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Interactive comment on "Effect of soil saturation on denitrification in a grassland soil" *by* Laura Maritza Cardenas et al.

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General remarks This paper presents results from a sophisticated laboratory experiment in which an agricultural soil was compacted and adjusted to 4 different moisture conditions. Glucose and nitrate was added and the formation, isotopic and isoptomeric composition of gasesous N was measured over a period of 12 days. Using those data the authors try to determine the contribution of different processes to N gas formation. The paper is a good example how much information you can get from experimental data if you spend a lot of energy in calculations and data analysis. However, in my eyes the paper has three critical weaknesses:

1.) The results are not really new. It is known for a long time that addition of nitrate and glucose stimulates denitrification in soils and that denitrification is favored under wet-

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terconditions. All the points in the conclusions are not new. If there is new knowledge obtained from the study, it has to be elaborated more clearly. R: we agree that some of the general points are known, for example the effect of soil moisture on emissions, but this is normally considered in relation to ranges of <60%, 60-75% and >75%. We have looked at a more detailed moisture adjustment, four levels at a relatively high moisture range, between 70 to 100% WFPS. We have also studied the isotopocules of N2O and found isotopic similarities at similar moisture levels. Moreover, for the first time we have conducted N2 +N2O flux measurements at defined saturation of pores size fractions as a prerequisite to model denitrification as a function of water status.

2.) The paper is lacking a clear story. It is not really clear to me what was the final purpose of all those detailed analysis. There are some hypothesis mentioned at the end of the introduction but the rest of the manuscript is not tailored to address those hypotheses. The hypothesis that wetter conditions reduce heterogeneity could be answered from just looking at the error bars in figure 1 – you do not need sophisticated analysis to prove this point. Aiming to understand what is going on in one0s own experiment (as stated in the last sentence of the introduction) is not a sufficient aim of a paper. R: We have done a detailed control of soil moisture in the soil and in order to do this we had to do the detailed analysis the reviewer refers to in terms of the moisture adjustment. In this way we ensured that the four moisture levels above 70% WFPS were as accurate as possible. We also used tools such as the isotopomers to confirm source processes, and this is the result of our research in the last 15 years, when we have built up a large database of isotopomers of N2O to improve the uncertainty in the determination of the sources. In this particular experiment we have been able to elucidate the effect of saturation on processes at relatively high moisture levels when combined with the measurements of N2O and N2 emissions.

3.) There are some problems with the experimental approach which limit interpretation of the data. First, moisture conditions were not constant but changed a lot during the experiment. The second treatment, for example at the end of the experiment had

the same water content as the third treatment in the beginning. They had changing substrate concentrations in parallel to changing moisture conditions. Thus, the interpretation of moisture effects during the course of the experiment is difficult. A way to minimize that effect would have been to moisten the supplied He/O2 gas. I would also expect that water loss was highest in the beginning, when the surface layer was drying. A way to get some information about temporal changes of water content would have been to weigh the incubation vessels during the incubation. Second, they measured gas emission – not gas production. They mention this problem in the paper but somehow ignore its consequences. The emitted gas probably originates from those sites which are physically linked to the atmosphere, while gas production, e.g. in the center of aggregates did probably contribute less to the emitted gas. So, the conclusions drawn from the analysis could be valid only for a part of the soil volume. R: we are aware there are limitations to the experimental approach. In order to moist the gas we would have to have an extra vessel where we flush the gas through. Measuring N2 is very difficult due to background atmospheric levels and any additions to the experimental system poses a risk of leaks. In addition, adding moist gas will likely block the tubing as these are very narrow (1/8"o.d.). The flow of the gas is very slow (10 ml/min) simulating a low wind speed so normally this would dry the soil in field conditions too. It would represent a rainfall event where the initial moisture differs between treatments but some drying occurs due to the wind flow. We believe the effect of drying will be more relevant (and significant relative to the initial moisture) later in the incubation. We also know that if drying is significantly affecting the microbes, we would see an increase in CO2 emissions which did not happen later in the incubation. We have introduced changes in the text to make the reader aware of this and have reflected this as 'the effect of initial soil moisture'.

