analysis (including analysis of N contents). Please see here: <https://www.raiffeisen-laborservice.de/biogas/analysen-guelle>.
- (ii) We agree that it would have been beneficial to get the manure analysed for the $\delta^{15}\text{N}$ content, especially for the interpretation of the event around 12 July. Unfortunately, we did not sample the manure that was applied at De-Fen for subsequent analysis.
- 20

RC2 Comment 8

Page 4, Line 16: This measurement-specific information seems not necessary here (“While. . .”) and could be deleted.

- 25 **Authors' response:** we agree. The sentence "While NH_4^+ was converted to an indophenol complex, NO_3^- was reduced to nitrite to produce the diazo chromophore." was deleted.

RC2 Comment 9

Page 5, Line 6: sentence could be shortened: “Then the gas was dried using a Nafion dryer. . .). . .also, delete: overpressured (the 4.5 bar already indicate that)

- 30 **Authors' response:** The sentence was adapted as follows "Then the sample gas was dried using a nafion drier [...]". We would like to keep the term "sample gas" in order to emphasize the gas type. The term "overpressure", however, was deleted.

RC2 Comment 10

Page 6, Line 3: Given the complexity of the pathways, this correlation criterion is not a sound argument for a valid measurement as it discards 18 out of 30 values while accepting 12 only leading to a bias in the results.

- 35 **Authors' response:** The main reason for discarding many Keeling plot derived source signatures, e.g. in the period 10 June to 21 June, was the low accumulation of N_2O . However, since both reviewers commented on the selection criteria for valid source signatures, we adopted the criteria as stated in our comment 2 to reviewer 1.

RC2 Comment 11

Page 9, Line 1. You could end the sentence after the Toyoda citation and delete after “, but. . .” as this does not add much.

Authors' response: We would like to keep this part of the sentence to highlight differences between agricultural and suburban sites.

RC2 Comment 12

On page 9, line 4 you write: “At night, within a stable nocturnal boundary layer, vertical wind speeds and hence tracer transport are low, while lateral wind speeds can be high and constituents like N₂O can be transported over larger distances. As a result, N₂O emissions from other land uses or land cover may have contributed to the observed N₂O isotopic composition. To assess the possible influence of other land use / land cover. Please omit may and possible in these occasions where you actually know that more distant emission contribute.

Authors' response: We agree, done.

10 RC2 Comment 13

- (i) Table 1: unit for bulk density is not % but rather g/cm³; pH is dimensionless
- (ii) Table 2: to prevent confusion with the units, provide all values for the mole fraction in ppm, i.e. for T 0.329 ppm.
- (iii) Table 3: Event no (a.u.)? did not get that for the three columns with SPKeeling and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$: one digit seems enough for the +- values, i.e. 1.9 instead of 1.91 for SP.
- 15 (iv) Table 4: caption: better: Characterization of the lower and upper range for. . . first column header “Source signature” should read parameter or signature

Authors' response:

- (i) Done
- (ii) Done
- 20 (iii) 'Event no. (a.u.)' was changed to 'Event number' and the number of digits was reduced to one as suggested
- (iv) We agree, done

RC2 Comment 14

- (i) Figure 3: Note, the x axis label for Fig. 3 and 4 and 5, 6 are all different (Date vs Datum in). Please select one for all, e.g. Date in 2016.
- 25 (ii) panel c with precipitation: 80 mm per hour seems a very high value, please check.
- (iii) please rewrite sentence to omit, respectively: “Blue and red dashed lines refer to a cutting event and to a manure application, respectively.” to: The blue dashed line indicates a cutting event and the red line manure application. (similar as you write in caption Fig. 4).

Authors' response:

- 30 (i) Done, 'Date in 2016 (dd.mm.)' was chosen for all
- (ii) Done
- (iii) Done

RC2 Comment 15

- (i) Figure 4: please explain the values given on the right side of the histograms
- 35 (ii) zoom panel a: at around 20.7. there is a weird magenta dot within the background values (black dot)
- (iii) caption: please replace y-axis with axes (plural) general observation at Fig. 4: In Fig. 3 it becomes apparent that the heavy precipitation events (around 12.7. and 22.7.) that lead to a progressive reduction of the WFPS are strongly connected with two prominent N₂O fluxes.

- (iv) While the first heavy rain event (around 12.7.) is connected with the manure application, the second rainfall event happens without manure application. It would be worthwhile to add these heavy rainfall events also in Fig. 4 with lines or other markers.

Authors' response: We agree and we will make the following changes:

- 5 (i) Done
(ii) Done.
(iii) Done.
10 (iv) The precipitation mentioned by the reviewer occurred on 23 July. As the scaling of Fig. 3 and 4 are different, the rainfall event occurs at the end of the N₂O isotope measurements and did not show up in the results. We therefore prefer to not add a vertical line on the last day of this plot, because possible consequences of this precipitation are not seen in the figure due to missing data in the next days.

RC2 Comment 16

- Figure 7: If possible adjust the colour legend to rounded numbers rather than 3.16e+02, e.g. 0.5; 1; 5; . . .300. Also, if possible, add the numbers (15, 30, 45 %) of the source sensitivities onto the isolines of figure itself (this is quicker than having this written in the caption).

Authors' response: the numbers in the legend of the Figure 7 are now written out.

References related to anonymous referee's review

- 20 Liu, X., Qi, Y., Dong, Y., Peng, Q., He, Y., Sun, L., Jia, J., and Cao, C. (2014). Response of soil N₂O emissions to precipitation pulses under different nitrogen availabilities in a semiarid temperate steppe of Inner Mongolia, China. *Journal of Arid Land*, 6(4), 410-422. doi:10.1007/s40333-013-0211-x
Peng, Q., Qi, Y., Dong, Y., Xiao, S., and He, Y. (2011). Soil nitrous oxide emissions from a typical semiarid temperate steppe in inner Mongolia: effects of mineral nitrogen fertilizer levels and forms. *Plant and Soil*, 342(1), 345-357. doi:10.1007/s11104-010-0699-1

c) Short comment by Klaus Schäfer

- 25 A very different method to determine N₂O emission fluxes was applied in Schäfer et al. (2012) to determine such fluxes from unfertilized grassland on the field scale. Fluxes in about the same amount, which are found and described here by Ibraim et al. in chapter 3.1 "N₂O fluxes and soil parameters", were determined in our investigations by a tunnel, coupled to an open-path Fourier transform infrared spectrometer, which covered 500 m², from 0 up to 14 µg N₂O-N m⁻² h⁻¹. The FLEXPART-COSMO simulations had source sensitivity which originates from areas within approximately 300 m to 700 m distance to the sample inlet representing an up-scaling similar to the tunnel method (100 m length). Peak emissions were detected in Schäfer et al. (2012) by concurrent chamber measurements after rainfall as described by Ibraim et al. also.

Authors' response

- 35 We agree that the two different approaches yielded similar results, which is very pleasant. Furthermore, while the FLEXPART-COSMO simulations were carried out to track the origin of the N₂O measured with the TREX-QCLAS instrumentation, the N₂O fluxes described in chapter 3.1 were obtained with a measurement system consisting of coupled flux-chambers and GC-ECS. Since the agreement between the TREX-QCLAS method and the GC-ECD method was good (SI Figure 2), we may conclude that all approaches, i.e. including the tunnel method, agreed well. We appreciate the short comment by Klaus Schaefer and acknowledge that the study mentioned therein is closely related to our work and, thus, we will refer to this work in our reviewed manuscript.

40

Attribution of N₂O sources in a grassland soil with laser spectroscopy based isotopocule analysis

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15 Abstract

Nitrous oxide (N₂O) is the primary atmospheric constituent involved in stratospheric ozone depletion and contributes strongly to changes in the climate system through a positive radiative forcing mechanism. The atmospheric abundance of N₂O has increased from 270 ppb during the pre-industrial era to approx. 330 ppb in 2018. Even though it is well known that microbial processes in agricultural and natural soils are the major N₂O source, the contribution of specific soil processes is still uncertain. The relative abundance of N₂O isotopocules (¹⁴N¹⁴N¹⁶N, ¹⁴N¹⁵N¹⁶O, ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁴N¹⁸O) carries process-specific information and thus can be used to trace production and consumption pathways. While isotope ratio mass spectroscopy (IRMS) was traditionally used for high-precision measurement of the isotopic composition of N₂O, quantum cascade laser absorption spectroscopy (QCLAS) has been put forward as a complementary technique with the potential for on-site analysis. In recent years, preconcentration combined with QCLAS has been presented as a technique to resolve subtle changes in ambient N₂O isotopic composition.

From the end of May until the beginning of August 2016, we investigated N₂O emissions from an intensively managed grassland at the study site Fendt in Southern Germany. In total, 612 measurements of ambient N₂O were taken by combining preconcentration with QCLAS analyses, yielding $\delta^{15}\text{N}^{\alpha}$, $\delta^{15}\text{N}^{\beta}$, $\delta^{18}\text{O}$ and N₂O concentration with a temporal resolution of approximately one hour and precisions of 0.46 ‰, 0.36 ‰, 0.59‰ and 1.24 ppb, respectively. Soil $\delta^{15}\text{N}\text{-NO}_3^-$ values and concentrations of NO₃⁻ and NH₄⁺ were measured to further constrain possible N₂O-emitting source processes. Furthermore, the concentration footprint area of measured N₂O was determined with a Lagrangian particle dispersion model (FLEXPART-COSMO) using local wind and turbulence observations. These simulations indicated that night-time concentration observations were largely sensitive to local fluxes. While bacterial denitrification and nitrifier denitrification were identified as the primary N₂O-emitting processes, N₂O reduction to N₂ largely dictated the isotopic composition of measured N₂O. Fungal denitrification and nitrification-derived N₂O accounted for 34 - 42 % of total N₂O emissions and had a clear effect on the measured isotopic source signatures. This study presents the suitability of on-site N₂O isotopocule analysis for disentangling source and sink processes in-situ and found that at the Fendt site bacterial denitrification/ nitrifier denitrification is the major source for N₂O, while N₂O reduction acted as a major sink [for soil produced N₂O](#).

1. Introduction

Nitrous oxide (N₂O) is the third most important greenhouse gas (GHG), accounting for 6 % of the total anthropogenic radiative forcing (Myhre et al., 2013), and is thus far the dominant stratospheric ozone depleting substance emitted in the 21st century (Ravishankara et al., 2009). Its globally averaged atmospheric concentration has increased since the preindustrial era from approximately 270 ppb (parts-per-billion, 10⁻⁹ mole mole⁻¹) at an average rate of 0.2 – 0.3% yr⁻¹ and reached 328.9 ± 0.1 ppb in 2016 (Prinn, 2016; WMO and GAW, 2016). While it is well known that natural and agricultural soils are the major N₂O sources on a global scale, the relative contributions of individual microbial and abiotic N₂O production and consumption pathways remain largely uncertain because different N₂O-producing and -consuming processes are active simultaneously in a soil. Until now, there were no direct methods that allow for the attribution of an emitted amount of N₂O to a given process in the field (Solomon et al., 2007; Billings, 2008; Butterbach-Bahl et al., 2013). However, a detailed understanding of the temporal and spatial variations in N₂O emissions and controlling processes is required to develop mitigation strategies and to better achieve emission reduction targets (Nishina et al., 2012; Cavigelli et al., 2012; Herrero et al., 2016; Decock et al., 2015). Atmospheric N₂O isotopic composition provides important information about N₂O production and consumption processes because distinct microbial and abiotic process pathways exhibit characteristic isotopic signatures (Toyoda et al., 2017; Decock and Six, 2013b; Verhoeven et al., 2018; Denk et al., 2017). Apart from ¹⁴N¹⁴N¹⁶O, representing 99 % of total atmospheric N₂O, the three most abundant N₂O isotopocules are ¹⁴N¹⁵N¹⁶O (¹⁵N at central α position), ¹⁵N¹⁴N¹⁶O (¹⁵N at terminal β position) and ¹⁴N¹⁴N¹⁸O (Toyoda and Yoshida, 1999; Kato et al., 1999). Abundances of isotopocules are usually reported in the δ-notation in per mil (‰) as δ¹⁵N^α, δ¹⁵N^β, δ¹⁸O, calculated according to the equation (1):

$$\delta X = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \quad (1)$$

where X denotes ¹⁵N^α, ¹⁵N^β or ¹⁸O and R refers to ¹⁴N¹⁵N¹⁶O / ¹⁴N¹⁴N¹⁶O, ¹⁵N¹⁴N¹⁶O / ¹⁴N¹⁴N¹⁶O or ¹⁴N¹⁴N¹⁸O / ¹⁴N¹⁴N¹⁶O, respectively, in a sample or standard (Toyoda and Yoshida, 1999; Brenninkmeijer and Röckmann, 1999; Werner and Brand, 2001). The international isotope reference scale for ¹⁵N / ¹⁴N is atmospheric N₂ (AIR-N₂) and for ¹⁸O / ¹⁶O Vienna Standard Mean Ocean Water (VSMOW). Thermal decomposition of isotopically characterized ammonium nitrate (NH₄NO₃) has been suggested as an approach to link the position-dependent nitrogen isotopic composition of N₂O to AIR-N₂ (Toyoda and Yoshida, 1999; Mohn et al., 2016). The total ¹⁵N content is usually reported as bulk ¹⁵N content (δ¹⁵N^{bulk}) according to equation (2):

$$\delta^{15}\text{N}^{\text{bulk}} = (\delta^{15}\text{N}^{\alpha} + \delta^{15}\text{N}^{\beta}) / 2 \quad (2)$$

while the site preference (SP) is used to denote the intramolecular ¹⁵N distribution according to the equation (3):

$$\text{SP} = \delta^{15}\text{N}^{\alpha} - \delta^{15}\text{N}^{\beta} \quad (3)$$

