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

Anonymous Referee #1

We thank the reviewer very much for the positive opinion and constructive comments on the manuscript.
The authors' answer is in italic font.

Referee: This manuscript investigated the effects of aggregate size and water saturation on N_2O and N_2 fluxes in two soils with contrasting SOM content by repacked soil cores based ^{15}N tracer incubation in combination with X-Ray computed tomography. The main outcome was that N-gases emissions could be well predicted by considering proxies for oxygen supply (anaerobic soil volume fraction, i.e., ansvf) and demand (CO_2 emissions), which linked the change of soil structure with N-gases emissions. Generally, this manuscript is well prepared and written, and the conclusions were supported by the results of the experiments.

Referee: One of my major concerns was that how could one time point (at the end of the incubation) microstructure analysis for the repacked soil cores represent the change of ansvf during the 192 h lasting incubation.

In theory the anaerobic soil volume fraction (ansvf) should be governed by O_2 supply imprinted by the distribution of air-filled pores and modulated locally by the O_2 demand through microbial respiration. The former was estimated from CT derived images after 192 h of incubation using the distance to air filled pores as an estimate caused only by physical conditions, i. e. pore structure (connected air), as explained in the method section (line 237 ff.).

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. However, in the discussion section variations of ansvf due to O_2 demand were mentioned (line 521ff. and line 597 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.

Another method was also used to estimate the ansvf ($ansvf_{cal}$, see Supplementary Material) by microbial denitrification activity only. We found accordance between both estimates for RM soil and discussed possible reasons for differences between ansvf and $ansvf_{cal}$ for GI soil.

In the revised version we will discuss in more detail how ansvf may be altered by O_2 demand (CO_2 release) and/or O_2 supply during the incubation time of 192 h.

Referee: In addition, why the aggregate size exhibit no obvious effects on CO2 and denitrification product stoichiometry should be discussed.

In the present study aggregate size did not affect CO_2 release or denitrification and we argued that aggregate radii (1-2 or 2-4 mm) were smaller than the thresholds of distances to connected air that were found to determine the ansvf. The critical distance to estimate the ansvf were selected from best correlations between ansvf and N_2O as well as (N_2O+N_2) fluxes. Results indicated that aggregate size might have been too small to provoke differences in CO_2, N_2O and (N_2O+N_2) fluxes. This point will be considered in more detail in the revised version.

So far we discussed this point in line 532 ff.: "The fact that aggregate size had no effect on denitrification indicates that critical distances were larger than the aggregate radii and rather controlled by air distribution in the macropore system. This is in contrast to the very short critical distances of 180µm for sufficient soil aeration estimated by Kravchenko et al. (2018) and Kravchenko et al. (2019) for intact soil cores containing crop residues for which soil respiration was not determined but likely to be much higher."

Referee: Specific comments Introduction
The challenge for direct measuring soil borne N_2 from soil cores should be mentioned. This info may also provide rational for the authors to use ^{15}N tracer to estimate N_2 flux.

We agree that this point could be better introduced and will be rephrased in the updated manuscript as (line 93):
"Since the N_2 background of air (78%) is very high, direct N_2 measurement from denitrification in soil is very challenging (Groffman et al., 2006, Mathieu et al., 2006). The ^{15}N labelling technique is a method successfully applied to determine N_2O and also N_2 production from denitrification from ^{15}N amended electron acceptors (NO_3^-) (Mathieu et al., 2006, Mosier et al., 1986, Parkin et al., 1985, Tiedje et al., 1989)."

Referee: Results
I suggest move the resulting regression equations from SI to text so that the reader could easily capture the key point of explanatory variables for denitrification.

This is a good remark and we will move the regression equation to the main text in the revised version (Result section, 3.4 Explanatory variables for denitrification, line 467).

Referee: Line 23,567 oxygen should be O2

We will replace oxygen with O_2 in the revised version.

Referee: Line 24, I suggest change the order of "ansvf" and "CO2" since "CO2" is more important in terms of explanatory based on the author's results.

We will change the order of CO_2 and ansvf in the revised version.

Referee: Line 119, comma in the sentence should be deleted.

We will delete the comma in the revised version.

Referee: Line 151, why additional nitrate solution was sprayed in the last two treatments? if the N substrates differed among the three treatments, how could the author compared the N2O and N2 flux among the tree treatments?

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."

Referee: Line 222, clearly

We will replace "clearaly" by "clearly" in the revised version.

"Only macropores twice this nominal resolution were clearly detectable in the soil core images."

Referee: clearly Line 444-445 the order of the sub figures for the two tested soils was reversed

We will correct this mistake:

"Figure 7: Average O_2 saturation (at the end of incubation experiment) measured with 4 sensors each located at the center of soil core as a function of distance to visible connected regression for soil from Gießen (GI, (a)-(c), blue) and Rotthalmünster (RM, (d)-(f), red), and for two aggregate sizes (2-4mm and 4-8mm). (a) and (d) show results for lowest (b) and (e) for medium and (c) and (f) for highest water saturation. The inset in (a), (b), and (d) shows a reduced distance range. The distance to visible connected air is averaged in a spherical region around the sensor tip (7.2 mm diameter). The Spearman's rank correlation coefficient (R) result from Spearman's rank correlation and indicate the extent of monotonic relation between the ranks of both variables. The associated p-values (p) were corrected for multiple comparison according to Benjamini and Hochberg (1995)."

Referee: Line 545 is?

We will write "as" instead of "is".

"However, there is always a trade-off between retrieving more information and disturbing the soil as little as possible."