Author's Responses to Reviewer Comments - soild-2-793-2015

R. Hüppi et al.

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1 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 have improved 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 application 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 (Felber et al., 2013, Case et al., 2014). 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% by weight (IUSS 2014). Our site has a moderate OC content of 2.6%.

Our results show that there are other effects than pH that have an impact on N_2O in a soil with high amounts of native soil carbon. We do not understand why the

reviewer thinks this effects to be less likely in our soil and what should be the point if we nevertheless see a kind of a large effect.. We define the purpose of this study in the abstract, to test effects of biochar on N_2O emissions in a temperate maize system. This is a practical scenario as there are many companies that sell biochar for this purpose in Switzerland, Germany and other countries with fertile temperate soils as the one we use.

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. We rephrased the second tested hypothesis in the last paragraph of the abstract to "we test...(ii) whether this possible reduction in N_2O emissions is similar when soil pH is increased by other means." It is known that pH exerts control on the N_2/N_2O ratio through influencing denitrifiers ability to synthesise N_2O reductase (Bergaust et al., 2010), and hence there is a mechanistic basis for our hypothesis. For the denitrification response, the pH-range from pH 6 to 7 is definitely of high importance: Bergaust et al., 2010 showed a large sensitivity of the assembly of N_2O reductase that is only optimal above pH 7. There can still be a significant decrease in N_2O emissions with increasing pH from 6.5–8. (Stevens et al., 1998).

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 (see figure 3).

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 have added more details about the method (principle of infrared filter technique, linearity of the instrument) to the text. 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 cited in the text that described and used the same system (Flechard et al., 2005, Felber et al., 2013); 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 reduced the aggregation span from 8 to 6 hours. This shows a higher resolution in time and does not affect cumulative emissions and differences between treatments.

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 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% in our case) chance, but it does not take the effect size of -52 % emissions into account. Hence, we decide to leave the dual interpretation of our data set, with respect to N_2O emissions, as it is. We omitted the part with the GLS modelling of the N_2O fluxes because the added value is limited. Hence there is also no need for transforming VWC values to WFPS.

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 account 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 have included those numbers in the manuscript. However, this enhanced the overall uncertainty owing to the variance in the yield data. Regarding the effect of biochar, yield based emissions are higher in effect size and the statistics shows lower p-values, see modified text.

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 have improved font size and scaling of the figures. We think more colours are helpful to the reader because many of them will read the paper in its online-version.

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 introduced a new table (table 3) with cumulated fluxes per area and per yield dry matter and also total N yield per plot. We also added a description of the yield based emissions to the result section.

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! We added this information to the manuscript.

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 as you assumed, hence 26.2/2.9 = 9.03. We corrected this in the manuscript.

799 Line 25: Where are the results from the CO_2 measurements? How were these used in your study?
Response: CO_2 data is only used for validating chamber functioning but we do not publish results or discuss them. There were no effects on treatment and we do not have a scientific question about CO_2.

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 provide now the numbers of fluxes calculated with each method in the last paragraph chapter 2.5. In general, 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, from observed concentrations in our chambers it becomes clear, 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 periods without fluxes or sudden temperature variations in the measurement container). Such events were not taken out manually from the raw dataset but at the described stage of the data processing script. It makes our procedure very transparent and objective without the need of visual screening of the raw data. Most data that were omitted by these rules were found in spring or autumn when fluxes were generally low. However, positive outliers were also checked for technical malfunctioning. We indicate the negative N_2O threshold of -10 instead of -50 ng/m2/s because it has been used that way in the calculation procedure before. However on the treatment effects the choice between -10 and -50 ng/m2/s does not make a difference. Overall only 2.5 % of the total numberall of fluxes waswere removed (mostly due to negative CO2 flux) and the cumulative total sum of all fluxes in the dataset was reduced by 0.61 % as indicated in the manuscript now. We added more details about this filtering to the manuscript.

803 Line 19: Please always include standard deviations or errors when providing mean values.
Response: We have added standard errors for mean cumulative emissions and soil bulk density. 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 have removed the figure about P uptake.

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
Stevens, R.J., Laughlin, R.J., Malone, J.P., 1998. Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biology and Biochemistry 30, 1119–1126. doi:doi: DOI: 10.1016/S0038-0717(97)00227-7

2 Author responds to anonymous review

We thank the anonymous reviewer for this review and the general agreement with our interpretation of the data. We are happy to include the helpful comments into our manuscript.

Comment: *The title is a bit confusing. It seems that biochar was applied in a limed soil. However, lime and biochar were added as different treatments. The title should be changed to make this clear.*
Response: We have changed the title also in the sense of A. Gattingers comment to: "Effect of biochar and liming on soil nitrous oxide emissions from temperate a maize field."

Comment: *Abstract. It is in general ok, but it could be improved. For instance, it is stated that "laboratory incubations have shown significantly reduced N_2O emissions from soil when mixed with biochar". This is true in average, but there are many laboratory studies that also found an increase in N_2O emissions after biochar treatment, and this seems to be linked to the specific mechanism leading to N_2O formation.*
Response: We are not aware about 'many' laboratory studies that found increased emissions. We talk about 'a number of laboratory incubations'; this is in line with published evidence. However, in our introduction, we explicitly mention increased emissions that are linked to nitrification pathways (Sanchez-Garcia et al., 2014).

Comment: *Please include in the abstract what kind of N fertilizer was applied, since this could have important implications for the N_2O formation pathway. Include also average soil pH before and after liming.*
Response: We have added that ammonium nitrate was used as fertiliser to the abstract. We also indicate now the treatment average pH in the abstract.

Comment: *The last statement of the abstract is a bit simplistic. This is a field study, there are many factors interacting and out of control, so it is difficult to distinguish which is the role of pH. It could be important for N_2O produced by denitrification pathways, but at the same time it could have promoted nitrification emissions. . . so I would not simplify the conclusions in this way. The discussion about pH in the subsequent sections reflects better the conclusions of the study, for instance, in the conclusions "there is no evidence that the reduction with biochar, relative to control is solely induced by a higher soil pH".*
Response: We agree on this comment and have changed the abstract to be inline with the conclusions.