The established technique for the analysis of N₂O isotopic composition is isotope-ratio mass-spectrometry (IRMS) (Toyoda and Yoshida, 1999), which is very sensitive and capable of providing highly precise analytical results (Toyoda and Yoshida, 2016). However, IRMS instruments are usually not suitable for field deployment. Recently, quantum cascade laser absorption spectroscopy (QCLAS) (Waechter et al., 2008; McManus et al., 2015), cavity ring-down spectroscopy (CRDS, Erler et al., 2015), and off-axis cavity output spectroscopy (OA-ICOS, Wassenaar et al., 2018) were introduced as alternatives for greenhouse gas (GHG) stable isotope analysis, with the capability for real-time, on-site analysis even at remote locations (Tuzson et al., 2011; Wolf et al., 2015; Eyer et al., 2016; Röckmann et al., 2016). Another advantage of spectroscopic techniques is their ability for direct selective analysis of intra-molecular isotopic isomers (isotopomers) such as ¹⁴N¹⁵N¹⁶O and ¹⁵N¹⁴N¹⁶O, while the determination of the SP using IRMS is only possible via a detour of measuring δ¹⁵N-NO⁺ in combination with δ¹⁵N^{bulk} and a correcting for scrambling (Toyoda et al., 1999). Several studies have successfully applied QCLAS and CRDS for N₂O isotope analysis in laboratory and field incubation experiments (Koster et al., 2013; Yamamoto et al., 2014; Erler et al., 2015;

Mohn et al., 2013; Winther et al., 2018), and more recently to analyse diurnal and seasonal isotopic variations in ambient N₂O (Mohn et al., 2012; Toyoda et al., 2013; Wolf et al., 2015; Harris et al., 2017). The isotopic composition of N₂O emitted from soils can be extracted from ambient air measurements using traditional two end-member mixing models, i.e. the “Keeling plot” approach (Keeling, 1961) or the Miller–Tans approach. While the Keeling plot approach requires stable background conditions, the Miller-Tans approach is also applicable if the stable background requirement is violated (Miller and Tans, 2003). However, the spatial attribution of the extracted N₂O isotopic composition has to date been neglected because atmospheric transport and turbulence needs to be considered.

The bulk isotopic composition of N₂O produced by biogeochemical source processes, i.e. $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$, is controlled by fractionation during N₂O production, the isotopic composition of N₂O precursors (i.e., NH₄⁺, NO₂⁻, NO₃⁻ and H₂O), and N₂O reduction. In contrast, the difference in ¹⁵N substitution between the central and terminal position within the N₂O molecule (SP) is independent of the precursor's isotopic composition and characteristic for specific reaction mechanisms or enzymatic pathways (Sutka et al., 2006). Therefore, SP provides distinct process information, which can be determined by pure culture studies and chemical reactions under laboratory conditions (Heil et al., 2014; Wei et al., 2017b; Toyoda et al., 2005). Decock and Six (2013a) and Toyoda et al. (2017) summarized that N₂O from hydroxylamine (NH₂OH) oxidation, fungal denitrification and abiotic N₂O production on the one hand and N₂O originating from nitrifier-denitrification and denitrification on the other hand display distinct SP values of $32.8 \pm 4.0 \text{ ‰}$ and $-1.6 \pm 3.8 \text{ ‰}$, respectively. Accordingly, SP values of N₂O from mixed microbial communities/ abiotic processes, may display large variations depending on the prominent reaction pathway and the respective study conditions.

With this study, we aim to improve the understanding of the temporal dynamics of N₂O isotopic composition, and to identify the relative contribution of the dominant N₂O producing and consuming microbial processes under field conditions. To achieve this, we i) applied a revised coupled TRace gas EXtractor (TREX) and a QCLAS-based instrumentation (TREX-QCLAS, Ibraim et al., 2018) for the first time during a field campaign for in-situ analysis of N₂O isotopocules from ambient air samples, ii) compared two approaches for the calculation of the isotopic composition of N₂O emitted from soils, namely the Keeling plot versus the Miller-Tans approach, iii) include the isotopic composition of a N₂O precursor, nitrate (NO₃⁻), to support the identification of dominant processes and iv) use local turbulence and wind profile measurements to outline the spatial extent for which the determined isotopic compositions of soil emitted N₂O are representative.

2. Material and Methods

2.1 Characterization of the research site Fendt

2.1.1 Study site

The TERENO-preAlpine Observatory (Kiese et al., 2018) research site Fendt (De-Fen), a typical montane grassland south of Munich (Germany) is situated at 595 m a.s.l. and has an annual mean temperature of 8.9 °C with 960 mm mean annual precipitation. The site is intensively managed, which includes up to five times of cutting per year for fodder production followed by manure application as well as occasional cattle grazing (Zeeman et al., 2017). Soil characteristics of the site are given in Table 1. These measurements were carried out between 29 May and 03 August 2016 as part of the ScaleX 2016 campaign (Wolf et al., 2017; <https://scalex.imk-ifu.kit.edu/>). During the measurement period, management activities included one cut (04 July 2016) and one manure application event (12 July 2016) with a load of 43.7 kg N ha⁻¹, of which 20 and 23.7 kg were in the form of organic and ammonium-N, respectively (Raiffeisen Laborservice, Ormont, Germany). [The average footprint area for N₂O flux and isotope measurements is given in Figure 7](#)

2.1.2 Environmental conditions

Rainfall was determined using four precipitation gauges (Rain collector, Davis instruments, Hayward, CA) as indicated in Figure 1 with triangles. The soil temperature was monitored at three locations across De-Fen (red squares in Figure 1) at three depths (5 cm, 10 cm and 15 cm) using PT100 sensors (IMKO, Ettlingen, Germany). Soil water content was determined within the area (locations are indicated by the dashed square in Figure 1) with five ThetaML2x probes (Delta-T Devices, Cambridge, UK), which integrate soil water content over a soil depth of 0 - 6 cm. Water filled pore space (WFPS) was calculated based on measured volumetric water contents and soil characteristics (Kiese et al., 2018). The atmospheric turbulence statistics were determined using the permanently installed micrometeorological instrumentation (Kiese et al 2018) and additional sonic anemometers installed at 6 m and 9 m above the ground. Vertical wind profiles were determined up to 1000 m above the ground in 20 m intervals using Doppler wind-lidar systems (StreamLine, Halo Photonics, Worcestershire, United Kingdom).

2.1.3 Concentrations of soil extracted NH_4^+ and NO_3^- and $\delta^{15}\text{N-NO}_3^-$

Soil samples (approx. 150 g, 2-7 cm depth) were collected twice per week in a sampling grid (mesh size 70 m) spanning the whole measuring area at the De-Fen site (dashed square Fig. 1; Wolf et al., 2017), extracted with 1M potassium chloride (KCl, Merck KGaA, Darmstadt, Germany) and stored at -18 °C. After the manure application, sampling was increased to daily time intervals (12 July 2016 – 15 July 2016), followed by further sampling on 19, 21 and 27 July 2016. The concentrations of NH_4^+ and NO_3^- were determined colorimetrically using a spectrophotometer (AGROLAB Agrarzentrum GmbH, Germany). ~~While NH_4^+ was converted to an indophenol complex, NO_3^- was reduced to nitrite to produce the diazo chromophore.~~

For 116 out of 298 soil extracts described above, $\delta^{15}\text{N-NO}_3^-$ was also analysed. This subset of samples was collected at the sampling nodes in the vicinity of the flux chambers and the TREX-QCLAS sample inlet. Soil extracts and 14 KCl blanks were analysed for $\delta^{15}\text{N-NO}_3^-$ at the Stable Isotope Facility of the University of California Davis, USA using the bacterial denitrification assay (Sigman et al., 2001; Casciotti et al., 2002). The reference materials USGS 32, USGS 34, and USGS 35, as supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD) were used for data correction and additional laboratory reference materials were included to monitor and correct for instrumental drift and linearity. The standard deviation for repeated measurements of reference material was < 0.2 ‰.

2.2 Measurements of soil N_2O fluxes

Soil N_2O flux rates ($f(\text{N}_2\text{O})$) were obtained using five replicated opaque static flux chambers coupled with a gas chromatograph with an electron capture detector (GC-ECD) and operated according to a pre-defined schedule. A detailed description of the method can be found for example in Rosenkranz et al. (2006). The chambers were alternately closed and opened for 60 minutes, and each chamber was sampled every 15 minutes, resulting in 4 headspace air measurements per chamber closure time. The chamber dimensions were $50 \times 50 \text{ cm}^2$ and either 15 or 50 cm in height, depending on vegetation height. All flux chambers were deployed south of the mobile laboratory within the dashed square in Figure 1. N_2O fluxes were calculated from the concentration increase over time according to Rosenkranz et al. (2006), taking into account local air pressure and the chamber headspace temperature.

2.3 Analysis of N_2O isotopocule by TREX-QCLAS

2.3.1 Analytical procedure

The TREX-QCLAS setup used in this study for the N_2O isotope measurements was developed and described in detail by Ibraim et al. (2018), based on a previous system developed for CH_4 isotope analysis by Eyer et al. (2014, 2016). In brief, N_2O from 5 L of ambient air is extracted using the TREX device and purged into the multi-pass (76 m) cell of the spectrometer (CW-QC-TILDAS-76-CS; Aerodyne Research Inc., Billerica, USA) by means of a low flow of synthetic air (20.5 % O_2 , 79.5

% N₂, Messer Schweiz AG, Switzerland). This approach is capable of measuring the four most abundant N₂O isotopic species (¹⁴N¹⁴N¹⁶O, ¹⁴N¹⁵N¹⁶O, ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁴N¹⁸O) at approx. 90 ppm with an Allan deviation of < 0.1 ‰.

The TREX-QCLAS was operated in an air-conditioned mobile laboratory (22 - 30 °C) situated at the north end of De-Fen (Figure 1). Ambient air was continuously sampled with a flow rate of approx. 900 mL min⁻¹ from 2 m above the ground at the Eddy Covariance (EC) tower and transported to the mobile laboratory using a SERTOflex tube (~ 20 m length, 6 mm OD, SERTO AG, Switzerland). ~~Then the sample gas was dried using a nafion drier~~~~The sample gas was then dehumidified using a permeation drier~~ (PermaPure Inc., USA) and subsequently pressurized to 4.5 bars ~~overpressure~~ using a membrane pump (PM25032-022, KNF Neuberger, Switzerland). Downstream of the pump the air was passed through a chemical trap for carbon dioxide (CO₂) and residual H₂O removal. After this pre-treatment, the air was passed into the TREX device for N₂O pre-concentration following the procedure as described in Ibraim et al. (2018).

Maintenance demand during field application was minimized by successively using a multi-position valve (Valco Instruments Inc., Switzerland) to switch between eight chemical traps for CO₂ and H₂O removal (Figure 2). Each of the traps consisted of a stainless steel tube (12 mm OD, 350 mm length) filled with 12 g Ascarite (10 - 35 mesh, Fluka, Switzerland), bracketed with magnesium perchlorate (Mg(ClO₄)₂, 2 × 1.5 g, Fluka, Switzerland) and silane-treated glass wool (Sigma-Aldrich Chemie GmbH, Switzerland). The CO₂ extraction capacity of the Ascarite traps was found to be sufficient for > 500 L at ambient CO₂ concentrations (unpublished). To avoid CO₂ breakthrough and particularly clogging of the trap under varying CO₂ and residual H₂O concentrations, the chemical trap was changed every day.

