Interactive comment on "Denitrification in soil as a function of oxygen supply and demand at the microscale" by Lena Rohe et al.

Anonymous Referee #3
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We thank reviewer #3 for constructive suggestions and comments on our manuscript.
The authors' answer is in italic font.

Reconsidering our data in detail revealed a mistake in calculating the fluxes of CO_2, N_2O and (N_2O+N_2). This error occurred because of wrong parentheses in the calculation. Correcting the calculation revealed increased values of fluxes by a constant factor compared to the previous values. All calculated fluxes have been corrected, having effects on CO_2, N_2O and (N_2O+N_2) fluxes, N loss and Figure 3, Figure 5 (will be removed to Supplementary Material), Figure S1, S3, Table S1 and S4, and the explained variability of N_2O and (N_2O+N_2) fluxes (calculated by the partial least square regression; PLSR) (Figure 8, Figure S7 and Table S2). We want to point out, that the values of fluxes are higher in the revised version, although the course of CO_2, N_2O and (N_2O+N_2) fluxes over incubation time did not change. We apologize very much for this mistake, but the changes made because of the increased fluxes did not affect the interpretation of data or statements of our study.

In the meantime we were able to calculate the ansvf ($ansvf_{cal}$) from parallel incubations using (N_2O+N_2) fluxes during oxic conditions and after switching to anoxic conditions (Supplementary Material). Therefore, instead of reporting $ansvf_{cal}$ based on the comparison between oxic and anoxic (N_2O+N_2) fluxes of two different incubation experiments, we now report values based on fluxes of the same experiment which we consider more reliable. Although $ansvf_{cal}$ values changed slightly our previous conclusions remain unchanged.

This study aimed to explore the controlling factors (soil organic matter, aggregate size, water saturation) of the denitrification process (N2O/N2) at microscopic scale using new approaches of X-ray computed tomography and 15N tracer incubation. They found that N2O/N2 fluxes could be well predicted by anaerobic soil volume fraction (ansvf, O2 supply) and CO2 release (O2 demand). This findings would expand our understanding of how the N2O and N2 are formed in soils. In general, the experimental design is clear, and the manuscript is well written. However, there are some concerns about the methodology and data interpretation.

Major comments

1.The authors selected two types of soils with contrasting soil properties, including soil organic matter contents, soil texture, soil pH and etc., so it is unclear why the authors concluded the differences in denitrification (N2O and N2O+N2 fluxes) between two investigated soils were triggered by different respiration rates due to different SOM content rather than other properties.

Main drivers for soil respiration are temperature, water saturation, oxygen saturation and nutrient content / availability. Soil types in turn affect soil structure, i. e. water saturation and oxygen saturation, and nutrient availability. The temperature was set at 20 °C during the incubation experiment and the water saturation was controlled in parallel experiments (70,

83, 90 % WHC). It is true, that soil texture or soil pH might affect the nutrient storage and thus availability for microbes, but nitrate as the electron acceptor for denitrification was supplied sufficiently in the presented experiment. Thus we could exclude the availability of nitrate, temperature effects, or water saturation in our analysis. In the revised version we included, that a recent study by Malique et al. (2019) investigated the denitrification potential of both soils (RM and GI) and found a higher denitrification potential with GI soil compared to that of RM soil. This finding emphasizes that soil texture and bulk density should mainly govern air content and thus O_2 supply at a certain water saturation, whereas SOM content should mainly govern microbial activity and thus O_2 demand and energy sources for denitrifiers. We fully account for bulk density differences through its effect on air content and air distribution at a given water saturation. This is assessed by proxies for O_2 supply.

We described controlled or excluded factors at the beginning of the discussion as follows (l. 496 ff.): "This study was designed to examine different levels of O_2 consumptions by comparing soils with different SOM contents and different levels of O_2 supply by comparing different aggregate sizes and different water saturations. Other factors that would have affected O_2 demand and energy sources for denitrifiers (quality of organic matter, temperature, pH, plant-soil interactions), O_2 supply (oxygen concentration in the headspace, temperature) or other drivers of denitrification (NO_3^- concentration, pH, denitrifier community) were either controlled or excluded in this study. "

However, experiments including variations in temperature, nitrate availability and/or other properties, like SOM or soil structure, would be very interesting and expand the knowledge on denitrification.

2. In the results section, the authors displayed the averages for the whole incubation, I feel it is better to show their results with incubation time. And of course, I also think it is not so reasonable to correlated average gas fluxes to the X-ray CT data of a specific incubation time, because the fluxes are not constant during incubation, neither for anaerobic soil volume fraction.

Structural measures were only analysed at the end of incubation. CO_2 and N_2O fluxes, O_2 consumption, and product ratios are presented as a function of time in the Supplementary Material (Figure S1, S2 and S5). Average values of CO_2, N_2O and (N_2O+N_2) release of the incubation period (24-192h) were used for correlations. Average O_2 saturation of the final 24h was taken for all subsequent analysis, as this probably best reflects the water distribution scanned with X-ray CT (see line 340 ff.).

Regarding the CT derived measures (e. g. connected air, diffusivity, distance to connected air, ansvf), the reviewer is correct in criticizing that we cannot rule out redistribution of water and air during 192 h of incubation. We assume that such redistribution events are typically associated with abrupt changes in local O_2 concentrations as well as CO_2 and N_2O release. The time series data (Figures S1 and S2) show that this may occur occasionally. However, taking several CT scans during incubation was just not an option due to methodological challenges. Likewise, variations of ansvf due to O_2 demand by local microorganisms (i.e. activity) and over incubation time cannot be estimated (line 523 ff.).