Detailed comments

l.17: remove "soils" R: removed

l.40: What do you mean with "benign" for the environment. Do you mean the process

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is important because it closes the global N cycle because it reverses N-fixation? R: no, it is benign because it does not cause harm to the environment.

l.64-73: I would move this paragraph to an earlier point, before talking about compactation. R: we have placed this paragraph after the compaction, as it follows from the previous paragraph where we discuss the effect of livestock on compaction. It also leads to the following text on effect of compaction on soil water: 'reducing the soil air volume and therefore increasing the WFPS'.

l.72: I would replace "powerful tool" by "basis". R: changed

l.81: If there are several references for one statement, present them in chronological order. R: changed

l.81-82: Remove sentence R: removed

l.83: ": : :under the conditions: : :" R: changed

l.92: Be more specific. What do you mean by "other steps of denitrification"? R: we agree that this sentence was not clear enough so we rewrote to: "Simultaneous occurrence production and reduction of N2O as in natural conditions presents a challenge for isotopic factors determination due to uncertainty on N2 reduction and the co-existence of different microbial communities producing N2O (Lewicka-Szczebak et al., 2014).
l.93: "reported here". R: changed

l.100: Does that mean that those results are only relevant at elevated C and N? R: We have modified the text as follows: 'The results generally confirmed the range of values of η (net isotope effects) and η18O/η15N ratios reported by previous studies for N2O reduction for that part of the soil volume were denitrification was enhanced by the N+C amendment. This did not apply for the other part of the soil volume not reached by the N+C amendment, showing that the validity of published net isotope effects for soil conditions with low denitrification activity still needs to be evaluated'.

l.108: Why CO2? R: we have changed the text: 'soil to assess the impact of different

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levels of soil saturation on N2O and N2 emissions after compaction. CO2 emissions were measured in addition as an estimate of respiration and thus of O2 consumption'. l.112: "controlled laboratory conditions" R: changed but this text is now in section 2.4 as recommended by another referee.

l.119: What do you mean by "heterogeneity in N emissions"? R: spatial distribution of emissions, text changed to clarify

l.120: I am not a soil scientist, but is that really new? R: prediction of N2O emissions is very difficult in part due to their spatial variability. We are trying to understand how this effect occurs in a relatively narrow range of moisture (70-100%). As far as we know there no other studies going to this level of detail. This has been included in the text (end of introduction section).

l.121: Aiming to understand what is going on in one0s own experiment is not a sufficient aim of a paper. R: we have changed the text: 'We aimed to understand changes in the ratio N2O/(N2O+N2) at the different moisture levels studied in a controlled manner on soil micro and macropores. Moreover, we used isotopocule values of N2O to evaluate if the contribution of bacterial denitrification to the total N2O flux was affected by moisture status'

l.137: Verb missing. "was applied"? R: the verb is early on in the paragraph. The paragraph is now split to make it clear.

l.228: "CO2 was measured: : :" R: changed

l.230: replace "pulled together in one sample" by "pooled" R: changed

l.232: Remove sentence. There is a similar sentence in the results section. R: removed

l.268: Were the data normal distributed? R: yes, all datasets were tested by fitting a Gaussian model resulting in Fprob<0.001. this was added in the results section.

l.275: "mixing model was then used" (use past tense) R: changed

l.283: When did this occur and what is a possible explanation? Wrong fractionation factors? We clarified the variability of endmember values and fractionation factors in the introduction: “The analysis comprised measurements of the N2O and N2 fluxes combined with isotopocule data. Net isotope effects (η values) are variable to a certain extent as they result from a combination of several processes causing isotopic fractionation (Well et al., 2012). The results generally confirmed the range of of η values and η18O/η15N ratios reported by previous studies for N2O reduction for the soil volume reached by the N+C amendment. This did not apply for the soil volume not reached by the N+C amendment.”

l.290: A TCD is an detector – not an analyzer. R: changed analysed for determined

l.303 Why was the gas stream not bubbled through water to saturate it with water? R: see our explanation above in point 3.

l.305: I would expect the highest water loss right in the beginning. R: the flowrate is very low so drying will take a while, we are assuming that the significant water loss will affect later in the incubation, later than the peaks appear. However, as explained earlier, we have now referred to the effect of the initial soil moisture in the treatments.

l.306. But they were similar between treatments in the end although different starting conditions. R: yes

l.314-316: There was a high variability in the data. R: but only for NH4+ it was not significant. A sentence was added

l.318: Remove “The results showed that” R: removed

l.329: I do not see that in Figure 1. In Unsat/sat the N2O maximum was at 12 kg N/ha d, not around 7. R: the referee is correct, we have now amended the text to reflect this: ‘The N2O maximum in the SAT/sat and HALFSAT/sat treatments was of similar magnitude (means of 5.5 and 6.5 kg N ha-1 d-1, respectively) and but not those of UNSAT/sat and UNSAT/halfsat (means of 7.1 and 11.9 kg N ha-1 d-1, respectively).