2.3.2 Calibration strategy and data processing

The isotopic composition of ambient air was referenced against a set of standard gases (Table 2) that were periodically measured (Figure 2) to ensure long-term repeatability. The measurement routine was implemented using a customized LabVIEW programme. Initially, two standard gases (S1, S2) were analysed for a two-point delta calibration and a target (T) gas was 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). This phase was followed by a series of four alternating S1 and ambient air sample (S) measurements. A full analytical cycle yielded 13 measurements, including four ambient air analyses, and required approx. four hours, corresponding to a measurement frequency of approx. 1 ambient air sample per hour.

Data processing was conducted as previously described by Harris et al. (2017) using Matlab (MathWorks, Inc., USA). Abundances of the four isotopocules (¹⁴N¹⁴N¹⁶O, ¹⁴N¹⁵N¹⁶O, ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁴N¹⁸O) were obtained with TDL Wintel (Aerodyne Research Inc., Billerica, USA), and isotope ratios were drift-corrected for changes observed in S1. Specifically, the isotope ratios of S1 were linearly fitted to cell pressure, cell temperature and to the goodness-of-the-TDL-fit. If this linear fit was significant (p-value < 0.05) the correction was applied to all data. These corrections were always relatively small and within the range of 0.05 – 0.2 ‰. In addition, a concentration correction was performed using a linear regression curve determined with S1 diluted in synthetic air. The concentration corrections were -0.20, 0.32 and -0.24 ‰ ppm⁻¹ for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and $\delta^{18}\text{O}$, respectively. Finally, delta values were calculated from isotope ratios using the two-point delta calibration based on S1 and S2. Since no international standards were available for N₂O isotopes, S1 and S2 were analysed against N₂O standards for which the isotopic composition was assigned at Tokyo Institute of Technology (Tokyo Tech) according to Toyoda and Yoshida (1999). In addition, past and ongoing inter-laboratory comparison measurements on pressurized air indicated a very good agreement with Tokyo Tech results (Mohn et al., 2014; Ostrom et al., 2018).

2.4 Source signatures of soil emitted N₂O

Source signatures of soil-emitted N₂O were interpreted using the Keeling plot approach (Keeling, 1958). Each analysis started at 7 pm on day n and lasted until 6 am on the consecutive day $n+1$ local time (UTC +1). 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. This procedure yielded a total of 30 source signatures, of which 12 displayed a significant correlation (p value < 0.05) between $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$, which was defined as the criterion indicating a valid measurement. The uncertainty of the calculated source signatures was assessed from the uncertainties in isotope delta values and N₂O concentrations using a Monte Carlo model with 200 iterations.

For comparison, the source signatures were also calculated with the Miller and Tans (2003) approach. An in-depth description of the implementation of the Miller-Tans method is provided by Harris et al. (2017). In brief, first, a baseline is determined by averaging the data points in the lowest 5 % of the diurnal N₂O concentrations with a 5-day moving window (see SI Figure 3). The same measurement points are also used to find the baseline of the isotope delta values – isotope values are not used to flag the baseline since deviations can be both positive and negative. Subsequently, the Miller-Tans equation (eq. 2 in Harris et al. (2017)) is used to derive the source isotope signatures based on a simple linear regression within a 24-hour moving window. The uncertainty in source isotopic composition is calculated by first propagating measurement errors into all terms used in the Miller-Tans equation and then running 200 iterations assuming a normal distribution of error in all terms.

2.5 Footprint analysis with FLEXPART – COSMO simulations

The Lagrangian Particle Dispersion Model FLEXPART (Stohl et al., 2005) was adapted for input from the numerical weather prediction model COSMO (Brunner et al., 2012, Oney et al., 2015 and Henne et al., 2016) and was used on a site-scale to determine the concentration footprint of our observations. For this purpose the model was adapted by locally nudging wind profiles and micro-meteorological observations at De-Fen into the COSMO model output. The latter was taken from the operation analysis and forecast runs by MeteoSwiss with a spatial resolution of approximately 1 km \times 1 km. Into these model fields observed profiles of the wind vector (composite of 2.5 m and 9 m sonic anemometer) were locally nudged using a tricubic nudging kernel with a width of 3 km, hence influencing approximately 3 grid cells around the observational site (further related information is provided by Wolf et al. (2017)). Turbulence statistics (friction velocity, Monin-Obukhov length) required by FLEXPART were taken from the observations and locally replaced the COSMO-simulated values. The effect of the nudging procedure was strongest at night and under stable boundary layer conditions, which COSMO often fails to reproduce correctly. FLEXPART was run in backward mode, tracing released model particles 24 h and generating hourly surface source sensitivities (τ_{50} (s m³ kg⁻¹); also called concentration footprint) for the location of the N₂O isotope observations. Source sensitivities were calculated on a regular longitude-latitude grid around the De-Fen site (47.825 – 47.845 °N and 11.50 – 11.51 °E) with a resolution of approximately 50 m \times 50 m and for model particles from the surface to 50 m above the ground, the latter of which was also the defined minimum of the model boundary layer height. Multiplication of the source sensitivities with a surface flux and summation over the whole model domain and time of the backward integration yields the concentration increment during the period of simulation. The map of source sensitivities was used as an indicator of the extent of the observed N₂O source. Average source sensitivities were calculated for the 12 accumulation events between 6 pm and 6 am the next day.

3. Results

3.1 N₂O fluxes and soil parameters

The initial phase of the measurement campaign (10 May 2016 – 21 June 2016) was characterized by low ambient air and soil temperatures (13.5 and 15.6 °C, respectively) along with high precipitation and high WFPS values (> 5 mm d⁻¹ and > 95 %, respectively, between 10 – 21 June; Figure 3). Soil extracted NH₄⁺ and NO₃⁻ values in this period were 0.27 to 8.32 mg N l⁻¹ and 0.12 to 3.15 mg N l⁻¹, respectively. This period was also characterized by the lowest N₂O flux rates ($f(\text{N}_2\text{O})$), i.e. the mean $f(\text{N}_2\text{O})$ of all five chambers was below 70 μg N m⁻² h⁻¹. After 21 June the N₂O fluxes increased, reaching a maximum of approx. 450 μg N m⁻² h⁻¹ on 24 and 25 June. $f(\text{N}_2\text{O})$ followed a diurnal pattern with slightly higher emissions during the day but also higher nocturnal $f(\text{N}_2\text{O})$ values compared to the initial phase of the campaign. Thereafter $f(\text{N}_2\text{O})$ decreased to around 200 μg N m⁻² h⁻¹ on 29 June, before it began to steadily rise from 30 June to 12 July. After the mowing-cutting event on 4 July, NO₃⁻ concentrations increased, while NH₄⁺ remained unaffected. In contrast, after the manure application on 12 July, the concentration of NH₄⁺ increased immediately, while NO₃⁻ only accumulated slowly over the course of the following week. In this period N₂O daytime emissions also peaked at > 900 μg N m⁻² h⁻¹ followed by a period of variable N₂O fluxes with very low but also very high emission rates, for example 17 July and 24 July at 290 and 2400 μg N m⁻² h⁻¹, respectively. Two weeks after the manure application the concentrations of NH₄⁺ and NO₃⁻ and N₂O fluxes were comparable to the period prior to manure application and mowing-cutting.

3.2 Ambient N₂O concentrations and isotopic variations

Figure 4 shows N₂O concentrations and isotopic composition ($\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, $\delta^{18}\text{O}$) analysed between 9 June and 23 July in ambient air 2 m above the ground. In total, 612 air sample measurements (S), 150 target gas (T), 1783 anchor gas (S1) and 164 calibration gas (S2) measurements were performed (concentrations and isotopic composition of T, S1 and S2 are given in Table 2). The data gap between 27 June and 8 July was caused by a hard disk failure of the system computer. The standard deviation for repeated in-situ T measurements (undergoing identical treatment compared to S) was 0.46 ‰, 0.36 ‰, 0.59‰ and 1.24 ppb, for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, $\delta^{18}\text{O}$ and N₂O concentrations, respectively.

Apart from a small nocturnal N₂O concentration increase on 11 June, no clear variations in ambient N₂O were observed in the first three weeks of the campaign, which is in accordance with the lowest soil N₂O fluxes, as described above. On 21 June the onset of a diurnal pattern with nocturnally enhanced N₂O concentrations accompanied by co-varying $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and $\delta^{18}\text{O}$ values was observed. Mean N₂O concentrations were 331.62 ± 1.41 ppb during the day and elevated at night with a maximum of 429 ppb observed on 23 June. During the day, mixed surface-layer isotopic compositions of N₂O were 15.22 ± 0.42 ‰, -2.78 ± 0.34 ‰, and 45.88 ± 0.43 ‰ for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and $\delta^{18}\text{O}$, respectively, thus yielding SP and $\delta^{15}\text{N}^{\text{bulk}}$ values of 17.95 ± 0.15 ‰ and 6.28 ± 0.30 ‰, respectively.

The nocturnal increase of N₂O concentrations was accompanied by a decrease in $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$, while $\delta^{18}\text{O}$ values generally increased at higher N₂O concentrations, but also showed the opposite behaviour for some events. The most extreme δ -values were 8.98 ‰, -9.66 ‰ and 50.61 ‰ for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and $\delta^{18}\text{O}$. Compared to the background values, this results in a difference of 6.24 ‰, 6.88 ‰ and 4.73 ‰ for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and $\delta^{18}\text{O}$, respectively.

3.3 Source signature of soil emitted N₂O and precursors

Source signatures of soil-emitted N₂O at De-Fen were calculated using the Keeling plot method (Keeling 1961, 1958) and the Miller-Tans method (Miller and Tans 2003), as shown in Figure 5. For periods complying with the quality criteria defined for the Keeling plot analysis, results of the two independent techniques agreed reasonably well, as shown in the correlation diagrams in Figure 5. Keeling plot-derived $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{18}\text{O}$ and SP values varied between -32.5 ‰ and -1.2 ‰, 38.0 ‰ and 65.0 ‰, and 8.4 and 36.8 ‰, respectively; the Miller-Tans analysis resulted in similar source signatures of -29.6 ‰ to 20.3 ‰

($\delta^{15}\text{N}^{\text{bulk}}$), 40.7 ‰ to 84.9 ‰ ($\delta^{18}\text{O}$) and 5.1 ‰ to 35.0 ‰ (SP) for the same period. The results of the Miller-Tans method were rather scattered for periods during when small changes in N_2O concentrations and N_2O isotopic composition precluded Keeling plot analysis (i.e. prior to 22 June). Values of individual Keeling plot derived source signatures can be found in Table 3.

The $\delta^{15}\text{N}\text{-NO}_3^-$ values ranged from 0.13 to 11.42 ‰. Spatial variations of $\delta^{15}\text{N}\text{-NO}_3^-$ across the De-Fen site were relatively large (Figure 5). In the first week of June $\delta^{15}\text{N}\text{-NO}_3^-$ was rather variable with very low values on 9 June but higher $\delta^{15}\text{N}\text{-NO}_3^-$ in the second week. Thereafter it decreased slowly from approx. 10 ‰ to values close to 0 ‰. After the manure application on 12 July a continuous increase of $\delta^{15}\text{N}\text{-NO}_3^-$ was observed, reaching a maximum of approx. 8 ‰ around 24 July.