Comment on Materials & methods: *I am not convinced about removing data. Even if this doesn't change the final conclusions, low CO_2 flux does not necessarily mean that the lids were not functioning properly.*
Response: We accept low and even slightly negative CO_2 fluxes, to capture measurement uncertainty around zero fluxes. But there is no mechanistic explanation for

substantial net CO_2 uptake from bare soil. Soil always emits CO_2 depending on temperature and substrate availability. CO_2 uptakes larger than 0.5 $\mu mol/m^2/s$ are a very robust indicator for technical problems not related to soil-atmosphere exchange. With this procedure we do not have to manually remove data points, what would have been difficult to document and justify. The procedure was also used in other studies using the same chamber system (Felber et al., 2013, Neftel et al., 2010). We stress that only c. 2 % of the data and 0.6 % of the total flux was removed by this. We improved the description of this filtering procedure in the manuscript and also explained, in the answer to R. Fuss, why N_2O data was filtered.

Comment on Results: *It is frequently mentioned in the manuscript that the differences between treatments are not significant. I would de-emphasize this point. P values are not the "absolute truth", and especially in N_2O studies (even more in the field) it is very infrequent to find "significant differences" according to traditional P values. Considering that you have calculated emission factors and also maize yields, it shouldn't be much work to include also yield-scaled emissions.*
Response: We have included yield-scaled emissions upon revision(also suggested by Andreas Gattinger). We are happy about your perception of statistical significance in our dataset. Indeed it is very rare to reach p¡0.05 significance with this setting, especially with only 3 replicates. We have added a remark on this in the result section on N_2O emissions. However, following the statistical convention we have to admit that we can't see a significant statistical effect of the treatment.

Comment on Discussion: *When you compare with other field studies where an increase in emissions was found after biochar addition, can you comment on the differences respect to your study? Were the soils different in these studies, and what about the biochars? Did the biochars used in these studies had low H:Corg atomic ratio and C:N ratio as in your case? These comparisons might be useful to define future field studies.*
Response: We can only speculate on possible mechanisms as long as we don't know about which properties are important. We added a comment to the manuscript that Cayuela et al., 2015 found low Corg:H ratios being linked to high effects. It is difficult to speculate whether or not this applies to the studies we mentioned because the effect of Corg:H ratio of biochars has not been tested systematically (mostly just one biochar is used).

Comment: *The clarity of the figures needs to be improved. It is rather difficult to discern between treatments: symbols/letters are very small and not clear.*
Response: Thank you for this comment. We have improved the clarity of the figures.

3 Author response to comments from Andreas Gattinger

General response: We greatly thank Andreas Gattinger for his fruitful comments and the appreciation for our work. We have improved the paper according his suggestions.

Comment 1. The treatment effect: *In its current version the N_2O emissions doesn't follow any significant treatment effect. This is due to the experimental design, where the treatments were defined according to its potential pH effect: "control", "biochar", "lime". In fact with the application of either biochar or lime, soil pH could be significantly increased relative to the control. However, for N_2O emissions the variations from the limed plots were that high that a final treatment effect on level $p = 0.05$ could not been determined. If the standard error of the N_2O flux curves from liming would have been in the same range as the flux curves determined for the control or biochar plots, there would have been an effect on N_2O emissions. Anyhow, high variations from N_2O fluxes from field measurements are a quite common feature. Therefore, I suggest to report the data in two ways. First, as it is now, along with the observed phenomena. Second, following an experimental design which considers only the treatments "control" and "biochar". For this, the statistical analyses need to be revised, as the treatment "lime" will be removed from the statistical model. This, however, impacts on the objectives and hypotheses, thus they need to be adapted as well. It could be done in a way by saying that this experiment follows two lines: one is to observe any biochar effect on N_2O mitigation, the other one goes for causative research (pH effect) by adding a lime treatment to the experiment. Considering the suggestions made by R. Fuss will be straightforward to improve the statistical approach in general. Adapting the paper in that way impacts on the overall context, meaning that the impact of biochar alone may deserve more attention in the discussions section and that statements for a possible pH effect should be done more cautiously. As a further consequence from the re-arrangement of the paper, I suggest another title: "Effect of biochar and liming on soil nitrous oxide fluxes from a maize field."*

Response: We have changed the title considering your suggestion. We also see the option to separate the two research questions more strictly. However, this suggestion is in clear contradiction to the review comment by R. Fuss. We therefore decided to give room for both interpretations and still point to the overall non-significance of treatments, also because this is in line with the generic use of statistical testing.

Comment 2. Crop yields: *The authors present crop yields from maize and its N and P uptake in figures 5 to 7. I suggest to replace the term "plant" by "above-ground biomass" to make it clearer. Furthermore, as already suggested by R. Fuss, I would report N_2O effects as 1) area-scaled and b) as yield-scaled N_2O emissions. This illustrations should ideally follow the same line as explained above, namely for the pH effect (control, biochar, lime) and for biochar effect (control, biochar). The yield-scaled illustration of N_2O emissions provides an even stronger argument for a possible GHG mitigation effect of biochar as it impacts apart from N_2O suppression also on crop growth. These aspects needs stronger consideration in the discussion of the revised paper as well.*

Response: We have included yield-scaled N_2O emissions. As explained above in response to other reviewer comments, this number comes with additional uncertainty from yield variability. Because there is no significant treatment effect on yield, but only a small tendence of biochar causing higher yields, yield-scaled emissions show higher effect sizes but not much smaller p-values. We have added the argument that yield-scaled emissions are a strong argument to judge biochar effects to the text.
We added above-ground biomass to the caption of the figures describing the plant yield.

