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Interactive comment on "Biochar's effect on soil nitrous oxide emissions from a maize field with lime-adjusted pH treatment" *by* R. Hüppi et al.

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Author response to review by Roland Fuss
We thank the reviewer for this well elaborated review. We appreciate that someone with great statistical skills and a thorough understanding of the method comments our work critically. His comments will improve our manuscript substantially.

Comment: *By far the most serious problem is the unfortunate selection of soil type for this study. Reduction of N_2O emissions is at most a co-benefit of biochar application. The potential application of biochars to agricultural soils aims at improving soil fertility and soil hydrology (and possibly as well at carbon sequestration). Thus, biochar ap-*

plication to a mollic gleysol is very unusual since a soil with such high carbon content cannot be expected to profit much from it in these regards. Furthermore, and even more importantly, if there are other effects than pH having an impact on N_2O they are less likely to occur in a soil with high amounts of native soil carbon. The authors need to justify their choice of soil and discuss in more details the implications on representativeness of their results.
Response: In contrast to the reviewer we argue that reduction of N_2O emissions is a major motivation for biochar application in temperate soils. Often, temperate soils are pedagogically young, only moderately weathered and thus fertile. This in particular applies to soils with relatively high clay content. Hence, improvement of soil fertility is not the major aim for biochar application and we cite studies that show a N_2O reduction potential also for temperate soils. Mollic Gleysols are commonly found in Switzerland with its high precipitation, positive water balance, and alluvial floodplains (55'000/300'000 ha of swiss cropland soils). Those soils are, as indicated also in our text, often drained for agricultural purposes, provide suitable production conditions and are intensively managed. We also stress that mollic Gleysols must not have 'high' carbon contents; the lower threshold being 0.6

Comment: *I do not believe that your experiment (on its own) could test the hypothesis that a reduction of N_2O emissions is due to a pH effect. If both treatments had reduced N_2O emissions (significantly) this wouldn't prove a pH effect.*
Response: The reviewer is right, it may not be a hard proof because not only pH itself, but also concentration of Ca ions and possibly soil aggregation change after liming and biochar application, and both factors may influence N_2O as well. However, a reduction effect after liming as strong as after biochar application would indicate, that one could use limestone instead of biochar to get the same effect in N_2O reductions, especially if there is the same soil pH effect from biochar and limestone. It is known that pH exerts control on the N2/N_2O ratio through influencing denitrifiers ability to synthesis N_2O reductase (Bergaust et al., 2010), and hence there is a mechanistic basis for our hypothesis. For denitrification response the range from pH 6 to 7 is definitely of high

importance as Bergaust et al., 2010 showed a large sensitivity of the assembly of N_2O reductase that is only optimal above pH 7. Generally there is a continuous decrease of N_2O/N2 ratio with increasing pH from 5-8.

Comment: *Your study also only observes relatively short term effects. It is known that liming can cause a short term increase of N_2O emissions due to enhanced N mineralization and nitrification. The long term benefits might be better than your results indicate.*
Response: As only one of the three chambers with lime shows high emissions the hypothesized effect on N mineralization seems not ubiquitous. Further, sufficient N was added in plant available form. Unfortunately, the reviewer provides no reference to his point. We reject the argument that we are only dealing with short term effects - we measured over more than a whole maize growing season, covering the complete warm period with major emissions after fertilisation.

Comment: *The description of the N_2O flux measurement method needs also to be more detailed. I'm unfamiliar with the type of analyzer used for measuring N_2O concentrations. Please provide a reference and/or briefly explain the measurement principle. You also need to give some numbers illustrating accuracy and precision of that instrument. I would also like to see more details regarding the temperature correction you applied. Also, please describe the chambers in more detail. E.g., did they include a fan or manifold to ensure mixing of the headspace air? Did they include a pressure vent? . . . Since apparently this is a chamber design were only the lid is closed and opened and the chamber walls are permanently on the plot, have you checked if there was an impact of this on soil humidity inside the chamber (compared to the surrounding soil)?*
Response: We will add some more details about the method. No fan was used, but chamber air was flushed with 1 l/min through the chamber to the analytical system. Pressure compensation was assured by the not totally gas tight chamber construction. However there are several publications listed that described and used the same system