4. Discussion

4.1 N_2O fluxes and WFPS

Throughout the measurement campaign, the N_2O flux rates were between 70 and 2400 $\mu\text{g N m}^{-2} \text{h}^{-1}$ at De-Fen, and thus of a similar order of magnitude as reported earlier for other intensively fertilized grasslands (Merbold et al., 2014; Wolf et al., 2015; Schäfer et al., 2012). $f(\text{N}_2\text{O})$ showed a clear dependence on the soil water content, with maximum emissions at 90 % WFPS (Figure 6). While for drier soils (WFPS < 60 %) lower but still substantial N_2O fluxes were detected, fluxes declined to their lowest values near water saturation, i.e. when WFPS was close to 100%. The observed relationship between $f(\text{N}_2\text{O})$ and WFPS ($R^2 = 0.92$) can be best described with an exponential function with two terms as given by equation 4:

$$(\text{N}_2\text{O})_{\text{Fitted}} = a \cdot \exp(b \cdot \text{WFPS}) + c \cdot \exp(d \cdot \text{WFPS}) \quad (4)$$

where the coefficients are best approximated by $a = -5.09\text{e-}06$, $b = 0.19$, $c = 15.86$ and $d = 0.04$. This relationship is a strong indicator that the activity of the main source process increases with the soil water content, which is characteristic for denitrification and nitrifier-denitrification (Wrage et al., 2001; Decock and Six, 2013a). Furthermore, the decline of N_2O fluxes at very high WFPS values is in line with this interpretation, because the last step of the denitrification pathway, N_2O reduction to N_2 , is only active under anoxic conditions. This shift from nitrification-dominated to denitrification-dominated N_2O production with increasing WFPS should be reflected in the isotopic signature of the residual N_2O . Indeed, there is a tendency towards high SP values under low (indicating higher nitrification contribution) and high WFPS values (indicating higher N_2O reduction to N_2 rates) (Figure 6). The peak $f(\text{N}_2\text{O})$ was observed on 23 July, a day after a severe precipitation event. The N_2O emission rate of this peak event was 2415 $\mu\text{g N m}^{-2} \text{h}^{-1}$ (average of five replicate flux chambers). Unfortunately, this event cannot be discussed in terms of N_2O isotopocules due to termination of TREX-QCLAS measurements after 22 July 2016.

4.2 On-site performance of TREX-QCLAS

The short term repeatability over 10 target gas (T) measurements was 0.25 ‰, 0.31 ‰, 0.30 ‰ and 0.25 ppb for $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, $\delta^{18}\text{O}$ and N_2O concentration, respectively. This is sufficient to track changes in ambient N_2O close to emission sources as described in this study and superior to most IRMS and laser spectrometer systems (Mohn et al., 2014), but slightly inferior to laboratory experiments using the same system (Ibraim et al., 2018) or earlier versions of preconcentration – QCLAS based approaches (Mohn et al., 2012; Harris et al., 2014; Wolf et al., 2015). The slightly lower repeatability was due to a more compact spectrometer design, which allowed for the integration of the system in a 19-inch rack at the cost of a higher optical noise level and larger drifts due to the harsher conditions in the mobile lab, i.e. higher temperature variations and vibrations.

4.3 Variability of N_2O concentrations and isotopic composition above De-Fen

During the day, the atmospheric boundary layer (ABL) and the lowest part of the ABL (surface layer) are well mixed due to turbulence arising from buoyancy and wind shear (Ibbetson, 1994). At night, stable stratification attenuates vertical mixing

processes, also leading to generally lower horizontal wind speeds. Both entail accumulation of local soil-emitted N₂O in the surface layer. For this reason, daytime N₂O concentrations and isotopic composition mostly reflect the atmospheric background, while the nighttime accumulation reflects the influence of soil-emitted N₂O.

Variations in N₂O, SP, δ¹⁵N^{bulk} and δ¹⁸O follow a diurnal pattern that is in agreement with the variations of N₂O concentrations depicted in Figure 4. Accordingly, average daytime N₂O concentrations, δ¹⁵N^{bulk}, SP and δ¹⁸O of 331.6 ± 1.41 ppb, 6.28 ± 0.30 %, 17.95 ± 0.15 ‰ and 45.54 ± 0.27 ‰, respectively, are in agreement with background measurements at other sites, such as Dübendorf, Switzerland (N₂O: 325.8 ± 3.3 ppb, δ¹⁵N^{bulk}: 6.53 ± 0.14 ‰, SP: 17.95 ± 0.40 ‰, δ¹⁸O: 44.41 ± 0.13 ‰ (Harris et al., 2017)), or Hateruma Island, Japan (decadal mean values for the northern hemisphere of δ¹⁵N^{bulk}: 6.65 ‰, SP: 18.44 ‰, δ¹⁸O: 44.21 ‰ (Toyoda et al., 2013)). Observed changes in N₂O concentrations and isotopic composition at night are within the range of previous studies from agricultural sites (Wolf et al., 2015; Toyoda et al., 2011), but clearly higher than variations measured at 13 m or 95 m above ground in an urban or suburban environment (Harris et al., 2014; Harris et al., 2017).

4.3.1 N₂O footprints

At night, within a stable nocturnal boundary layer, vertical wind speeds and hence tracer transport are low, while lateral wind speeds can be high and constituents like N₂O can be transported over larger distances. As a result, N₂O emissions from other land uses or land cover ~~may~~ have contributed to the observed N₂O isotopic composition. To assess the ~~possible~~ influence of other land use / land cover, the concentration footprint calculated with FLEXPART-COSMO was assessed for periods where the Keeling plot and Miller-Tans approaches were applied. The FLEXPART-COSMO simulations indicate that between 15 % and 45 % of the source sensitivity originates from areas within approximately 300 m to 700 m distance to the sample inlet, respectively (isolines in Figure 7). Highest source sensitivities which amounted to 30% of the total sensitivity were calculated for areas predominately covered by grassland or pasture. Although sources outside this local area contributed more than half of the total emissions and included other land cover such as arable land and forest, the impact of individual source areas was smaller by several orders of magnitude, hence having much less impact on the isotopic source signature. While more than 95 % of the area covered by the 15 % isopleth (bold isolines in Figure 7) corresponds to grasslands, the residual 5 % belongs to a wetland to the northeast of the De-Fen (Figure 7). Furthermore, the 30 and 45 % isopleth's surfaces include approximately 20 % of mixed forest and 5 % wetland along with around 75 % under grassland, underlining further that sensitivities were highest for grassland emitted N₂O.

In addition to the N₂O footprint, the temporal trend of the N₂O concentration at the sampling point was simulated using individual source sensitivities and assuming a homogeneous N₂O flux identical to measured local N₂O fluxes (see section 2.2). Simulated N₂O concentrations were in very good agreement with N₂O concentrations measured by the TREX-QCLAS (SI Figure 10), indicating that the simulated footprint, attributing a substantial part of the emissions to the De-Fen grassland, is representative of the measurement site. Furthermore, N₂O concentration measurements obtained with TREX-QCLAS were in a good agreement with the local N₂O flux measurements (SI Figure 2).

4.4 N₂O source signatures and implicated processes

4.4.1 Comparison Miller-Tans and Keeling plot techniques

Figure 5 shows the temporal trends of the N₂O source signatures, illustrating the potential of this quasi-continuous dataset to identify process changes induced by management events or changing environmental parameters. The dataset also enables a direct comparison of two approaches for extracting the isotopic composition of N₂O emitted from soils based on surface layer measurements, namely the Keeling plot and the Miller-Tans approach. In the first three weeks of the campaign, i.e. under conditions of low N₂O fluxes, the Keeling plot results did not pass the quality criterion ($R^2 > 0.2$ for δ¹⁵N^α and δ¹⁵N^β versus ~~inverse concentration~~), and the source signatures, i.e., the extracted/calculated 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) the extracted isotopic composition of N_2O emitted from soil ($\delta^{15}\text{N}^{\text{bulk}}$, SP, $\delta^{18}\text{O}$) derived from the Miller-Tans method showed relatively large uncertainties (shaded area in Figure 5). Thereafter, N_2O source signatures as estimated with the Keeling plot and Miller-Tans approaches show a comparable trend and mostly agree within the indicated uncertainties without systematic deviations. Overall, the agreement (R^2 value) between the Miller-Tans and Keeling plot results is best for $\delta^{15}\text{N}^{\text{bulk}}$ (0.84), intermediate for SP (0.57) and weakest for $\delta^{18}\text{O}$ (0.39) (Figure 5). The weaker correlation for $\delta^{18}\text{O}$ - N_2O can be explained by a lower analytical data quality as compared to $\delta^{15}\text{N}^{\text{bulk}}$ and SP, exemplified by a higher standard deviation for repeated measurements of the target gas (0.59 ‰ for $\delta^{18}\text{O}$ and 0.41 ‰ for $\delta^{15}\text{N}^{\text{bulk}}$ and SP). The reasoning behind this effect might be that the calibrated range of $\delta^{18}\text{O}$ values (S1, S2) does not cover the isotopic composition of the target and sample gases, because no suitable calibration gas was available. A difference of 7 ‰ in $\delta^{18}\text{O}$ between the two calibration gases is rather small, leading to a relatively high uncertainty in the respective calibration factors.

The base calculation for both the Keeling plot and Miller-Tans is identical and the two methods would yield identical results if every term was known perfectly. However, the uncertainty term is treated differently in the two approaches. The Miller-Tans approach calculates source signatures for individual sample gas measurements (SI Figure 3) and, thus, may be the better choice when the source process or the background N_2O isotopic composition changes rapidly, i.e. during a 24 hour period. However, the large fluctuations of the source signatures (up to 100 ‰, Figure 5) extracted by the Miller-Tans approach prior to 22 June indicate that the uncertainty estimated for the Miller-Tans approach is too optimistic and needs to be reassessed. In addition, it is noteworthy that the Keeling plot approach as presented here, implicitly considers changes in background N_2O concentration from day to day, since one Keeling plot (comprising both N_2O background and N_2O variations) was carried out per day. Therefore, we conclude that the Keeling plot method remains a robust way of estimating source signatures of N_2O emitted from a predominantly agricultural landscape as the one presented here, where variations in background N_2O compared to source contributions can be neglected and changes in source processes generally occur only on long timescales as a response to changes in environmental conditions (e.g. WFPS).

4.4.2 Range of N_2O source signatures

Typical source signatures of biologically produced N_2O are approx. -40 – 0 ‰ and 0 – 40 ‰ for $\delta^{15}\text{N}^{\text{bulk}}$ and SP, respectively, while $\delta^{18}\text{O}$ - N_2O are around 40 ‰ and 70 ‰ for N_2O emitted through grasslands or wetlands, respectively (Toyoda et al., 2017). Accordingly, the $\delta^{15}\text{N}^{\text{bulk}}$ values found in our study are well within literature values of grassland emitted N_2O , while the SP values are rather high. Interestingly, the obtained $\delta^{18}\text{O}$ values were strongly elevated on some occasions and close to those found by Ostrom et al. (2007) in a pure culture experiment in which approx. 80 % of produced N_2O was reduced to N_2 . A correlated increase of the SP, $\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ values, with SP values potentially larger than the endmember value of $32.8 \pm 4\%$, can be explained by N_2O reduction to N_2 , which is particularly active under wet and anaerobic soil conditions (Wrage et al., 2004; Lewicka-Szczebak et al., 2017). Thus, isotopic fractionation during partial N_2O reduction must be taken into account in order to apportion isotopic source signatures of soil-emitted N_2O (Lewicka-Szczebak et al., 2017; Verhoeven et al., 2018). The fractionation factors $\epsilon^{18}\text{O}/\epsilon^{15}\text{N}^{\text{bulk}}$, $\epsilon^{18}\text{O}/\epsilon_{\text{SP}}$ and $\epsilon^{15}\text{N}^{\text{bulk}}/\epsilon_{\text{SP}}$ have been determined in a number of incubation experiments and it has been suggested that their ratios (2.4, 2.8 and 1.2, respectively) 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). Furthermore, $\delta^{18}\text{O}$ - N_2O of denitrification is affected by oxygen exchange between reaction intermediates (NO_3^- , NO_2^-) and soil water as a function of WFPS. Unfortunately, the interpretation of $\delta^{18}\text{O}$ - N_2O is further complicated by oxygen exchange between NO_3^- and soil water (Well et al., 2008; Kool et al., 2011).