4 Author response to editor comment by Karsten Kalbitz

Response: Dear Editor,
we thank you for the editorial work and the balanced comments on the review. We can surely enhance the argumentation regarding the choice of soil type and put it into the perspective of agriculturally used soil types in Switzerland; please see our response to the first review. Our research question focuses on whether or not biochar is a viable option for the fertile agricultural soils we find in many regions of temperate Europe. In addition, biochar is proposed and increasingly used as soil amendment in Switzerland. There is an ongoing legislative effort for its wider application and companies already sell biochar for agricultural use.

Comment: *As R. Fuß I do not understand the motivation for selecting a Eutric Mollic Gleysol for this field experiment. In the introduction you mentioned the positive effects of biochar application for soils with "a small cation exchange capacity and low organic carbon content"*
Response: Correctly, the main reason for applying biochar in temperate agriculture is not to improve soil fertility, but more so to positively influence the soil's greenhouse gas balance. We explicitly address this point in our introduction now. Eutric Mollic Gleysol is a very common soil type in Switzerland, often used for agriculture, and the pH is supposed to be typical according to standard agricultural practice. In fact; we have selected the soil around our Institute with the lowest pH. Also high CEC and high organic C content is very usual for Swiss agricultural soils. The question is, whether also in these soils, biochar has an effect on N_2O emissions. Seeing some effects even in such soils would be an even stronger argument to promote biochar use in temperate agriculture.

Comment: *You have to explain what kind of pH effect you expected at such a soil with a pH of 6.3. I would expect that effects might be different comparing an increase in pH from 5 to 6 with 6 to 7. I would suggest to discuss differences in potential mechanisms as well.*
Response: We explained possible mechanisms of biochar at higher soil pH in our manuscript and in the response to reviewer R. Fuss. To our knowledge there is little

knowledge about mechanisms involved in biochar functioning in soil with respect to N_2O. We have stressed scientific evidence in the manuscript that show the relevance of the pH for N_2O emissions between pH 6-8, hence our increase from 6.1 to 6.5 is in the most pH sensitive range.

5 List of all relevant changes made to the manuscript

- The title was changed.
- negative N2O threshold is properly described as -10 ng-N/m2/s like it was applied to the dataset before
- Reduction effect has changed from 53 to 52 % (biochar vs control), due to consistent use of flux thresholds.
- time average interval was changed from 8h to 6h
- yield based emissions were introduced per kg-N uptake and t dry matter yield
- Results and discussion of yield based emissions was added.
- The part with GLS modelling of VWC and emission was skipped. The VWC data was just analysed by anova on a half-daily mean basis (8.7 % showing significant treatment effects, with higher VWC in biochar).
- The abstract was modified as suggested by reviewer 2 (mention fertiliser type, formulate the last sentence as in the conclusion)
- Reference from Bakken et al., 2012 about pH effect on N_2O reductase was added to the introduction
- More information about the N_2O measurement was added and the temperature correction is described in more depth.
- Chosen method (robust linear or HMR) was indicated as summary of the whole dataset.
- For mean results, standard errors were added.
- A table with plot wise emissions per area, yield and N uptake was added.
- The discussion and calculation of IPCC emission factors is omitted.
- Soil pH of the treatments is now indicated in the abstract and it was made clear in the result section about pH that the most significant difference with the 0.4 pH increase was from 6.1 to 6.5 in June during the highest emission phase.
- We added more details about pH sensitivity of soil N_2O emissions (Stevens et al., 1998, Bakken et al., 2010).
- Figure font size was changed

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~~Biochar's effect~~ Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize field~~with lime-adjusted pH treatment~~

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Abstract

Biochar, a carbon-rich, porous pyrolysis product of organic residues may positively affect plant yield and can, owing to its inherent stability, promote soil carbon sequestration when amended to agricultural soils. Another possible effect of biochar is the reduction in emissions of nitrous oxide (N_2O). A number of laboratory incubations have shown significantly reduced N_2O emissions from soil when mixed with biochar. Emission measurements under field conditions however are more scarce and show weaker or no reductions, or even increases in N_2O emissions. One of the hypothesized mechanisms for reduced N_2O emissions from soil is owing to the increase in soil pH following the application of alkaline biochar. To test the effect of biochar on N_2O emissions in a temperate maize system, we set up a field trial with a 20 t ha^{-1} biochar treatment, a limestone treatment adjusted to the same pH as the biochar treatment (pH 6.5), and a control treatment without any addition (pH 6.1). An automated static chamber system measured N_2O emissions for each replicate plot ($n = 3$) every 3.6 h over the course of 8 months. The field was conventionally fertilised at a rate of 160 kg N ha^{-1} in 3 applications of 40, 80 and 40 kg N ha^{-1} as ammonium nitrate.

Cumulative N_2O emissions were ~~53~~52 % smaller in the biochar compared to the control treatment. However, the effect of the treatments overall was not statistically significant ($p = 0.~~26~~.27$) because of the large variability in the dataset. Limed soils emitted similar mean cumulative amounts of N_2O as the control. ~~This indicates that the observed~~ There is no evidence that reduced N_2O ~~reduction effect of biochar was not~~ emissions with biochar relative to the control is solely caused by a ~~pH effect~~higher soil pH.

1 Introduction

Agriculture faces major challenges regarding world food security because of climate change, continued population growth and resource-depleting practises (IAASTD, 2009). Accounting for roughly 12 % of anthropogenic greenhouse gas (GHG) emissions per year, agriculture is a sector with a considerable mitigation potential and, at the same time, is highly vulner-

able to the consequences of a changing climate (IPCC, 2014). With its 300 fold warming potential compared to CO_2, nitrous oxide (N_2O) from soil is a downside of the large productivity increase in agriculture, mostly due to synthetic nitrogen fertiliser application. Reducing agricultural N_2O emissions would reduce the GHG induced radiative forcing (IPCC, 2014), improve the stability of the stratospheric ozone layer (Ravishankara et al., 2009) and reduce agriculture's energy intensity when achieved with a lower nitrogen fertiliser use (IAASTD, 2009).