We assume that there are substantial variation during the first 24 h of incubation, which are omitted from the analysis, but only minor variations after all the genes for denitrification have been expressed and the soil has reached a dynamic equilibrium of O_2 supply and demand and a rather static distribution of water and air. Although microbial activity could affect the ansvf, ansvf largely contributed to explanation of N_2O and (N_2O+N_2) fluxes, in combination with CO_2 release.

3. From the detailed information showed in supplementary file, the variation between three replicates is very large (eg. Figure S1), the reasons for this large variation as well as the effects on the data reliability need to be clarify.

We can only assume possible reasons for the observed variations between replicates, but since the replicates were treated very similar according to the described protocol we cannot clearly identify reasons. The only explanation that we found was that small differences in repacking the moistened soil aggregates occurred between replicates (i. e. compaction, distribution of pores, and connectivity of pores), and possibly heterogeneity in the content of organic matter fractions in the aggregates. These small differences may largely affect soil aeration und thus microbial activity.

As can be clearly seen in Figure S4, repacking the aggregates in 2 cm intervals affected the visible air content and also ansvf. Both measures largely differed among replicates incubated at medium saturation for GI and RM soil. This was also pointed out in the result section 3.2, l 380 ff..

For CO_2 emission it was discussed in line 322 ff.: "The variability in CO_2 fluxes between replicates is much higher than the temporal variability during incubation. This is probably explained by small differences in packing of the columns that can have large consequences for soil aeration."

The same explanation was given for N_2O and (N_2O+N_2) emissions in line 328 ff.: "The huge variability between replicates is again higher than the temporal variability (e.g. in Figure 4d and time series in Supplementary Material, Figure S1) and the effect of aggregate size is inconsistent due to the large variability among replicates." Additionally, small variations in N_2O emissions may result from co-occuring N_2 emissions during denitrification.

Regarding the O_2 saturation averages of the last 24h of incubation were used for correlations and statistical analysis, because we assumed best accordance of the O_2 averages and water distribution (connected air content and ansvf) analysed by CT image analysis at the end of the experiment. The reliability of O_2 saturation data was discussed in the results section (l. 337 ff.): "Average O_2 saturation was lowest with highest water saturation and roughly the same for saturations <80%WFPS (Figure 3b). Some sensors showed a gradual decline in O_2 concentration, whereas some showed a drastic reduction or increase in a short period of time, probably due to water redistribution (Supplementary Material, Figure S2). The average of the final 24h was taken for all subsequent analysis, as this probably best reflects the water distribution scanned with X-ray CT. Standard errors among the seven O_2 microsensors were high in each treatment due to very local measurement of O_2 that probed very different locations in the heterogeneous pore structure."

We think that the data are reliable and comparable, because CO_2, N_2O and (N_2O+N_2) emissions and O_2 saturation as well as the other explanatory variables of the present study were measured for each replicate. Thus, small variations in connected air content or ansvf affect denitrification and respiration in one soil core.

4. And of course it would have been of interest to see the variations in denitrifying communities at microscopic scale.

We agree that this information would be very interesting and helpful for interpretation of results. However, we have presented a very comprehensive experimental setup, combining gas flux measurements, isotopic analysis, image analysis of CT derived data as well as simulating the diffusivity. These were very time consuming methods, especially the demanding image analysis. Methods to analyse the denitrifying communities in soil are not established in our lab and unfortunately we were not able to perform genetic analysis. In the revised version the

microbial community is added to the other factors altering denitrification under field conditions in the section 4.3 "Future directions and implications for modelling" (l. 645 ff.).

Minor comments:

L125: The soil depth of topsoil should be define, 0-20 cm?

This information was included as follows: "Fine-textured topsoil material was collected from two different agricultural sites in Germany (from a depth of 10 - 20 cm in Rotthalmünster (RM) and 3 - 15 cm in Gießen (GI), (Table 1)."

L141: How much soil is used for each column?

The target bulk density was 1.3 g cm^{-3} for RM soil and 1.0 g cm^{-3} for GI soil (Table 1). Thus 902 g dry weight of RM soil and 694 g dry weight of GI soil were used per column.

In line 144, we included: "This packing resulted in 902 and 694 g dry weight of RM and GI soil, respectively."

How about the soil depth of the repacked soil cores?

The height of the repacked soil cores was 10 cm. This information is provided in line 142 and Figure 1.

How to control the compactness of filling?

We repacked the soil in five 2 cm intervals.

"This ^{15}N-labelled soil was filled in 2 cm intervals into cylindrical PVC columns (9.4cm inner diameter x10cm height) (Figure 1) and compacted to a target bulk density that correspond to site-specific topsoil bulk densities (Jäger et al., 2003; John et al., 2005)."

L150-151: Why spray additional nitrate solution in 83% and 95% WHC treatments but not in 70% WHC?

We agree that this should be clarified and explained in more detail. All treatments contained the same amount of nitrate per mass of soil (50mg/kg soil). Hence the total amount of nitrate per column differed between the two soil types due to different bulk densities. However, the total amount of nitrate did not differ between three saturation levels. 50mg/kg N-KNO_3 was added to the respective amount of water. Hence, for higher water saturations the nitrate concentration in the solution was lower, so that the total amount was the same. This solution was used for moistening the soil. We will rephrase as:

"A ^{15}N solution was prepared by mixing 99 at% ^{15}N-KNO_3 (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) and unlabelled KNO_3 (Merck, Darmstadt, Germany) to reach 50 mg N kg^{-1} soil with 60 at% ^{15}N-KNO_3 in each water saturation treatment. Hence, for higher water saturations the stock solution was more diluted in order to reach the same target concentration is the soil. In a first step the soil was adjusted to 70% WHC before packing. […] Three different saturation treatments were prepared for subsequent incubation experiments: 70%, 83% and 95% WHC. For the latter two saturation levels the rest of NO_3^- solution was sprayed sequentially onto each layer after packing."