(Flechard et al., 2005, Felber et al., 2013) and we have not developed a new technique here.
Indeed, the chambers were permanently on the plot and we have not checked the impact of soil humidity inside the chamber. We expect only a minor increase in humidity within the chambers also because the lids are opened most of the time and only close for 15 min within 3 hours. However, of course these measurements have the same constraints as most other static chamber measurements. These effects are empirically small, unavoidable and the same for all treatments and replicates.

Comment: *I'm not convinced that the statistical treatment of the data is correct/optimal. First of all, I don't understand why the data was smoothed as a first step. This shouldn't be necessary and needs more justification. Then, for modeling cumulated fluxes I would suggest to at least try a mixed effects model with random effects corresponding to rows and columns of the plots (although this might be precluded by the low number of plots). Your post-hoc decision to compare only two treatments with a t-test is dubious (read: not allowed). Regarding modeling N_2O fluxes in dependence on explanatory variables: Again I don't understand why you work with weekly averages. Also, you write that a GLS has been used. However, a GLS model is only preferable over an ordinary least squares model if you model variance heterogeneity or autocorrelation of residuals. But you do not mention doing that. Also, since you have repeated measures you should definitely use a mixed effects model. Furthermore, you should at least try using WFPS instead of VWC as an explanatory variable. Finally, an assumption of linearity is probably not really appropriate. We know that the relationship between N_2O fluxes and soil humidity is usually not linear, but some kind of optimum curve. I suggest using a generalized additive (mixed) model instead of a linear model (see R package mgcv). This model should probably also consider Nmin concentrations.*
Response: Smoothing was done to reduce gaps in the dataset and the need for interpolation. The synchronous data was needed to get comparable cumulative flux estimates.

We did not apply mixed models because the number of replicates hamper their statistical power. Considering the available resources and sample size of our study, it is most meaningful to show the data as it is with its obvious pattern. We also refer to a previous study (Felber et al. 2014) where the statistical power, using the same chambers, n = 3, and a similar experimental design, was sufficient to show significant effects. Obviously, the statistical power in our experiment with such a large variability but small sample size is very small. With regard to the unequal variance between treatments, a simple comparison of control and biochar treatment has more power and should be valid with the reservations we discuss. Furthermore, the p-value only suggests that we can reject the null hypothesis with 1-p (74

Comment: *You should avoid discussing non-significant differences.*
Response: Knowing the apriori high variability in soil, biochar and N_2O measurements, a 0.05 significance level may be debatable. Still, it is interesting to discuss a large effect size.

Comment: *It's unfortunate that you didn't measure over a whole year. This does not allow comparing you data to IPCC emission factors, which are based on annual data. However, instead you should calculate and compare N_2O emissions per yield, which are actually more important for the GHG balance than emissions per area unit.*
Response: IPCC emission factors accounts for emissions from fertiliser addition by subtracting background emissions from unfertilised soil over one year (IPCC, 2014). Therefore, we do not call our EFs' 'IPCC emission factors' any further, but 'N_2O emissions per unit N applied' Calculating per yield emissions is definitely a good idea and we will include those numbers in the manuscript. However, this will enhance the overall uncertainty owing to the variance in the yield data.

Comment: *The quality of the graphs needs improving. The font size is too small and you use colors even when they are not necessary.*

Response: We will use different scaling for the graphs to improve font size. We think more colours are helpful to the reader as long as it's still easy to grasp the message.

Comment: *I would also appreciate if you could provide cumulated N_2O fluxes and crop yields for each plot, e.g., as supplementary material. This might be useful for possible meta studies.*
Response: We will include these numbers in the revised manuscript or supplementary material.