Biochar is produced by thermal decomposition of organic material in a low-oxygen environment, called pyrolysis. This stable charcoal-like material has the potential to contribute to the mitigation of climate change by increasing soil carbon (C) (Lehmann, 2007; Woolf et al., 2010; Lal et al., 2011). In addition, biochar can increase crop yields (Jeffery et al., 2011; Biederman and Harpole, 2013; Crane-Droesch et al., 2013) and reduce water stress, which helps to adapt to climate change (Mulcahy et al., 2013). Its application to soils that have a small cation exchange capacity and low organic carbon content is associated with higher crop yields (Crane-Droesch et al., 2013) with an overall mean response of 10 % (Jeffery et al., 2011). For fertile, temperate soils improvement of soil quality is not key to biochar application. Rather, biochar effects on soil-borne GHG emissions, N_2O in particular, has become a strong argument for its amendment.

Biochar also controls nitrogen (N) cycling (Clough et al., 2013). Biochar can reduce N leaching (Steiner et al., 2008; Güereña et al., 2013) and soil-borne N-containing GHG ~~(van Zwieten et al., 2015). Especially nitrous oxide (~~(Van Zwieten et al., 2015) . Especially N_2O ~~)~~ emissions from soil are reduced on average by 54 % in lab studies and 28 % in field measurements (Cayuela et al., 2015). In field situations, N_2O reduction effects are typically difficult to verify because of less uniform conditions and a large spatial and temporal variability of fluxes (Felber et al., 2013; Schimmelpfennig et al., 2014). A few field experiments indicated an increase in N_2O (e.g., Verhoeven and Six, 2014; Liu et al., 2014), many showed no significant effects ~~(Angst et al., 2014; Karhu et al., 2011; Scheer et al., 2011; Suddick and Six, 20~~ other studies indicated decreasing N_2O emissions (e.g., ~~Felber et al., 2013; Van Zwieten et al., 20~~ Only few studies with biochar have looked at N_2O emissions beyond 120 days (Verhoeven

and Six, 2014), hence there is a large uncertainty about longer term effects of biochar addition.

Biochars are often alkaline and therefore increase soil pH after application (Joseph et al., 2010). Denitrifying bacterial communities have the potential to increase their N_2O-reducing activity with increasing pH, which may reduce N_2O emissions from soils (Cavigelli and Robertson, 2001; Simek and Cooper, 2002; Čuhel et al., 2010). Low pH possibly impedes the synthesis of a functional N_2O reductase enzyme (Bakken et al., 2012) . Some authors suggest that the elevated soil pH is responsible for reduced N_2O emissions following biochar application through increased activity of N_2O reducing bacteria (Van Zwieten et al., 2010; Zheng et al., 2012). In contrast, Yanai et al. (2007) argue that the suppression of N_2O emissions by biochar is not through increased N_2O reduction activity because biochar ash also increases soil pH but does not reduce N_2O emissions. Cayuela et al. (2013) showed that biochar's acid buffer capacity was a more important factor in denitrification than the pH shift in soil. There are indications that biochar enhances nosZ expression, the gene responsible for the transcription of the N_2O reductase in denitrifying microorganisms (Harter et al., 2014; Van Zwieten et al., 2014). This could be a mechanistic link to the observed reduction in N_2O emissions through biochar increasing soil pH and microbial activity. In contrast, under conditions favouring nitrification and not being as sensitive to pH as total denitrification, biochar addition increased N_2O emissions in the lab (Sánchez-García et al., 2014) and possibly in the field (Verhoeven and Six, 2014).

In this study, we test (i) whether N_2O emissions are reduced following the application of biochar to soil of a temperate maize cropping system and (ii) whether this possible reduction in N_2O emissions is ~~due to an increase in pH~~ similar when soil pH is increased by other means. The latter was tested by a treatment where limestone was added to increase soil pH to the same level as that from the addition of $20\,\mathrm{t\,ha^{-1}}$ biochar. N_2O emissions and maize yield were quantified during one growing season in the field.

2 Method

2.1 Field site

The experiment was established on a cropland field near the Agroscope research station in Zurich, Switzerland (47.427° N, 8.522° E, 437 m a.s.l.). The climate is temperate with a mean annual air temperature of 9.4 °C and mean annual rainfall of 1054 mm (Climate data 1981–2010, Meteoswiss, 2013 from the MeteoSwiss station Zurich Affoltern 500 m from the experimental site). The field was under conventional management with maize in 2013, the year prior to the experiment.

The soil is a clay loam with a particle size distribution of 37 % sand, 27 % silt and 36 % clay. ~~According to the world reference base for soil resources (?) it~~ The soil is a Eutric Mollic Gleysol (Drainic) (IUSS Working Group WRB, 2014) . The untreated soil has a pH of 6.3 in water ($1 : 2.5$ w/v), total organic carbon content of 26.2 g kg^{-1}, total N of ~~0.29~~2.9 g kg^{-1} and bulk density of 1.3 g cm^{-3}.

2.2 Biochar

Several biochars were screened in advance to pick one with a high liming capacity and with properties in agreement to the guidelines for contents of polycyclic aromatic hydrocarbons (PAHs), ~~C- and N-content~~ C and N of the European Biochar Certificate (EBC, 2012). The chosen biochar was produced in a Pyreg reactor (Pyreg GmbH, Dörth, Germany) by Verora in Edlibach ZG, Switzerland in late 2013 (see chapter 30, case study 2 in Lehmann and Joseph, 2015). Pyreg reactors use slow pyrolysis in a continuous system with an average residence time of circa 25 min and a peak temperature of approximately 650 °C. The feedstock was green waste mainly from tree pruning. The biochar has the following properties: 64.9 % total C; 62.1 % ~~Corg~~C_{org}, pH 9.8 ($1 : 10$ in water); liming capacity 17.2 %$CaCO_3$, 148 m^2 g^{-1} BET surface area and ash content 20 %. Elemental ratios are 0.11 O / C and 0.33 H / C molar and 94 C / N by mass. Moisture content at the time of application was 12 %. Biochar was sieved < 3 mm shortly before it was spread on the field.

