

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

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The paper aims to quantify N₂O and N₂ production process in grassland soils and its dependence on compaction. N₂O and N₂ emissions and their isotopic signature have been monitored over a period of 12 days after amendment of KNO₃. The presented laboratory studies simplify the complex soil pore system into macro and micropores and uses four stages in a rather narrow range of 70 to 95% “mean” WFPS. The experimental setup is described in detail. The results agree with the expected values, i.e. domination of bacterial denitrification processes for the higher water content and an increasing share of other contribution for when part of the pores is dry. The measurement of the isotopic signature allows to distinguish different production processes and their dependence on the water status of the macro and micropores. I had difficulties to follow the argumentation and get quickly lost in too many in details. I also

C1

miss a discussion of the significance of the presented findings for the characterization of the emissions of N-species for real grassland systems, although in the introduction (e.g. lines 62 and 63) the study is set in this context. The used soil stem from a long-term permanent grassland. But the preparation of the samples (a necessary step for the laboratory study) destroys the specific characterization of a grassland soil. Roots and the organization of the aggregates are removed and there is no plant growth that greatly influence the distribution and availability of N substrate as well as the oxygen supply. It should also be mentioned that a large share of N-input in agricultural system occurs in reduced N-form (excrement's, urea or ammonium nitrate). In grazed system, spatial heterogeneity is related to the urine patches with a very high N-input on a very limited area. Also, compaction (trampling by animal, tractor tracks) is spatially very heterogeneous and likely uncoupled to N-substrate input. R: the authors agree that soil structure is destroyed, but as the referee says himself, this is a laboratory study, so we are not trying to reproduce the field conditions but to understand soil processes. In fact, we are assessing the potential for this soil to emit N₂O and for this reason we have optimised the conditions for denitrification. The plant is not included for the same reason, as we aim to understand the processes in the soil, although we agree that the plant plays a major role in modifying these processes. The soil used in this study is not sourced from a grazed grassland, but a grassland that is cut, so the effect of the animal, via grazing, soil compaction and excreta deposition is not relevant. The results from the present study shows for N₂O as well as (N₂O and N₂) emission a remarkably low variability among the four treatment, much lower as typically experienced in field measurements. Below are given specific comments as a guideline to improve the manuscript Abstract: Lines 16 and 17: The soil emitted N₂O is predominantly derived from denitrification and to a smaller extent, nitrification in soils, This is a too crude generalization. There are many ways to produce N₂O and the share between them depends in a complex manner from the main driver, such as oxygen content, substrate availability, etc. R: the authors agree with the referee point and in fact the sentence goes on to say: ‘both processes controlled by environmental factors and their interac-

C2

tions, and are influenced by agricultural management'. We have however made it clear that it is a generalisation. Lines 20 and 21: Soil water content expressed as water filled pore space (WFPS) is a major controlling factor of emissions and its interaction with compaction, has not been studied at the micropore scale. This is slightly misleading as the experimental setup can only measure net fluxes across the surface of the entire soil samples and naturally does not allow to determine N₂O production/consumption in and out of the micropores. R: yes, the referee is right in that we are not looking at production and consumption separately; but we only claim the control is on emissions (not production and/or consumption) and we are controlling moisture at the micropore scale. Introduction Lines 210 and 211: concentration) for 24 h, or until the system and the soils atmosphere were emitting low background levels of both N₂ and N₂O (N₂ can get down to levels of 280 ppm much smaller than atmospheric values). Please indicate these "background" values. R: the flushing goes on until there is no further decrease in the background signal. This normally occurs within 24 hours. Values can reach a few gN/ha/d (much lower than atmospheric values of 70%). Lines 222 and 223: Flushing was carried out with He for half an hour before the solution was required for application to the soil cores and continued during the application process to avoid atmospheric N₂ contamination (a total of one and a half hours). How this affects the oxygen availability? R: the flushing is done to the amendment outside the incubation vessel, so we remove N₂ from the liquid before application. The incubation vessel on the other hand continues to receive He/O₂ so it should not affect O₂ availability, in fact the increase in CO₂ in later experiments supports this assumption. Lines 304 and 305: We accepted these as unavoidable features of the experimental set-up, but we suggest that the main response of the gaseous emissions occurred under the initial conditions, prior to the loss of water over subsequent days. "We suggest" is a strange formulation, either the time course of the emissions clearly shows this, or it is an assumption. R: this statement came after a comment from a previous reviewer. We have changed the text now to say 'we assume'. Results Lines 311 UNSAT/halfsat (50-100 N kg- dry soil) Unit of NO₃- seems incorrect. Also, the header of Table 2 is wrong (twice UNSAT/SAT)

C3

R: the referee is correct, units and heading have been amended. Lines 349 to 351: The results showed that the total N emission (N₂O+N₂) (Table 3) had a consistent decreasing trend, with decreasing soil moisture i.e. from 63.4 for SAT/sat (100% WFPS) to 34.1 kg N ha⁻¹ (71% WFPS) for UNSAT/halfsat. I don't see a consistent decreasing trend. Only the driest treatment shows a lower emission. R: we have modified the text to reflect this properly: 'The results showed that the total N emission (N₂O+N₂) (Table 3) decreased between the highest and the lowest soil moistures i.e. from 63.4 for SAT/sat (100% WFPS) to 34.1 kg N ha⁻¹ (71% WFPS) for UNSAT/halfsat' It also would make more sense to use the same reference for the mineral N content as well as the cumulative gaseous emissions (e.g. per g soil). R: we agree this is a good suggestion. So we have included this extra information in table 3. Lines 351 and 352: The maximum cumulative N₂O occurred at around 80% WFPS as Fig. 2 shows. This is an overinterpretation. There are four values and a fit with three unknown is applied. R: we agree that there are no many points, but the value of this analysis is that for a narrow soil moisture range (70-100%) there seems to be a linear response for the N₂ but not for the N₂O and the total flux. Those shown were the best fits. Noticeable emissions of N₂O and N₂ occur in all four treatment only up to day four. Bacterial denitrification is identified as the main production pathway. This is due to the experimental setup with a combined amendment of KNO₃ and glucose, a setup that produce good conditions for denitrification irrespective of the specific treatment. R: as mentioned earlier, we optimised conditions for denitrification, except for soil moisture that is the factor we are studying.

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C4