4.4.3 N₂O source partitioning using SP and $\Delta\delta^{15}\text{N}^{\text{bulk}}$

An SP-versus- $\Delta\delta^{15}\text{N}^{\text{bulk}}$ (Figure 8(a)) mapping approach as originally presented by Koba et al. (2009) was used to interpret the Keeling plot-derived source signatures with respect to the possible underlying N₂O producing and consuming processes. Here, $\Delta\delta^{15}\text{N}^{\text{bulk}}$ denotes the $\delta^{15}\text{N}$ difference between the product N₂O and its substrate (NO₃⁻). While Koba et al. (2009) applied this approach in the framework of a groundwater study where NO₃⁻ was the only available N₂O substrate, the grassland research site De-Fen showed rather high NH₄⁺ concentrations (Figure 3(b)). Therefore, the N₂O substrate at De-Fen might be either NH₄⁺ for N₂O emitted by nitrification (N) and nitrifier-denitrification (ND) or NO₃⁻ from fungal denitrification (FD) and bacterial denitrification (BD). Within the framework of this study, it was assumed that $\delta^{15}\text{N}\text{-NH}_4^+$ and $\delta^{15}\text{N}\text{-NO}_3^-$ values were in a similar range, i.e. approx. 0 – 15 ‰, in agreement with the literature (Mook, 2002; Holland, 2011). We thus used only the $\delta^{15}\text{N}\text{-NO}_3^-$ values for the substrate isotopic composition. For periods where N₂O emissions were present but no $\delta^{15}\text{N}\text{-NO}_3^-$ values were obtained, the $\delta^{15}\text{N}\text{-NO}_3^-$ values were approximated by linear interpolation. In addition, the concept of Koba et al. (2009) was modified for the two N₂O-emitting domains FD/N and BD/ND using literature values as provided in Table 4. For simplicity, in the remaining part of this section the flux-weighted average values of SP and $\Delta\delta^{15}\text{N}^{\text{bulk}}$ are discussed, while values of individual events can be found in Table 4. Plotting SP vs. $\Delta\delta^{15}\text{N}^{\text{bulk}}$ revealed that there was a trend of increasing SP with decreasing $\Delta\delta^{15}\text{N}^{\text{bulk}}$ values. As indicated in Figure 8 with orange crosses, the flux-averaged SP, $\Delta\delta^{15}\text{N}^{\text{bulk}}$ and $\delta^{18}\text{O}$ values were 23.4, 19.0 and 62.3 ‰, respectively. The slope of the SP-versus- $\Delta\delta^{15}\text{N}^{\text{bulk}}$ linear regression line of -0.85 (solid red arrow in Figure 8(a)) is in agreement with literature values (-0.83 and -1.1) given by Koba et al. (2009) and Toyoda et al. (2017) for partial N₂O-to-N₂ reduction. This observed negative slope, which is in contrast to the grey shaded area anticipated for mixing of N₂O produced by BD/ND and FD/N indicates a major contribution of BD/ND and N₂O reduction to N₂, the final reaction step in the anoxic reduction of NO₃⁻ to N₂. The suspected predominance of denitrification agrees with previous field studies presented by Opdyke et al. (2009), Wolf et al. (2015) and Mohn et al. (2012). SI Figure 9 illustrates contributions of FD/N on the total N₂O emissions for individual accumulation events.

A semi-quantitative source partitioning can be calculated assuming average SP (-0.9 ‰) and $\Delta\delta^{15}\text{N}^{\text{bulk}}$ (18.5 ‰) values for N₂O production by BD/ND and a fixed SP/ $\Delta\delta^{15}\text{N}^{\text{bulk}}$ ratio of -0.83 for N₂O reduction to N₂ (Figure 8(a)). Correspondingly, the simultaneous SP increase and $\Delta\delta^{15}\text{N}^{\text{bulk}}$ decrease during N₂O reduction to N₂ can be interpreted in terms of the N₂O/(N₂O+N₂) product ratio using the Rayleigh fractionation approach of Mariotti et al. (1981). Accordingly, a 90 % reduction of N₂O translates into an increase in SP by 13.6 ‰ assuming an SP fractionation factor (ϵ_{SP}) of -5.9 ‰ 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 N₂O/\(N₂+N₂O\) product ratio \(Lewicka-Szczebak et al., 2015\).](#) A deviation of source signatures from the SP/ $\Delta\delta^{15}\text{N}^{\text{bulk}}$ line can then be interpreted in terms of addition of N₂O produced by additional processes, e.g. FD/N. This interpretation is supported by the relationship between SP and WFPS (Figure 6). Accordingly, the lowest SP values were found at intermediate to high soil water contents (80 – 90 % WFPS) along with maximum N₂O fluxes, while SP values increased towards lower WFPS values, due to the increasing contribution of nitrification, and towards higher WFPS values, due to increasing N₂O reduction to N₂. Furthermore, the fraction of FD/N-derived N₂O increased with NH₄⁺ fertilization, also in agreement with the literature (Toyoda et al., 2011; Koster et al., 2011).

A semi-quantitative interpretation of isotope signatures can be done assuming average source signature values (SP and $\Delta\delta^{15}\text{N}^{\text{bulk}}$) and considering two scenarios (see also SI Figure 4): in scenario 1, BD/ND-produced N₂O is partially reduced to N₂ and the residual N₂O (rN₂O; remaining N₂O after N₂O reduction to N₂) is then mixed with N₂O derived from FD/N (path of solid arrows in Figure 8(a)). In scenario 2, N₂O from BD/ND is mixed with FD/N-derived N₂O before a part of the mixed N₂O is reduced to N₂ (path of dashed arrows in Figure 8(a)). While these scenarios result in equal source signatures, they assign a different relative contributions of the processes involved. The respective N₂O to N₂ reduction rates can be calculated based on the associated shift in SP, which corresponds to the y component of each of the red arrows in Figure 8(a).

For convenience, here we only discuss the reduction rates and source partitioning of the two scenarios for flux-averaged SP and $\Delta\delta^{15}\text{N}^{\text{bulk}}$ values (23.4 and 19.0, respectively), while those of individual events could be estimated analogously (related results given in Table 3). Assuming scenario 1, the SP shift caused by N_2O reduction is equal to 18.0 ‰; resulting in a reduction rate of approx. 95 % assuming $\epsilon_{\text{SP}} = -5.9$ ‰. The remaining 5.4 ‰ SP shift can be explained as the result of mixing the rN_2O with FD/N-derived N_2O . A 5.4 ‰ SP shift corresponds to approx. 38 % contribution of FD/N-derived N_2O with the residual N_2O emitted by BD/ND. Note that the FD/N contribution is less than 1 % when accounting for the total N_2O production, i.e. the N_2O before partial reduction to N_2 . In contrast, in scenario 2, the FD/N-derived N_2O is mixed with BD/ND-derived N_2O first. This mixing induces a SP shift of approx. 13.0 ‰, which is given by the y-coordinate of the intersection of the mixing-line and the reduction line of the mean source signatures. However, since no N_2O reduction to N_2 has occurred yet at this point, this shift corresponds to 39 % contribution of FD/N to total N_2O production. The remaining 10.4 ‰ SP shift is then subject to reduction of N_2O to N_2 , corresponding to approx. 83 % reduction of N_2O to N_2 .

4.4.4 N_2O source partitioning using SP and $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$

Identification of the processes producing and consuming N_2O was also done using an adapted SP-versus- $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ mapping approach (Figure 8(b)) as previously presented by Lewicka-Szczebak et al. (2017). This approach was suggested because the values of $\delta^{18}\text{O}-\text{N}_2\text{O}$ from BD/ND and FD/N are less variable than those of $\delta^{15}\text{N}-\text{N}_2\text{O}$. The lower variability is indicated by the smaller BD/ND and FD/N boxes in Figure 8(b) compared to Figure 8(a); thus, 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 \(Kool et al., 2011\).](#) The approach was slightly modified using the values presented in Table 4 to match the FD/N and the BD/ND domains according to Figure 8(a) with regard to SP values. In this approach, $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ represents the difference between the $\delta^{18}\text{O}$ values of the product (N_2O) and the substrate (H_2O). Since no measurements for $\delta^{18}\text{O}-\text{H}_2\text{O}$ were available, we used a value of -8 ‰ in accordance with Xiahong et al. (2009). Values obtained for $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ were clearly higher than previously observed in grassland soils (Wrage et al., 2004; Wolf et al., 2015; Snider et al., 2012) but particularly close to $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ values from studies related to wetland ecosystems (Toyoda et al., 2017; Snider et al., 2009), likely reflecting the fact that the study site was in the vicinity of a wetland (see section 4.3.1 and Wolf et al., 2017) and often flooded due to extraordinary precipitation events throughout the measurement period.

In the mapping approach suggested by Lewicka-Szczebak et al. (2017), two scenarios are considered to estimate the shift in N_2O isotopic composition due to N_2O reduction to N_2 . In Figure 8(b), the y-component of the red arrows represents the SP shift that was caused by N_2O reduction to N_2 . Knowledge of the degree to which SP has been changed due to fractionation during N_2O reduction is a prerequisite for determining the relative contributions of the process groups BD/ND and FD/N using a simple mixing model and the SP values given in Table 4. Scenario 1 assumes that BD/ND-derived N_2O is partly reduced to N_2 before mixing with N_2O originating from FD/N, while scenario 2 assumes the reverse order (i.e. first mixing, then N_2O reduction). The two scenarios yield different reduction rates and proportions of BD/ND- versus FD/N-derived N_2O , although final N_2O source signatures are identical. A quantitative estimate of source contributions was conducted for the flux averaged mean values of 23.4 and 62.3 ‰ for SP and $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ as follows: using scenario 1 (depicted with solid arrows in Figure 8(b)), N_2O reduction to N_2 has led to an SP shift of approx. 17.3 ‰, which corresponds to approx. 95 % N_2O reduction. The residual SP shift of 6.1 ‰ would be caused by the mixing of FD/N-derived N_2O with the rN_2O , corresponding to approx. 19 % FD/N-derived N_2O compared to BD/ND. The 19 % mentioned here only accounts for the mixing with the rN_2O but not for the initially produced N_2O . Taking into account that 95 % of the N_2O initially produced was reduced to N_2 reveals that the FD/N contribution to total N_2O production was below 1 %. In contrast, in scenario 2, the FD/N-derived N_2O is mixed into the

N₂O pool before N₂O reduction to N₂ has occurred. Therefore, approx. 29 % FD/N-derived N₂O is needed to account for a 16 ‰ SP shift in the produced N₂O. In this case, the residual SP shift of 9 ‰ is due to N₂O reduction, corresponding to a 79 % reduction rate with $\epsilon_{SP} = -5.9 \text{ ‰}$.

4.4.5 Comparison of the results obtained with the SP-vs.- $\Delta\delta^{15}\text{N}^{\text{bulk}}$ and SP-vs.- $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ approaches

5 In summary, the two scenarios lead to different calculated relative amounts of N₂O produced by BD/ND and FD/N as well as the emissions ratio of N₂O to N₂. The average contribution of FD/N to the N₂O emissions was 42 and 34 % according to the SP-vs.- $\Delta\delta^{15}\text{N}^{\text{bulk}}$ and SP-vs.- $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ approaches, respectively (distributions given in SI Figure 8, temporal trend given in SI Figure 9). However, regardless of the approach and scenario, the obtained rN₂O values were very low, indicating that N₂O reduction played a major role. The median of the rN₂O values obtained with the SP-vs.- $\Delta\delta^{15}\text{N}(\text{NO}_3^-/\text{N}_2\text{O})$ approach
10 was 0.02 for scenario 1 and 0.10 for scenario 2. Utilizing the SP-vs.- $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ approach, those values were even slightly lower and corresponded to 0.01 in scenario 1 and 0.02 in scenario 2 (SI Figure 5). Interestingly, the two rN₂O values calculated for scenario 1 with the two approaches were highly correlated, while those for scenario 2 were not correlated (SI Figure 5). This indicates that scenario 1 more likely occurred at our site.

The rN₂O values were also compared to the WFPS (SI Figure 6) and to the ambient temperature (SI Figure 7). A positive
15 correlation should be expected between WFPS and the N₂O reduction rates, resulting in a negative correlation between WFPS and rN₂O values. However, observed rN₂O values did not reflect this hypothesis. Similarly, one could expect a positive correlation between rNit (the fraction of measured N₂O originating from fungal denitrification or nitrification, therefore with high SP values) and rN₂O, since the contributions of fungal denitrification and nitrification should be higher under conditions that are disadvantageous for N₂O reduction. However, also this hypothesis was refuted by these results.

20 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)~~Our findings confirm that natural abundance isotope studies of N₂O provide an effective way to disentangle N₂O production pathways and to estimate N₂O reduction rates.~~ However, the complexity of N₂O production pathways could not be fully accounted for, in particular abiotic processes; for example, N₂O production by NH₂OH oxidation (Heil et al., 2014) or NO₂⁻ reduction
25 (Wei et al., 2017a) were not considered. These reactions yield N₂O with high (34 – 35 ‰) or variable (8 – 12 ‰) SP and might therefore be falsely interpreted as nitrification-derived N₂O. In addition, the approach cannot resolve individual processes with high SP, i.e. fungal denitrification versus nitrification, or low SP, i.e. heterotrophic versus nitrifier denitrification, due to overlapping source signature regions. Furthermore, nitrite (NO₂⁻) and nitric oxide (NO) could have acted as the substrate instead of NO₃⁻, leading to different fractionation factors from those incorporated for NO₃⁻.