2.3 Experimental setup

Three different treatments were introduced; 20 t ha^{-1} biochar, control without additions and a limestone treatment to increase the soil pH to the same level as with biochar. The field was split into 3×3 plots with a size of 2 by 3 m (6 m^2 per plot and 3 replicates for each treatment). One meter buffer zones were established between plots on all sides. The 3 different treatments were arranged in a randomized complete block design with the 3×3 grid accounting for spatial variability. The whole field, including the buffer zones, were planted with maize (zea mays). Initial pH values were not different among treatment plots (see pH measurement ~~in January on~~ from January 2014 in Fig. 2).

2.4 Field management

The field was ploughed in autumn 2013 after the maize harvest. In January 2014, 20 t ha^{-1} biochar and 2 t ha^{-1} limestone, respectively, were spread on the wet, ploughed field surface. Freshly applied biochar was gently mixed with the first 1–3 cm of soil by hand at the same time. In mid-February 2014, the automated GHG chamber system was installed and in March the field was harrowed by a rototiller to a depth of circa 15 cm. The chamber frames were reset into the soil again and Decagon TE5 temperature and humidity sensors (Decagon Devices Inc., Pullman~~Wa~~, WA, USA) were placed at a depth of 8 cm in the centre of each plot. The TE5 sensor measures the volumentric water content (VWC) in soil by time domain reflectometry (TDR) at 70 MHz.

In May, potassium (K) and phosphorus (P) fertiliser was applied at a rate of 41.4 kg P ha^{-1} and 132 ~~kgK~~kg K ha^{-1}. Nitrogen was applied in 3 portions of 40, 80 and 40 kg N ha^{-1} on the 26 May, 16 June and 16 July, respectively, as ammonium nitrate (LONZA-Ammonsalpeter 27.5 % N). The fertiliser doses were spread on each plot of 6 m^2 and chamber frame of 0.03 m^2 separately to ensure equal distribution. On the 5 May, two of the three lime replicates were treated with another 1 t ha^{-1} of limestone because the pH was not in the same range as the biochar plots. Maize (Padrino from KWS SAAT AG, Einbeck, Germany) was sown on the 8 May with 0.14 m distance within rows that were 0.6 m apart from each other.

For plant protection only one herbicide application was conducted on the 19 June with 1 L ha^{-1} Dasul (Syngenta, Basel, Switzerland), 1 L ha^{-1} Mikado (Bayer CropScience, Germany) and 1 kg ha^{-1} Andil (Omya AG, Switzerland). Despite manual weeding and herbicides a considerable amount of weeds emerged. Plots were harvested on the ~~13th of~~ 13 October.

2.5 Nitrous oxide measurement

N_2O and CO_2 emissions were measured with static chambers of a fully automated measurement system (Flechard et al., 2005; Felber et al., 2013) consisting of nine stainless steel chambers ($30 \times 30 \times 25$ cm). These chambers were placed on PVC frames inserted 3 cm deep into soil. ~~Two frames were placed on each plot at a similar distance to the plot borders. These~~ The frame positions were moved three times during the growing season to obtain a better spatial representation of each plot. After maize had been sown, the chamber positions were between rows and no vegetation was grown within the chamber frame. Each of the 9 chamber lids were automatically closed and opened sequentially (over a period of ~~3.5~~3.6 h) allowing N_2O and CO_2 to accumulate in the chamber headspace for 15 min. Chamber headspace air was circulated (1 L min^{-1} air flow) through an inlet and outlet line from each chamber through polyamide tubes (4 mm I.D.) to the analytical system and back to the chamber headspace ~~continuously~~ after sample analysis. The analytical and chamber control instruments were installed in a nearby field cabin under temperature controlled air conditioning. N_2O concentrations were continuously measured and stored every minute using a gas filter correlation ~~technique~~ analyser (TEI Model ~~46C, Thermo Environmental Instruments Inc., Sunnyvale, CA~~46c, Thermo Fisher Scientific, Waltham, MA, USA). The gas stream is exposed to infrared light from specific bands (filtered), both from N_2O absorbing and non absorbing bands. From this difference a gas specific and concentration sensitive signal is retrieved. The instruments linearity is described with ± 2 % with negligible interference of H_2O, CO_2 or CO. CO_2 was measured with an infrared sensor from Liston Scientific Corp. (Irvine, CA, USA). The system was calibrated every 11 h with three different concentrations from certified gas standards (Carbagas, Rümlang, Switzer-

land). The N_2O analyser showed a drift with room temperature variations that the air conditioning could not avoid completely. Hence a temperature correction factor was applied to the raw data from a regression of the device temperature with data during calibrations in May. The temperature correction factor used was about 9.1 ppm per °C temperature change from the 37 °C device reference temperature. The mean N_2O analyser device temperature in June–July was 37.4 ± 2 °C (± 1 sd).

