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

Anonymous Referee #2

We are thankful to the reviewer #2 for constructive comments on the 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.

Denitrification process is of critical importance because it is closely related with agricultural sustainability, environmental quality, and human health. However, the denitrification process in particular N_2O/N_2 generation and emission is poorly understood at microscopic scales. This study provides very useful information towards understanding the complete denitrification process with X-ray CT imaging analysis, and gives new insights how the N_2O and N_2O+N_2 are formed in soils at microscopic scales.

Major issues/concerns

The authors selected two different land use types of soils when investigating soil organic matter contents. The grassland soil has a SOM up to 4.5%, much higher than that of arable soil. I feel that it is difficult to compare the denitrification process between soils with different land use types. The authors had better use arable soils with different gradients of SOM to investigate the effects of SOM on the complete denitrification process.

We acknowledge that grassland and agricultural soil have vastly different soil structure and different input of plant residues. However, these effects are removed after sieving and removal of particulate organic matter and long-term storage. In other words, we did not work with differently managed soil, but rather with soil material with similar texture, but different SOM content, artificially repacked to some target bulk density, so that potential management effects are ruled out.

In our experiment we controlled the nitrate content, temperature and water saturation, but could include other measures for oxygen supply and demand, such as soil structure measures that are influenced by the soil texture (i. e. proportion of sand, silt and clay in soil), or CO_2 fluxes that indicate microbial activity. Possibilities to explore complete denitrification with soil organic matter (SOM) were described in detail in the discussion section (l. 619 ff.).

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

It is unclear why the authors set up these three different water saturation (70, 83 and95%). The 60% water saturation is widely used when setting up the soil microcosm experiments. I feel that 60% water saturation is needed as the control when setting up the gradients of water saturation experiment in this study.

It is true, a lower water saturation is widely used, especially in studies focussing on nitrification or on co-occuring processes like nitrification, nitrifier denitrification and denitrification. It is known from previous studies, that N_2O is produced during nitrification in soil at approximately 70% WFPS (Davidson 1991, Cardenas et al. 2017). This paper focuses on denitrification only. So with a series from 63% to 95% WFPS we capture the transition from low N_2O production through denitrification due to sufficient oxygen supply all the way to low N_2O emission due to further reduction to N_2. Another treatment would not have brought about any additional insights into the microscale mechanisms at play. Moreover, we conducted pre-test with varying WFPS, finding that with these soils, minimum saturation of 75% WFPS was necessary to ensure robust N_2 flux detection.

Moreover, the flooded paddy soils are widely distributed all over the world, in particular Asian areas. The authors had better include such kind of soil in their experiments to gain a full picture of water saturation effects on the complete denitrification process. The flooded paddy soil usually has a low N_2O emission but a high N_2 emission. It may be an excellent material when investigating the effects of water saturation on the complete denitrification.

It is true, that water saturation effects on complete denitrification of paddy soils, in particular differences in N_2O and N_2 emissions following different saturations, is very interesting to analyse, especially when regarding effects of climate change on such anthropogenic systems. However, naturally these flooded or ponded tropical or subtropical soils are exposed to completely different climatic conditions than the selected soils of the presented study. Thus it might be very interesting to include such soils in comparable experiments with temperature gradients as an additional factor for denitrification activity. The current study focussed on disentangling structural effects of mineral soils on O_2 supply and O_2 demand, without considering of temperature effects.

We have touched this comment in the section 4.3. (Future directions and implications for modelling) and included in the updated version at the end of this section (l. 645 ff.):

"It would thus be very interesting to include also different soil types and land-use types from various climate zones in future studies, e.g. paddy soils having high water saturation and are known to show a high denitrification activity with N_2 emissions exceeding that of N_2O emissions."

The authors have shown very detailed information in Results section. However, it is difficult for reader to follow in this section. So the authors need to improve this section and lead the readers to pay attention to their important findings.

Thank you for the suggestion. We tried to sharpen the results section by removing the regression analysis of ansvf with different gases into the supporting information and only

keeping the essential findings of this regression analysis in the main text. By this, we have removed one figure and one paragraph from the main paper.

The authors showed their results based on different gradients of distance, water saturation and so on. I feel that they need to show their results with incubation time, at least in supplementary files. They should clarify why they show the results of a specific incubation time in the main body of this manuscript.

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 (24-192 h) were used for correlations. Average O_2 saturation of the final 24 h was taken for all subsequent analysis, as this probably best reflects the water distribution scanned with X-ray CT (see l. 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. 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 average N_2O and (N_2O+N_2) fluxes, in combination with CO_2 release.

Minor issues/concerns

P4 L119: delete the comma after N2O.

We will delete the comma in the revised version.

P5 L150-151: is added nitrate amounts equal for each treatment?

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 (l. 137 ff):

"Three different saturation treatments were prepared for subsequent incubation experiments (70%, 83% and 95% WHC) to control the O_2 supply and thus provoke differences in denitrification activity. For this ^{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. […]For the latter two saturation levels the rest of NO_3^- solution was sprayed sequentially onto each layer after packing."

P24 L630-633: please clarify this sentence.

This sentence was clarified by deleting "more involved".