30 4.4.6 Effect of manure application on the source signatures

In addition to the mapping approaches discussed above, N₂O source signatures can be interpreted with respect to management events. After the manure application on 12 July and rainfall events in the days thereafter a strong shift to lower SP and $\delta^{15}\text{N}^{\text{bulk}}$ values was observed (Figure 5). The negative shift in $\delta^{15}\text{N}^{\text{bulk}}$ might be explained by changes in the isotopic composition of the applied precursors, by an enhanced fractionation due to higher substrate availability or changes in process conditions (e.g.
35 WFPS, see sections above)~~or by an enhanced fractionation due to higher substrate availability.~~ However, since SP is considered to be process-specific and substrate-independent (Yoshida and Toyoda, 2000), it should not change as a response to a change in the substrate isotopic composition or by enhanced fractionation. There are two alternative explanations for the lower SP and $\delta^{15}\text{N}^{\text{bulk}}$ values. The increase in NH₄⁺ concentration after manure application was followed by an increase of NO₃⁻ concentration. This indicates a stimulation of nitrification. An increase of N₂O production due to nitrification would be associated with
40 higher SP values. However, the nitrate produced during nitrification may have been used as substrate for denitrification, given

the increase in WFPS due to intensive rainfall events. While N_2O is an obligatory product of denitrification, and only a by-product of nitrification, the N_2O yield of denitrification may have been higher, and the increase of SP due to nitrification may have been outweighed by the decrease of SP due to denitrification. Secondly, N_2O reduction to N_2 could be slightly reduced due to an elevated NO_3^- availability (Wang et al., 2013). A parallel increase in WFPS and N_2O flux rates after the manure application combined with low FD/N fraction in the period 17 July to 22 July supports the hypothesis that both effects might have contributed to a decrease in SP values.

5. Conclusions

Real-time and in-situ N_2O concentration and isotope measurements were successfully performed at a temperate humid grassland site in Southern Germany with a coupled preconcentration technique and quantum cascade laser absorption spectroscopy (TREX-QCLAS) based method in a two-month period between June and July 2016. Concentrations of soil-extracted NH_4^+ , NO_3^- and $\delta^{15}N-NO_3^-$ values were taken into account to interpret the N_2O measurements. This study provides new insights into the isotopic composition of grassland-emitted N_2O under changing soil environmental and management conditions. Our results support previous observations that bacterial denitrification/ nitrifier denitrification (BD/ND) is the dominant N_2O -emitting source in permanent grassland soils. The measured N_2O isotopic composition, in particular the intramolecular isotopic composition, or site preference (SP), can be explained by taking into account partial N_2O reduction to N_2 . Two distinct approaches were used to estimate the relative contributions of BD/ND and FD/N as well as the N_2O reduction rates. The average FD/N contribution to the total N_2O emissions was 42 and 34 % with the SP-vs.- $\Delta\delta^{15}N^{bulk}$ and SP-vs.- $\Delta\delta^{18}O$ approaches, respectively, indicating that denitrification dominated the N_2O emissions. N_2O reduction rates were estimated by calculating the residual N_2O fractions (rN_2O), i.e. the fraction of remaining N_2O after N_2O reduction to N_2 has occurred. Two distinct scenarios were considered for each of the two approaches, resulting in the four rN_2O values of 0.02, 0.10, 0.02 and 0.01. The low values underline both the dominant role of denitrification in N_2O production at the grassland site and the large extent to which N_2O reduction occurred during the measurement period.

This study demonstrates the suitability of the TREX-QCLAS for in-situ analysis of the isotopic composition of soil-emitted N_2O in terrestrial ecosystems. While the observations presented here integrate N_2O fluxes and thus source processes at the plot scale, the interpretation of source processes in future studies will be resolved at smaller spatial scales, for example by a combination of TREX-QCLAS with static flux chambers and the implementation of an isotopic biogeochemical soil model. In particular, an approach based on the combination of the TREX-QCLAS method with static flux chambers would allow us to distinguish between the two scenarios (reduction then mixing vs. mixing then reduction) discussed in this study.

6. Acknowledgements

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7. Tables and Figures

7.1 List of table captions

	Table 1 Soil characterization of the research site Fendt. Values are given for the topsoil (0-10 cm) according to Kiese et al. (2018).	26
5	Table 2 Mole fractions and isotopic compositions of standard 1 (S1), standard 2 (S2) and target (T) gas cylinders that were used in this study. N ₂ O mole fractions were analysed at Empa against standards from commercial suppliers (S1, S2) or from the National Oceanic and Atmospheric Administration/ Earth System Research Laboratory/ Global Monitoring Division (NOAA/ ESRL/ GMD) (T). N ₂ O isotopic composition was also analysed at Empa against standards previously analysed by Sakae Toyoda/ Tokyo Institute of Technology. The standard gas S1 is used for drift correction, and standard gas S2 for a span-correction of measured δ values. The indicated error is one standard deviation for replicate sample measurements and does not include the uncertainties of the calibration chain.....	26
10	Table 3 Characterization of the accumulation events. Columns refer to date, water filled pore space (WFPS), observed N ₂ O fluxes ($f(N_2O)_{GC-ECD}$), Keeling plot-derived SP values, obtained net isotope effect for $\Delta\delta^{15}N(NO_3^- - N_2O)$, obtained net isotope effect for $\Delta\delta^{18}O(N_2O/H_2O)$, fraction of remaining N ₂ O after N ₂ O reduction (rN ₂ O. sc11 = SP vs. $\Delta\delta^{15}N(NO_3^- - N_2O)$ approach scenario 1, sc12 = SP vs. $\Delta\delta^{15}N(NO_3^- - N_2O)$ approach scenario 2, sc21 = SP vs. $\Delta\delta^{18}O(N_2O/H_2O)$ approach scenario 1, and sc22 = SP vs. $\Delta\delta^{18}O(N_2O/H_2O)$ approach scenario 2). Results are sorted by descending WFPS.	27
15	Table 4 Characterization of the lower and upper range for SP, $\Delta\delta^{15}N$ and $\Delta\delta^{18}O$ for the two N ₂ O-emitting domains fungal denitrification/ nitrification (FD/N) and bacterial denitrification (nitrifier denitrification (BD/ND) according to literature. All values are given in per mil (‰).	28

7.2 Tables

Table 1 Soil characterization of the research site Fendt. Values are given for the topsoil (0-10 cm) according to Kiese et al. (2018).

Soil type	Texture sand/ silt/ clay (%)	Bulk density ($g \cdot cm^{-3}$)	pH (a.u.)	Total nitrogen (%)	Soil organic carbon (%)
Cambic stagnosol	27 ± 2 / 43 ± 1/ 30 ± 1.3	1.1 ± 0.1	5.7 ± 0.3	0.43 ± 0.01	3.9 ± 0.4

Table 2 Mole fractions and isotopic compositions of standard 1 (S1), standard 2 (S2) and target (T) gas cylinders that were used in this study. N₂O mole fractions were analysed at Empa against standards from commercial suppliers (S1, S2) or from the National Oceanic and Atmospheric Administration/ Earth System Research Laboratory/ Global Monitoring Division (NOAA/ ESRL/ GMD) (T). N₂O isotopic composition was also analysed at Empa against standards previously analysed by Sakae Toyoda/ Tokyo Institute of Technology. The standard gas S1 is used for drift correction, and standard gas S2 for a span-correction of measured δ values. The indicated error is one standard deviation for replicate sample measurements and does not include the uncertainties of the calibration chain.

Gas Type	$\delta^{15}N^{\alpha}$ (‰)	$\delta^{15}N^{\beta}$ (‰)	$\delta^{18}O$ (‰)	N ₂ O mole fraction (μ ppm)
S1	15.51 ± 0.30	-3.25 ± 0.20	34.97 ± 0.16	90.15 ± 0.005
S2	-63.08 ± 0.78	-59.81 ± 0.48	27.99 ± 0.28	90.84 ± 0.024
T	15.25 ± 0.09	-3.37 ± 0.13	43.80 ± 0.17	329.250 <u>329</u> ± 1.04 <u>0.001</u>

*Values of S1 and S2 given in ppm, value of T given in ppb

Table 3 Characterization of the accumulation events. Columns refer to date, water filled pore space (WFPS), observed N₂O fluxes ($f(\text{N}_2\text{O})_{\text{GC-ECD}}$), Keeling plot-derived SP values, obtained net isotope effect for $\Delta\delta^{15}\text{N}(\text{NO}_3^--\text{N}_2\text{O})$, obtained net isotope effect for $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$, fraction of remaining N₂O after N₂O reduction (rN₂O. sc11 = SP vs. $\Delta\delta^{15}\text{N}(\text{NO}_3^--\text{N}_2\text{O})$ approach scenario 1, sc12 = SP vs. $\Delta\delta^{15}\text{N}(\text{NO}_3^--\text{N}_2\text{O})$ approach scenario 2, sc21 = SP vs. $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ approach scenario 1, and sc22 = SP vs. $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ approach scenario 2). Results are sorted by descending WFPS.

5

Event number (a.u.)number	WFPS (%)	Date (2016) (dd.mmm.)	$f(\text{N}_2\text{O})_{\text{GC-ECD}}$ ($\mu\text{g N m}^{-2} \text{ h}^{-1}$)	SP _{Keeling} (‰)	$\Delta\delta^{15}\text{N}(\text{NO}_3^--\text{N}_2\text{O})$ (‰)	$\Delta\delta^{18}\text{O}(\text{N}_2\text{O}/\text{H}_2\text{O})$ (‰)	rNit1 (a.u.)	rNit2 (a.u.)	rN ₂ O_sc11 (a.u.)	rN ₂ O_sc12 (a.u.)	rN ₂ O_sc21 (a.u.)	rN ₂ O_sc22 (a.u.)
1	98.2 ± 0.2	22.Jun.	50	31.9 ± 1.9 4	5.3 ± 6.8 4	74.2 ± 2.6 4	0.38	0.43	< 0.01	0.03	< 0.01	0.13
2	97.2 ± 0.6	23. Jun.	147	36.7 ± 2.0 2	14.4 ± 4.6 0	73.0 ± 2.3 2	0.50	0.58	< 0.01	0.04	< 0.01	0.18
3	95.3 ± 0.9	24. Jun.	318	22.4 ± 4.2 0	11.3 ± 5.3 4	51.3 ± 7.0 0	0.36	0.36	0.04	0.10	0.14	0.41
4	93.2 ± 0.8	17. Jul.	259	8.4 ± 3.7 2	24.6 ± 6.2 9-3	65.6 ± 3.3 6-4	0.35	0.25	0.76	0.81	0.11	0.21
5	90.2 ± 1.2	18. Jul.	260	34.3 ± 5.2 0	18.7 ± 6.3 4	70.7 ± 2.0 9-1	0.51	0.53	< 0.01	0.07	< 0.01	0.19
6	87.7 ± 12.6	12. Jul.	236	13.0 ± 4.4 6-5	25.9 ± 4.0 6-1	46.6 ± 3.3 7-4	0.37	0.14	0.51	0.64	0.26	0.36
7	85.5 ± 2.7	19. Jul.	560	25.7 ± 0.7 8	16.9 ± 4.6 2	74.5 ± 2.7 4	0.41	0.24	0.02	0.12	0.02	0.01
8	73.8 ± 9.3	20. Jul.	411	16.7 ± 1.99 <u>2.0</u>	18.7 ± 5.6 59	56.7 ± 2.4 8-5	0.36	0.15	0.16	0.28	0.12	0.21
9	70.3 ± 1.6	09. Jul.	596	29.6 ± 1.0 4	11.5 ± 5.0 4-95	70.7 ± 3.5 6	0.40	0.39	0.01	0.06	0.01	0.15
10	66.0 ± 1.9	21. Jul.	475	33.2 ± 7.8 6-9	21.1 ± 3.9 85	62.3 ± 6.6 4	0.53	0.58	< 0.01	0.10	< 0.01	0.32
11	64.1 ± 2.1	10. Jul.	340	33.0 ± 3.9 0	10.1 ± 5.0 4-97	60.0 ± 2.0 4	0.42	0.60	< 0.01	0.04	< 0.01	0.38
12	58.8 ± 1.3	11. Jul.	251	15.7 ± 0.7 4	29.5 ± 4.8 2	55.8 ± 1.0 6-1	0.41	0.13	0.53	0.70	0.14	0.21

Table 4 Characterization of the lower and upper range for $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{18}\text{O}$ boundaries for the two N_2O -emitting domains fungal denitrification/ nitrification (FD/N) and bacterial denitrification (nitrifier denitrification (BD/ND) according to literature. All values are given in per mil (‰).