N_2O and CO_2 fluxes from soil were calculated from the continuous concentration measurement (resolution 1 per min) when chamber lids were closed. Data from the first 3 min of the total 15 min closure time were omitted from the flux calculation to remove signal noise due to gas exchange from the system during chamber switching and closing (Felber et al., 2013). The same flux estimation procedure (R-script by R. Fuss on bitbucket.org, see Fuss, 2015) was used as in Leiber-Sauheitl et al. (2014). It is a modification of the HMR package (Pedersen et al., 2010) that chooses between exponential curvature for non-linear chamber behaviour (Hutchinson-Mosier regression) and robust linear regression (Huber and Ronchetti, 1981). The exponential HMR scheme considers non-linear concentration increase in the chamber due to a possibly decreasing concentration gradient, chamber leakage and lateral gas transport. Robust linear regressions provide a more reliable flux estimate for low fluxes when there is a lot of variation due to limited measurement precision and outliers. Following the flux script's recommendation, non-linear HMR was used for 1034 fluxes, whereas for all the other 13 034 fluxes the robust linear regression was chosen. The resulting flux estimates from this procedure were then filtered for implausible large N_2O uptake by soil (i.e. when the ambient N_2O concentration suddenly drops with increased mixing in the boundary layer). N_2O fluxes smaller than -5010 ng ng $N_2O\ m^{-2}\ s^{-1}$ (Neftel et al., 2010) were removed as well as data associated with a likely invalid chamber functioning (i.e. frozen lids) when indicated by CO_2 flux fluxes $< -0.5\ \mu mol\ m^{-2}\ s^{-1}$ (Felber et al., 2013). In total 302 and 351 data points from From the entire dataset (of 14 068 points) were rejected fluxes, 302 were rejected due to the CO_2 flux threshold and additional 49 fluxes due to N_2O (2.5 % of the total number of fluxes removed). Considering a cumulative sum of all fluxes in the dataset, filtering reduced this number by 0.61 %.

2.6 Yield

The yield was separated into grain (kernels) and above-ground plant material. Cobs were threshed and dried whereas the plants were weighed freshly on the field, chaffed and a sub-sample was then dried to measure water content and for further plant nutrient analysis. From both plant and grain, dry matter total N~~and P~~, P, K, Ca and Mg content were measured (FAL, 1996). For yield based N_2O emissions, cumulative N_2O emissions in kg N_2O-N ha^{-1} were related to total dry matter (DM) yield in $t\ ha^{-1}$ (from harvested plant and grain together, see Table 3) and to total above-ground plant N uptake in $kg\ N\ ha^{-1}$ (see discussion).

2.7 Soil sampling and analysis

Soil samples for pH, ammonium (NH_4^+) and nitrate (NO_3^-) measurements were taken on the 31 January, 31 March, 26 May, 16 June and 4 September 2014. At each sampling, five randomly distributed soil cores per plot were taken (0–10 cm) and pooled. Soil pH was determined in moist soil samples using water at a ratio of $1:2.5\ w/v$ and measured with a PH100 ExStik pH meter (Extech Instruments Corp., Nashua, NH, USA). Soil bulk density was measured on the 27 June at a depth of 3–8 cm using 100 cm^3 steel cores, 3 per plot.

For soil NO_3^- and NH_4^+ concentrations, 20 g of moist soil were mixed with 100 mL 0.01 M $CaCl_2$ solution. The suspension was shaken for 30 min, filtered and then analysed by segmented flow injection analysis on a SKALAR SANplus analyser (Skalar Analytical B.V., Breda, ~~the~~ Netherlands).

2.8 Statistical analysis

The obtained fluxes from the automated chamber system were aggregated to ~~8~~6 h means producing a regular, smoothed dataset. The system was able to measure each chamber three times for every 11 h calibration cycle during regular operations, hence on average ~~2.2~~ 1.6 measurements for each chamber were included in each a ~~8~~6 h mean. Still missing values after this aggregation step were linearly interpolated for each chamber. Treatment averages and standard ~~deviations~~ errors were calculated from the 3 chambers on the repli-

cated plots. If not indicated otherwise, treatment means are shown with $\pm$ 1 standard error.

Statistical analyses were performed with R (version 3.0.1, The R Project, 2014). Significance level was chosen at $p < 0.05$ for all procedures, unless indicated otherwise. Significant treatment effects for cumulated fluxes were determined using ANOVA from rbase package (treatments: control, biochar and lime; $n = 3$). Bartlett test of homogeneity of variances showed conflicting ANOVA assumptions for the cumulative fluxes. This could be ~~solved~~ improved by log transformation of the flux data.

~~In addition, a generalized least squares model (GLS) was constructed with weekly cumulated emissions as dependent variable, and weekly averages of soil volumetric water content (VWC) and the treatments (control, biochar, lime) as explanatory variables. A restricted maximum likelihood generalised linear model from nlme R package was used to calculate the GLS.~~

3 Results

3.1 ~~Meteorological data on the~~ Environmental field conditions

The year started with above average temperatures and low rainfall (Fig. 1). End of May to June was dry with high temperatures being on average for Switzerland 1.5 °C above the 1981–2010 norm (Meteoswiss, 2015). The soil's volumetric water content fell to circa 20 %, inducing high water stress on the young maize seedlings. The lack of soil moisture presumably hampered the dilution of the first application of 40 ~~kgN~~ kg N ha^{-1} in the soil solution. Along with the 2nd N fertilisation the field was therefore irrigated with 33 mm water (shown as green bar in ~~the precipitation dataset~~Fig. 1). The summer months ~~following (~~July and August ~~)~~ were rather cold and wet with daily mean air temperatures below 20 °C (Meteoswiss, 2015).

~~The GLS model indicated a significant, treatment specific ($p = 0.0202$) effect of weekly mean soil VWC on weekly cumulated fluxes ($p = 0.0034$). Biochar plots had significantly~~

higher soil water content than lime and control plots ($p < 0.001$).However, there is no interaction between treatment and VWC on a weekly basis ($p = 0.542$) Soil VWC tended to be higher in biochar plots (Fig. 1) with 37 out of 423 (8.7 %) half-daily means showing a statistically significant treatment effect.

