Interactive comment on "Alteration of nitrous oxide emissions from floodplain soils by aggregate size, litter accumulation and plant soil interactions" *by* Martin Ley et al.

Y. A. Teh (Referee)

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GENERAL COMMENTS

This is a creative and interesting process-based experiment that uses different aggregate treatments (i.e. micro- versus macro-aggregate dominated) and plant-soil treatments (i.e. a gradient of "plant influence," from rhizosphere to detritus-affected soil to plant-free soil) to determine how differences in soil structure and various levels of plant influence potentially influence N2O dynamics in soil. The factorial experimental design is powerful because it enables the investigators to assess not only main effects, but also evaluate the potential importance of synergistic effects among different treat-

ments. Overall, it is my view that this paper was clearly written, with a well-justified experimental design, and a logical analysis of the data. The introduction to the paper clearly explains the basis and wider significance of this research, while the methods section explains the overall approach taken with clarity. The results section documents the main findings of the work succinctly, while the discussion takes a reasonable (and not overly speculative) approach to data interpretation, informed by the authors' grasp of the current literature. The investigators' comprehensive measurement of a range of environmental parameters is to be commended and enables them to make logical inferences about the role of different treatments and environmental factors in regulating N2O dynamics during different parts of the simulated water cycle. In particular, the investigators make good use of redox potential measurements to evaluate how changes in redox/O2 availability could be driving N dynamics along the "plant influence" gradient that they have created in the laboratory.

However, while I am generally supportive of this research and believe it will make a valuable contribution to the wider body of knowledge on this topic, I do have a few general remarks that I believe need to be addressed before this paper can go forward to publication. First, I think the authors need to be open and transparent about the potential limitations of their research. For example, the soil structure treatments represent two extremes (large versus small aggregates), whereas in reality micro- and macro-aggregates would be mixed together. The authors need to explain how their experimental treatment could relate or correspond to real-world conditions, drawing if possible on pre-existing field or laboratory data (see points 1 and 5 below). Likewise, the authors need to be clearer about the limitations underlying their rhizosphere (Salix) treatment. It is difficult to generalise more widely about the effects of plant rhizospheres on N dynamics without examining a range of different plants (including single and multi-species mixtures), in order to tease-apart individual species effects from generic rhizosphere effects (see point 6 below); I think it is important, in the revised version of this text, that the authors acknowledge this limitation and spend a bit more time exploring what they believe could be more widely generalisable from their

study, rather than what is species-specific.

Second, I do not believe that the authors have fully exploited their experimental design in the analysis of their data, and sincerely believe that more could be done to examine these data in greater depth. For example, as mentioned above, one of the strengths of a factorial experimental design is that the investigators can establish if there are synergistic interactions among different experimental treatments (e.g. aggregate X rhizosphere effects). However, the investigators do not appear to have examined if interactions among treatments occurred, or at least these findings are not reported if these tests were conducted. Moreover, I would suggest that the authors try more complex multivariate models to analyse their data; for instance, using approaches such as analysis of co-variance (ANCOVA), generalized linear models, or mixed effects models. The benefit of these more comprehensive multivariate models is that they enable the investigator to establish the relative importance of different treatments and continuous environmental variables in regulating flux.

Third, I agree with the first referee that the authors need to spend a bit more time clearly highlighting what knowledge gaps this paper fills. As the first referee indicates, there are already existing studies that have examined the individual effects of all the variables discussed here. In order to make this paper more impactful, the authors need to articulate how this specific study is unique or advances our current state-of-knowledge (e.g. does the factorial design add knowledge or insight?).

Specific comments are provided in the section below.

SPECIFIC COMMENTS

1. Lines 136-137: For experimental purposes, the investigators have created quasi-artificial system conditions, with treatments either containing macro- or micro-aggregates. While I fully understand why this was done, it would be useful to understand (even qualitatively) how close or far from reality these treatments are. For example, what was the proportion of macro- and micro-aggregates under natural con-

ditions?

2. Line 173: Clarity of expression; consider revising this section to read "The mesocosm experiment had a factorial experimental design consisting of two factors (model soil and plant-soil treatment), with the first factor containing two levels (macroaggregates, microaggregates) and the second factor containing three levels (unamended, litter added, plant present). This experimental design resulted in six treatments, each replicated six times."

3. Line 179-180: What was the rationale for autoclaving the leaves? Under natural conditions, these leaves would contain their own microbial community which could contribute to N2O dynamics, and autoclaving means that the results will be biased towards the activity of the soil community (or, spore-forming phyllosphere microbes able to resist the effects of autoclaving).

4. Lines 221-232: Further detail on the statistical analyses are required here. For example, what were the independent variables used in the ANOVA? Did the model include interaction terms? Given that sampling was conducted over different periods of time, did the authors use a repeated measures ANOVA, to account for the effects of time?

5. Lines 300-353: This is an interesting and well-written part of the discussion. However, I do think that this part of the discussion could be improved by trying to link back the findings from the experiment to natural conditions (see point 1). For example, under natural conditions, what is the relative distribution of macro- or micro-aggregates? Based on your understanding/knowledge of the natural aggregate distributions, what patterns or processes do you think will dominate in a natural setting? While I realise this might be somewhat speculative (unless other data, such as field measurements, are available), I think it's an important talking point, as it will enable the reader to relate these findings (derived under somewhat artificial conditions) to the real world.

6. Lines 380-406: The discussion of potential direct and indirect effects facilitated by

the presence of an active root system is interesting and well-reasoned. However, I was left wondering as to how generalizable these findings are, given the wide range of traits displayed by different plants? I.e. to what extent are the trends identified here unique to Salix, and to what extent are these patterns more widely generalizable? I think it is important that the authors develop this section a bit further, in particular acknowledging this limitation more frankly.

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