Parameter	FD/N	BD/ND	Literature
SP	29.8 ⁱ – 34.5 ⁱ	-5.0 ⁱⁱ – 3.2 ⁱⁱ	i) Denk et al. (2017); ii) Lewicka-Szczebak et al. (2017)
$\Delta\delta^{15}\text{N}$	30.9 ⁱⁱⁱ – 68.0 ^{iv}	0.0 ^{iv} – 37 ^{iv}	iii) Rohe et al. (2014) and iv) Koba et al. (2009)
$\Delta\delta^{18}\text{O}$	35.6 ^v – 55.2 ^v	17.4 ^v – 26.5 ^v	v) Lewicka-Szczebak et al. (2017)

Notes: i) 29.8 and 34.5 refer to the 0.25 and 0.75 quantiles of all values compiled by Denk et al. (2017) in Table S12 for the indices 1 – 3. iii) Lowest absolute isotope effect (η) of NO_3^- reduction to N_2O by fungal denitrification as found by Rohe et al. (2014). iv) Taken from Koba et al. (2009) (referring to Yoshida (1988))

7.3 List of figure captions

Figure 1 Map of research site De-Fen with nearby farm buildings (yellow), streets and country lanes (grey), ditches (blue), the mobile laboratory (filled red square), 2-m sample inlet for TREX-QCLAS measurements, (red dot), area of flux chambers and soil water content measurements (dashed red square), precipitation gauges (open triangles) and location of PT-100 sensors (open squares)..... 30

Figure 2 Instrumental setup for semi-continuous analysis of N_2O isotopes by coupled preconcentration laser spectroscopy (TREX-QCLAS) (Ibraim et al., 2018), including peripherals for the conditioning of the sample gas. Consecutive sample gas treatments include dehumidification by permeation drying, adjustment of sample gas pressure with a pressure release valve after the membrane pump, and CO_2 / H_2O removal using Ascarite / $\text{Mg}(\text{ClO}_4)_2$ traps and filtering for particles using a sintered metal filter. An automatized multiposition valve enables us to switch between eight different Ascarite traps and thus reduces the maintenance effort to one visit per eight days. The indicated gases are: target gas (T), synthetic air (SA), standard gas 1 (S1) and standard gas 2 (S2). CO is removed from the analyte gases using a Sofnocat catalyst (type 423, Molecular Products LTD). At the top right, a full measurement cycle is given. Letters on the y axis correspond to different gas types: standard 1 (S1), standard 2 (S2) sample (S) and target (T) gases. The x axis gives the elapsed time in minutes. The full measurement cycle lasts approx. four hours, which results in a frequency of approx. 1 hr^{-1} for ambient air measurements. 30

Figure 3 (a) Average N_2O flux ($f(\text{N}_2\text{O})$) as measured by five replicate flux chambers coupled to a GC-ECD. (b) Concentration of NH_4^+ - N and NO_3^- - N. Up to 8 nodes across the De-Fen site were sampled twice per week, except in the week of fertilization, when sampling frequency was increased. Therefore, variability within a sampled day refers to spatial variability across the De-Fen site on the given day. (c) Observed WFPS (red) and precipitation (blue). (d) Observed ambient (2 m above ground) and soil (2 - 6 cm below ground) temperature. The blue dashed line indicates a cutting event and the red line indicates a manure application Blue and red dashed lines refer to a cutting event and to a manure application, respectively. 31

Figure 4 Time series of N_2O concentrations (a), $\delta^{15}\text{N}^\alpha$ (b), $\delta^{15}\text{N}^\beta$ (c) and $\delta^{18}\text{O}$ (d), respectively. The left axes give concentrations and isotope delta values on the respective scales, and while the right axis shows axes depict the difference to background conditions values ($\Delta X = X_{\text{measured}} - X_{\text{background}}$, where X refers to N_2O , $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ or $\delta^{18}\text{O}$, respectively). At the top right, histogram plots of the four quantities are given. Coloured symbols indicate ambient air samples (S) from 2 m above ground, and black symbols refer to the corresponding measurements of the target gas (T, Table 2). Shaded areas indicate one standard deviation (σ) as calculated for three consecutive measurements of T. Standard deviations for the complete measurement period are given on the right, in coloured font for S and in black for T. The data gap in the middle of the campaign was due to a hard disk failure of the TREX-QCLAS system computer. The vertical blue dashed lines indicates a mowing cutting event on 04 July 2016, while the red dashed line indicates a manure application on 12 July 2016. 32

Figure 5 Temporal trend of $\delta^{15}\text{N}$ of soil-extracted NO_3^- at eight different nodes (N1-N8) near the N_2O flux and isotope measurements at De-Fen, indicating large spatial variability. In (a), concentration-weighted average values (red filled symbols) and their uncertainty (one standard deviation) are given. Source signatures (b) $\delta^{15}\text{N}^{\text{bulk}}$, (c) SP and (d) $\delta^{18}\text{O}$ of soil-emitted N_2O derived from the Miller and Tans (2003) approach (red crosses) and the Keeling (1961, 1958) plot approach (blue filled symbols) are given. Uncertainties are indicated as pale red shaded areas for the Miller-Tans approach and error bars for the Keeling plot approach (one standard deviation with a Monte Carlo model). The blue dashed line shows the cutting event, while the red dashed line indicates the manure application. Three panels on the right: correlation diagram of results derived from the Miller and Tans (2003) and the Keeling (1961, 1958) plot approaches. The dashed line corresponds to the 1:1 slope. 33

Figure 6 (a) Noon-to-noon average N_2O flux rates $f(\text{N}_2\text{O})$ versus water filled pore space (WFPS) from the grassland site De-Fen. Indicated uncertainties represent variations of $f(\text{N}_2\text{O})$ and WFPS within one day. Results for individual chambers are given in SI Figure 1. N_2O fluxes were highest at 85 – 92 % WFPS. The red dashed line corresponds to a two-term exponential fit of the data shown here. (b) SP as a function of WFPS. Lowest SP values were found in the range of 85 – 95 % WFPS, which corresponds to the highest N_2O fluxes. The red dashed line depicts a double exponential fit of the data shown here (this fit was not significant). The model used to fit the data corresponds to $y = a \cdot \exp(b \cdot x) + c \cdot \exp(d \cdot x)$ (coefficients a , b , c and d are given in the main text). 33

Figure 7 The average footprint area as calculated by the FLEXPART-COSMO model. The model was driven with local wind vectors and observed N_2O flux rates, and indicates a major contribution of soils south-west of the ambient air inlet. The source sensitivities Tau (τ) are given as the product of residence time (in seconds) and inverse atmospheric density (in $\text{m}^3 \cdot \text{kg}^{-1}$). Isolines enclose the areas of largest source sensitivities summing up to 15, 30 and 45 % (decreasing thickness of lines) of the total simulated source sensitivity. The dashed rectangle indicates the area depicted in Figure 1. Individual source signatures for the 12 events are provided in the supplements (SI Figure 11). 34

Figure 8 Source signatures of soil-emitted N_2O for 22 to 24 June (stars 1-3), July 9 to 12 (squares 6, 9, 11 and 12) and July 17 to 21 (hexagons 4, 5, 7, 8 and 10). The colour coding of the symbols refers to WFPS, where blue corresponds to high and red corresponds to low WFPS (exact values in Table 3). The source signatures of fungal denitrification- and nitrification-derived N_2O (FD/N) and bacterial denitrification- and nitrifier denitrification-derived N_2O (BD/ND) are highlighted with rectangles according to the values given in Table 4, and the shaded area represents the mixing region of the two domains. The orange cross indicates the flux averaged values of the respective source signatures. Red arrows denote the path of partial N_2O reduction to N_2 , while black arrows indicate the direction of mixing with FD/N-derived N_2O . Solid arrows indicate scenario 1 (first reduction, then mixing), while dashed arrows indicate scenario 2 (first mixing, then reduction). (a) SP versus $\Delta\delta^{15}\text{N}$ map according to Koba et al. (2009), where $\Delta\delta^{15}\text{N} = \delta^{15}\text{N}\text{-NO}_3^- - \delta^{15}\text{N}\text{-N}_2\text{O}$ (b) SP versus $\Delta\delta^{18}\text{O}$ of soil-emitted N_2O according to Lewicka-Szczebak et al. (2017), where $\Delta\delta^{18}\text{O} = \delta^{18}\text{O}\text{-N}_2\text{O} - \delta^{18}\text{O}\text{-H}_2\text{O}$, 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. SP versus $\Delta\delta^{18}\text{O}$ of soil-emitted N_2O according to Lewicka-Szczebak et al. (2017), where $\Delta\delta^{18}\text{O} = \delta^{18}\text{O}\text{-N}_2\text{O} - \delta^{18}\text{O}\text{-H}_2\text{O}$. An exemplary illustration is provided in the supplementary (SI Figure 4). 34

7.4 Figures

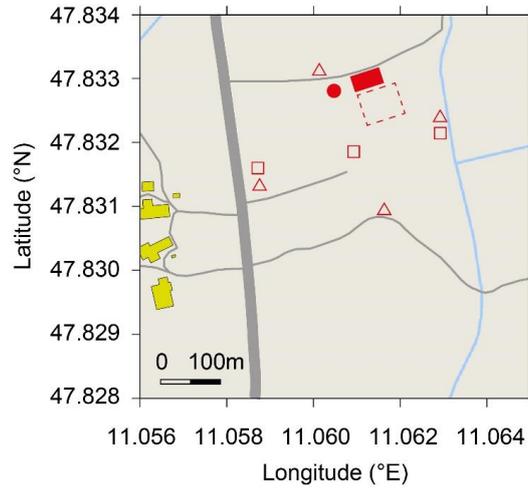


Figure 1 Map of research site De-Fen with nearby farm buildings (yellow), streets and country lanes (grey), ditches (blue), the mobile laboratory (filled red square), 2-m sample inlet for TREX-QCLAS measurements (red dot), area of flux chambers and soil water content measurements (dashed red square), precipitation gauges (open triangles) and location of PT-100 sensors (open squares).