3.2 Soil pH and nitrogen

Soil pH increased with limestone and biochar addition in medium terms by circa 0.4 pH units (Fig. 2). During the time with major emissions in June, the pH between control and biochar/lime soils significantly ($p < 0.001$) increased from 6.1 to 6.5 with. The initial soil pH was on average 6.3 and not different among treatments. Following biochar application soil pH increased to up to 7.4 whereas with addition of limestone soil pH increased to up to 6.9 (averages across replicates). The pH sharply decreased after the initial peak, especially in those two liming plots, which were treated subsequently with another 1 t ha^{-1} in May. Soil pH of biochar and lime treatments were not significantly different at any sampling time, whereas soil pH of the control treatment was systematically below that of the amended soils.

Mean soil bulk density was not statistically different between treatments (1.31 $\pm$ 0.03 g cm^{-3} in the control, 1.29 $\pm$ 0.07 g cm^{-3} in biochar and 1.36 $\pm$ 0.04 g cm^{-3} in the liming treatment).

Soil mineral N was not statistically different between treatments at any sampling date (Tables 1 and 2).

3.3 N_2O fluxes

Emissions were characterized by peak events, particularly in summer, and by background emissions in spring and autumn (Fig. 3). Main emissions occurred after the second fertilisation event of 80with the highest dose around early August. Afterwards, there were only emissions from one of the lime plots but almost none until the end of October from all the other plots. This also corresponds to the low amounts of available soil N, indicating that the plants had taken up most of it. All treatments revealed similar temporal N_2O emis-

sion dynamics but the height of the peaks differed. During peak events emissions from the biochar treatment were often lower than those from the other treatments, especially compared to the control. This resulted in an increasing difference in cumulative fluxes (Fig. 4) between control and biochar. Mean cumulative emissions for the entire growing season were 170~~, 357 and 360 mg~~ $\pm$ 16.5, 353 $\pm$ 31.7 and 359 $\pm$ 164 mg N_2O-N m^{-2} for biochar, control and lime treatments, respectively (see Table 3 for plotwise results). Relative to the control, mean cumulative N_2O emissions were ~~53~~52 % smaller in the biochar treatment. The whole treatment effect was, however, not statistically significant ($p = 0.26$) due to the large variability in the dataset. Emission means from control and lime ~~are~~ were very similar. With lime, N_2O emissions were highly variable and this treatment included both the chamber with the highest and also the one with the lowest cumulative emission. ~~We therefore~~ Alternatively, we also calculated p ~~values for~~ -values comparing only biochar and control treatments ~~only~~ with a Welch Two-Sample t test~~resulting~~. This resulted in a significant difference ~~with~~ ($p = 0.022$). All p-values have to be treated with caution because they were produced with a minimal number of replicates. Furthermore a large treatment effect size is not reflected in the p value.

~~Emission factors~~ N_2O emissions per unit N applied calculated from the 160 ~~applied~~ kg N ha^{-1} with the mean cumulative emissions during the growing season, resulted in ~~0.67~~1.06 % for biochar, ~~1.42~~2.21 % for control and ~~1.43~~2.25 % for the lime treatment~~, but~~. In analogy to cumulated emissions these values were not significantly different ~~. For comparison with with IPCC emission factors, background emissions need to be subtracted. We estimated background emissions by cumulating only emissions that were directly influenced by the N-fertiliser applied (between 26 May and 13 August = approx. 3 months) and subtract half of the cumulative emissions from the residual period measured (approx. 6 months). This resulted in IPCC emission factors of 0.58for biochar, 1.28for control and 1.25for the lime treatment~~among treatments and have the same statistics.

3.4 Maize yields and plant growth

Maize yields were not significantly different between treatments, for both grain and ~~plant dry matter~~ above-ground plant DM (Fig. 5). Nitrogen ~~and P uptake~~ uptake by maize did not differ among treatments (~~Figs~~Fig. 6~~and 7).~~ , Table 3). Table 3 shows cumulated N_2O emissions for each plot and per area as well as per DM yield. Yield based emissions (Table 3) resulted in 0.128 ± 0.010 kg N_2O-N per t-DM, for biochar, 0.319 ± 0.036 kg N_2O-N per t-DM for control and 0.306 ± 0.148 kg N_2O-N per t-DM for the liming treatment. Although the yield based emission with biochar is 60 % lower compared to the control, overall there is no significant treatment effect ($p = 0.19$). There was no difference between treatments for any of the measured nutrients in the yield (data not shown).

4 Discussion

4.1 N_2O emissions

Our high-frequency automated N_2O chamber measurements give a detailed picture of the emissions from a ~~biochar-lime~~ biochar and lime field trial. Neither soil NO_3^- nor NH_4^+ concentrations can explain N_2O emission patterns at any point in time. ~~Estimated IPCC emission factors are at the lower end of the range of the IPCC guidelines for cropland soils of 0.3–3(IPCC, 2006) .~~ Although cumulative N_2O emissions were not significantly different among the three treatments, emissions with added biochar were ~~53~~52 % below the control treatment. The magnitude of reduction is in agreement with the meta-analysis of Cayuela et al. (2015) who showed a general reduction of N_2O emissions by biochar of 49 ± 5 % (lab and field experiments) but it is larger than the reduction found by the same authors under field conditions (28 ± 16 %). In our temperate maize field, N_2O emissions ~~thus decreased~~ can thus decrease with biochar addition as much as they have been shown to be reduced under controlled lab conditions.

Our results show no ~~a~~ decrease in N_2O emissions when limestone is used to increase the soil pH to the same level as that with biochar. This finding does not support the hypothesis that biochar's N_2O reduction effect is ~~solely due~~ similar to a geochemical ~~manipulation~~ adjustment of soil pH. However, it must be considered that the large variability among the three replicates hampers the power of this conclusion. A post-hoc power analysis showed a 23.4 % probability of accepting a true alternative hypothesis considering the obtained results in cumulative N_2O emission. To have at least a power of 80 % we would have needed 10 replicates for each treatment. The high variability solely in the liming treatment might be due to additional lime application to the field in May 2014 and the high spatial-temporal variability of that soil property in general. The two replicates that received additional limestone were the ones that emitted more N_2O than the other plot. Hence, instead of reducing emissions by increasing the pH, the additional limestone application could have provoked local arbitrary disturbance to soil chemistry leading to emission hotspots. To determine the biochar effect on N_2O emissions, we therefore also compared only the biochar and control treatments ~~;~~ (see results); according to the analysis of that reduced dataset, the cumulative emissions in the biochar amended plots ~~are~~ were significantly lower (by ~~53~~52 %) than in the control treatment.