Some specific comments
796 Line 3ff: Low pH possibly impedes the synthesis of a functional N_2O reductase enzyme (Bakken et al. 2012, doi: 10.1098/rstb.2011.0321).
Response: Thanks for the helpful reference!

797 Line 12/13: Something is not correct here: C/N = 26.2/0.29 = 90.3. I assume that the N content was actually 2.9 g/kg.
Response: Right! N content is factor 10 higher hence 26.2/2.9 = 9.03. We correct that in the script.

799 Line 25: Where are the results from the CO2 measurements? How were these used in your study?
Response: CO2 data is only used for validating chamber functioning but we do not publish results or discuss about it. There were no effects on treatment and we do not have a scientific question about CO2.

800 Line 7: I'm not sure whether my bitbucket repo should be used as a reference.
Response: If you are not sure and do not have other suggestions it's in our opinion

the best thing to cite it that way, because everyone can access the code, use it as well or start a discussion on the very useful bitbucket plattform.

800 Line 7ff: With the relative high number of concentration-time points there might be better decision criteria for (robust) linear vs HMR (e.g., it might be sufficient to rely on Akaike's information criterion with finite sample correction, AICc). Mine were developed for fits to low numbers of points and more research is needed here. Please give information how many fluxes were calculated with which method and some measure of the distribution of flux standard errors. (Note that the HMR package recently corrected the calculation of standard errors and my package includes a function that calculates them correctly.) Anyway, I'm happy that you used a reproducible method.
Response: We will provide the numbers of fluxes calculated with each method. There are many ways of how to calculate these static chamber fluxes. We think it is important that the method is well documented, consistent and reproducible. Our analysis show that there are only minor differences from different calculation approaches. In general it is clear from observed concentrations in our chambers, that non linearities have to be taken into account to not underestimate the true flux. But non linear methods (as HMR that is used) often introduce a large variability from the uncertainty of the estimated nonlinear flux parameter. So far there is no optimal procedure described to balance the calculation between those two standpoints. The approach by you (Roland Fuss, the reviewer) is promising but not yet sophisticated and studied enough. It is still not settled how to adjust the parameter of whether or not HMR/robust linear is used (maximal factor to allow HMR to blow up the flux estimate; i.e. 4) to each user's specific measurement system (number of concentration measurements, deployment time, chamber size, precision of the quantification, soil properties, chamber characteristics etc.). This could be done by a comparison of larger datasets from different measurement systems. But this is far beyond the scope of this study. We just keep going with the most simple approach that is well documented and open source.

800 Line 17: I'm somewhat concerned by this. If you have implausible low values it stands to reason that you also have too high values. Only removing the low outliers could result in bias.
Response: Not necessarily! These high N_2O uptakes are technically due to certain chamber malfunctioning (frozen lids, upcoming turbulence after a night with no wind and a stable atmospheric layering, not closing lid, unfortunate variations of the N_2O analyser during no flux period or sudden temperature variations in the measurement container). Such events were not manually taken out from the raw dataset but at the described stage of the data processing script. But the same effects are not as pronounced in situation with positive and especially high fluxes. However, positive outliers were also checked for technical malfunctioning.

803 Line 19: Please always include standard deviations or errors when providing mean values.
Response: Standard errors will be provided in the revised script. They are also shown in figure 4.

808 Line 5ff: How does the discussion of P uptake contribute to answering your hypotheses? Omit Fig. 7 or provide it as supplementary material.
Response: okay! we put it into supp.

Cited references:
IUSS Working Group WRB. 2014. World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.
Bergaust, L., Mao, Y., Bakken, L.R., Frostegård, Å., 2010. Denitrification Response Patterns during the Transition to Anoxic Respiration and Posttranscriptional Effects of Suboptimal pH on Nitrogen Oxide Reductase in Paracoccus denitrificans. Appl.

Environ. Microbiol. 76, 6387–6396. doi:10.1128/AEM.00608-10

Interactive comment on SOIL Discuss., 2, 793, 2015.