l.348. Right. But what are the consequences of this for your experiment and its interpretation? R: this belongs to the discussion (4.1) so have been moved in there to explain the potential underestimation of the production due to low diffusion.

l.354: You probably mean “CO2 fluxes”. Why was CO2 measured? R: yes, added fluxes in the sentence. CO2 indicates aerobic respiration and as explained above (l.108) is also affected by the soil moisture and level of compaction.

l.360: The carbon budget is interesting but complicated. Could you calculate recovery rates for the added glucose? It looks as if there are recoveries higher than 100%. Can this be interpreted as a priming effect? A problem with using CO2 for carbon budgeting is, that depending on pH you also have other IC species in the soil solution. Do you know the pH in your soils? R: pH is 5.63 as shown in Table 1. We did not do a C budget, but it is possible that soil C would have also contributed to the CO2 emitted but to a lower extent compared to the added glucose.

l.370: Add article before “period” R: added

l.375: The SP data have a high standard deviation. Are the differencers discussed in this paragraph real? R: we think the larger variation (high SD) of SP around day 3 corresponds to the with highest variation of N2 and N2O fluxes (which is evident from Figs

l.391:You may consider adding these data to the plot. R: data added to figure

l.394: Separate into two sentences. Start second one with “In our data, maximum : : :.” R: changed

l.404 So what is the message ofthis paragraph with respect to the first sentence of the paragraph? R: we have rewritten: ‘the question arises to which extent the relationships between the d18O and d15Nbulk and between d18O and SP within the individual treatments denitrification dynamics. We checked this to evaluate the robustness of isotope effects during N2O reduction as a prerequisite to calculate the percentage of bacterial

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denitrification in N2O production."

l.405: Why was this done? R: we have found that the isotopologues seem to be potentially more powerful than initially thought. By looking at these relationships we have learnt how the responses relate to the sources of these gases.

l.428: Why was this plot done? R: the same reason as above

l.441: I do not see data within those areas in the plots. R: we have not been so clear, and we refer to the vectors more than the areas. Text has been changed to reflect this.

l.456: "sat" page 19: It is difficult to detect the storyline on this page. R: we are explaining that from our results we are providing a refinement in the soil moisture (WFPS) thresholds previously established as borderline for nitrification-denitrification. We are also proposing that WFPS which was previously established as a normalised parameter for these type of soil moisture thresholds, might actually change with soil type.

L513: Could it be that there was C limitation in the dryer treatments because glucose was metabolized aerobically? R: if glucose was metabolised we would have expected C to have been less limiting

l.534-537: The message of the CO2 paragraph is not really clear. Are the CO2 data helpful in this manuscript? R: we have deleted the paragraph as suggested.

l.539: How much is the unacounted N-loss in comparison to the accounted gasesous losses? R: we added: " unaccounted-for N loss is two to three times the total measured gas loss (Table 3)".

l.541: NO: What are typical NO fluxes in the literature? Can the NO flux have a significant magnitude? The same applies to microbial biomass: Is the microbial biomass potentially formed from the unaccounted N-loss in a realistic order of magnitude? R: we are now able to measure NO fluxes in the system. Loick et al reports a ratio N2O/NO of 0.4 for example, so yes, it can be significant. We did not do microbial biomass in this instance.

l.567: How should nitrification contribute to BDEN? Do you mean nitrifier-denitrification? R: thus large contributions to the total N2O flux from nitrification were not probable

l.636: I do not understand the content and purpose of this paragraph. R: text changed to: The question arises, if the poor coincidence of Pool 2 isotopologue fluxes with previous N2O reduction studies reflects the variability of isotope effects of N2O reduction or if the contribution of other processes like fungal denitrification could explain this (Lewicka-Szczabek et al, 2017). The latter explanation is evaluated in section 4.3.

l.719: Don0t you have 4 periods in the figure? Table 3: Unit missing for Total emitted N. Tables 5 and 6: I wonder whether these data could be presented better in figures. R: no, only three. Units included. Yes, figures can illustrate better, but as we explained in the initial review, this data is very useful for models and we think providing the values will be more useful.

Figure 5: the four sub-graphs are quite similar. Isn0t a conclusion that the results were not much influenced by soil moisture? Do you really need 4 graphs? R: we concluded that there were similarities between the 2 high moisture and 2 low moisture treatments. We believe this is an important finding due to the relatively narrow range of soil moisture we have studied, above 70%, in which we still find differences in fluxes. Davidson stated that the threshold for nitrification-denitrification lies at about 60%, in our case we have managed to refine this.

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