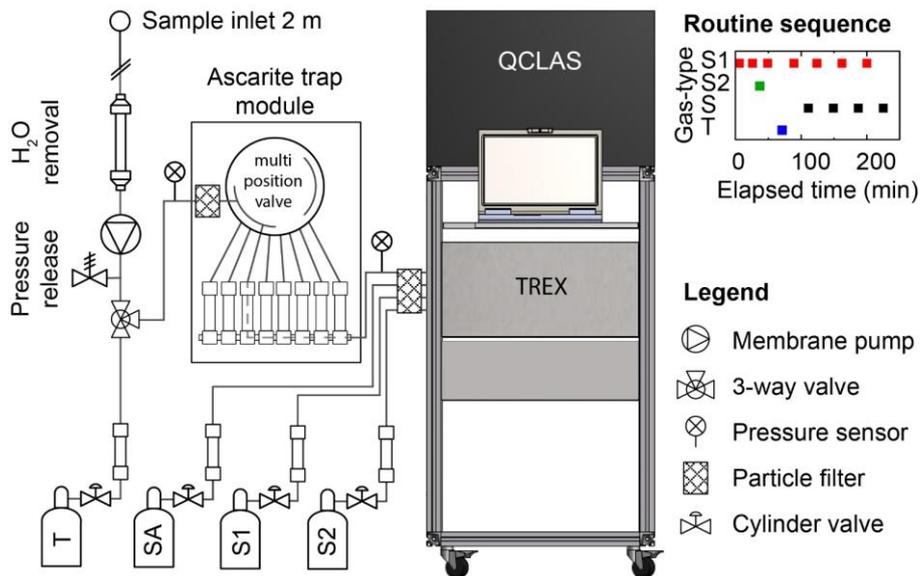
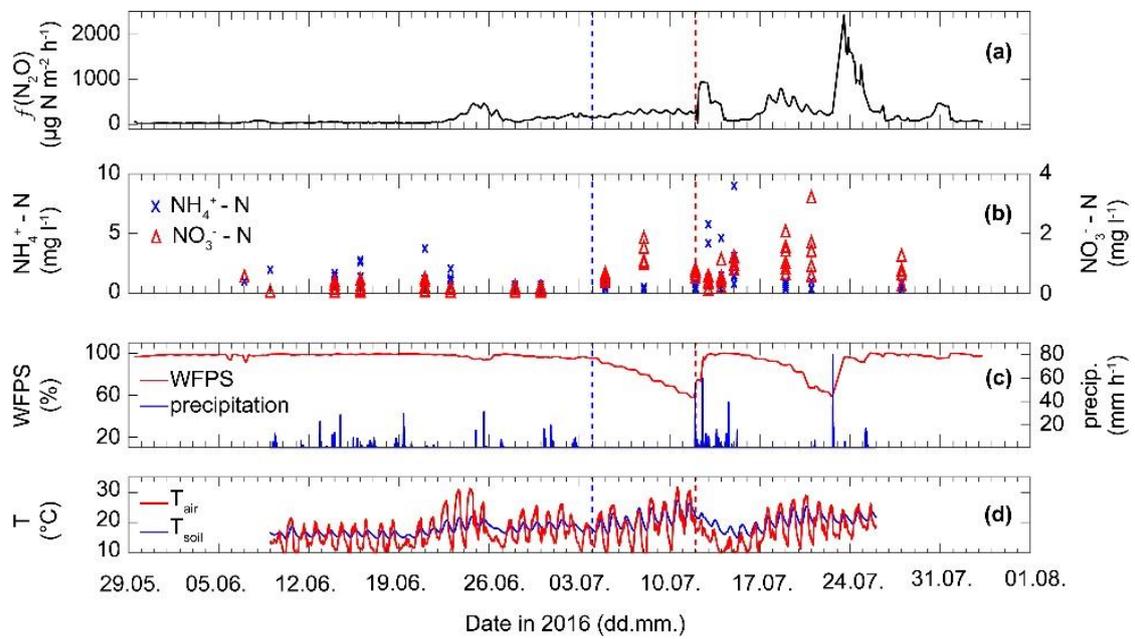


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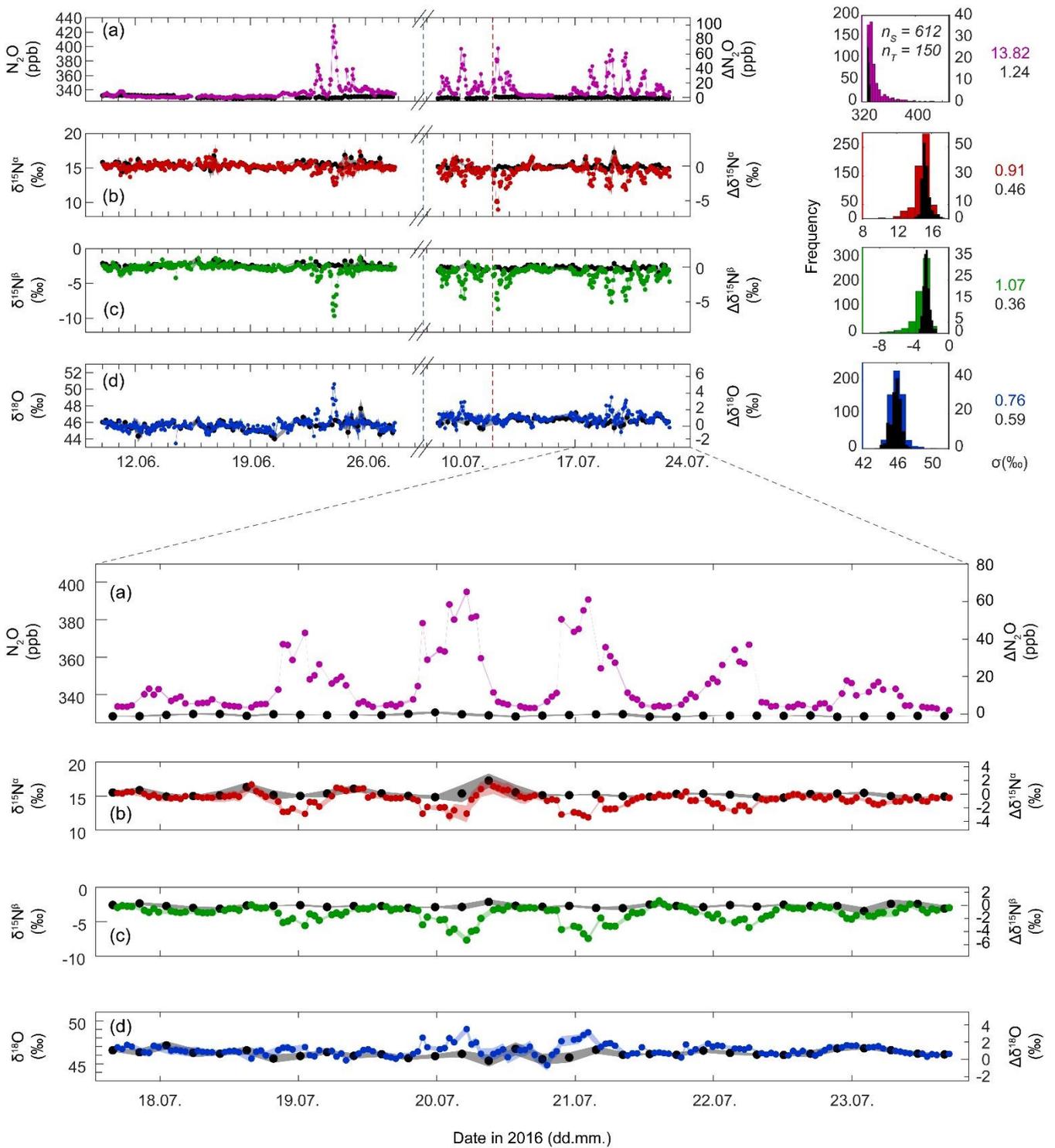


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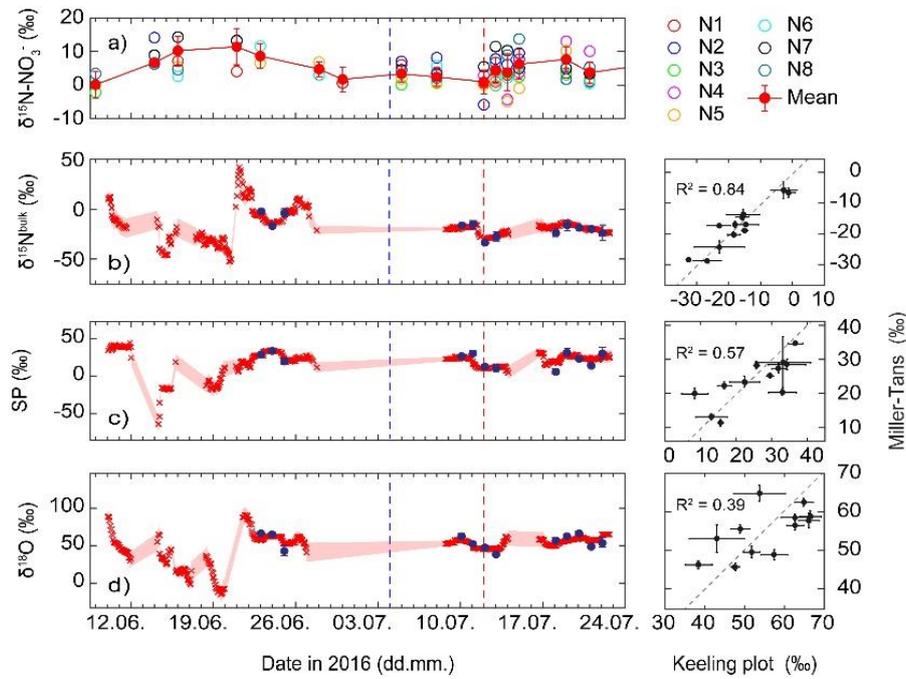


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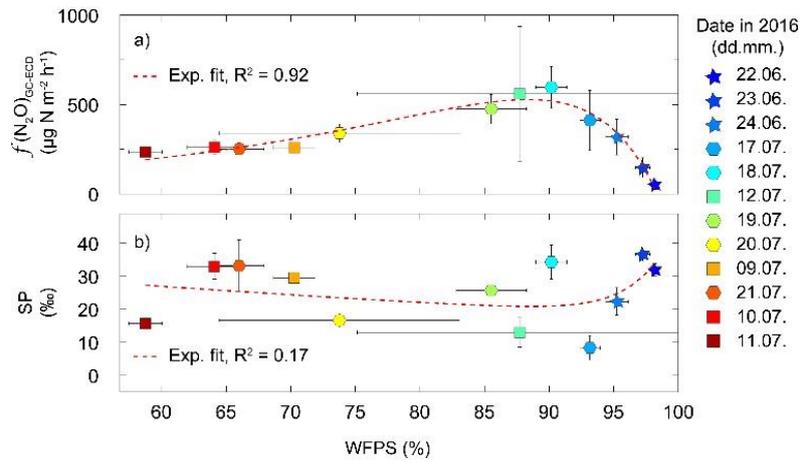


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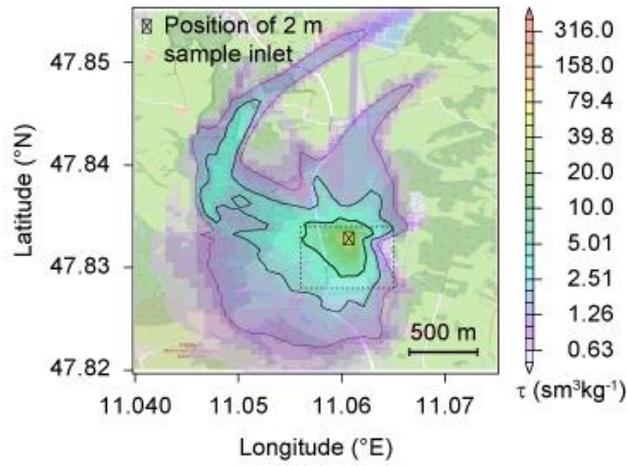


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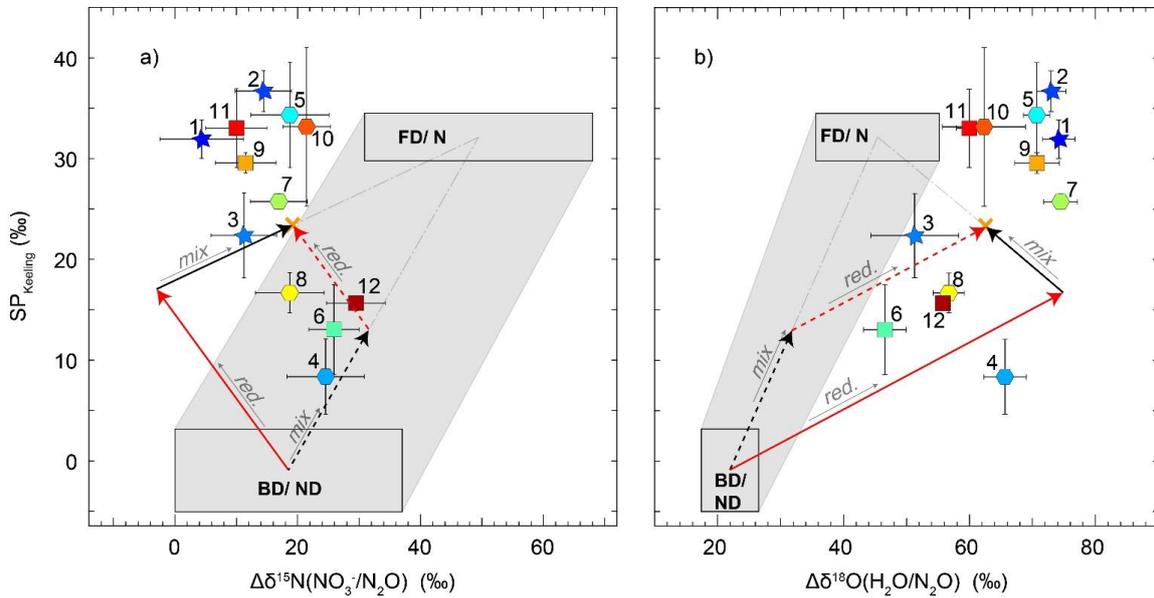


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