~~The GLS model shows that not only treatment but also water content affects soil emissions. However, the mechanism behind the overall negative feedback of VWC on emissions (i.e. higher VWC leads to lower emissions) can not be derived from our data. Biochar effects on soil physical properties have been shown to increase water-holding capacity, reduce bulk density and increase soil sub-nanopore surface together with a 92decrease in emissions (Peake et al., 2014; Mukherjee et al., 2014) . This suggests that increased soil aeration by biochar dominates the effect of increased water content and hence does not favour denitrification (Van Zwieten et al., 2010) .~~

Using the same measurement technique, application rate and similar biochar properties ~~we find much higher emission reductionsin cropland than Felber et al. (2013) in a grassland field~~Felber et al. (2013) also reported N_2O emission reductions, but smaller as compared to the difference we saw between biochar and control. In line with our results other field stud-

ies have ~~also~~ shown significant reductions in N_2O emissions following biochar amendment ~~(Van Zwieten et al., 2010; Taghizadeh-Toosi et al., 2011; Liu et al., 2012)~~ (Taghizadeh-Toosi et al A number of studies found no significant effect of biochar addition in the field (Schimmelpfennig et al., 2014; Angst et al., 2014; Scheer et al., 2011; Karhu et al., 2011; Anderson et al., 2014). Often the much higher variability in the field and the low number of replications make it difficult to reproduce reduction effects observed in laboratory studies. In particular, Angst et al. (2014) found no significant difference but there was a tendency for lower emissions with biochar addition, suggesting that the variability in the field was too high to get significant effects. However there are also studies that showed increased emissions from biochar application in the field (Verhoeven and Six, 2014; Shen et al., 2014). There is a large variability of biochar properties and effect size between these field studies. Since the driving mechanism of how biochar reduces N_2O emissions from soil are still unknown, it can only be shown by meta-analysis that a low $H : C_{org}$ ratio seems to beneficial for N_2O suppression (Cayuela et al., 2015) .

Biochar has been shown to increase water-holding capacity and reduce bulk density (Peake et al., 2014) . Mukherjee et al. (2014) showed that 92 % decrease in N_2O emissions by biochar coincided with reduced bulk density by 13 % and increased soil nanopore surface area by 12 % relative to the control. In our experiment there are some situations where increased VWC with biochar coincide with reduced N_2O emissions (Fig. 1 and 2). Although there was no significant difference in bulk density, supposed increase in nanopore surface provides both suitable pores for water retention and oxygen air. Overall the improved soil aeration by biochar dominates the effect of increased water content and hence does not favour denitrification (Van Zwieten et al., 2010) .

Sánchez-García et al. (2014) found that biochar increases soil N_2O emissions produced by nitrification-mediated pathways. In our study, the water content (Fig. 1) was high during periods of high emissions~~and~~ , suggesting that during periods of high water content denitrification dominates the N_2O production in soil. The high emissions were thus often triggered by large precipitation events. There are many indications from lab experiments that biochar can reduce N_2O emissions in denitrifying conditions at high water content

~~(Felber et al., 2013; Harter et al., 2014; Singh et al., 2010; Yanai et al., 2007)~~ (Yanai et al., 2007). Under denitrification conditions, the pH between 6 and 8 exerts control over the $N_2O : N_2$ ratio (Stevens et al., 1998) , especially with a pH of the soil below 7, when the reduction of N_2O to N_2 is inhibited by acid conditions (Simek and Cooper, 2002). Various studies have suggested that an elevated soil pH is responsible for reduced N_2O emissions following biochar application through increased activity of N_2O reducing bacteria (Van Zwieten et al., 2010; Zheng et al., 2012). In contrast, Yanai et al. (2007) argued that the suppression of N_2O emissions by charcoal is not due to increased N_2O reduction activity with increased soil pH because biochar ash increased the pH to the same degree as biochar, but did not reduce N_2O emissions. ~~Also~~ In the lab, Cayuela et al. (2013) found no N_2O mitigation when soil pH was increased to the same level as biochar did but with $CaCO_3$ addition. They also showed that biochar's buffer capacity but not biochar pH was highly correlated with lower N_2O emissions compared to pH-adjusted biochars (Cayuela et al., 2013). In our case, we used a biochar with rather high liming capacity (17.2 % $CaCO_3$) and pH (9.8). We can confirm that with this kind of biochar N_2O emissions can effectively be reduced also in real field conditions, although the high variability in the ~~pH adjusted control~~ limed treatments does not allow us to reject the hypothesis of soil pH being the major driver of N_2O emission reductions. ~~A post-hoc power analysis showed a 23.4probability of accepting a true alternative hypothesis considering the obtained results in cumulative emission. To have at least a power of 80we would need 10 replicates for each treatment.~~

More recent studies show that biochar enhances nosZ abundance in soil bacteria, which can lead to lower N_2O emissions (Harter et al., 2014; Van Zwieten et al., 2014). Some authors relate this enhancement of N_2O reducing bacteria to biochar's redox activity that facilitates electron shuttling for the sensitive process of N_2O reduction (Kappler et al., 2014; Cayuela et al., 2013). This shuttling might be the connection between reduced N_2O emissions and low ~~H:Corg~~ $H : C_{org}$ ratios (Cayuela et al., 2015) in biochar that refers to condensed aromatic structures and its quinone/hydroquinone moieties being electro-active by allowing electron transfer across conjugated pi-electron systems (Klüpfel et al., 2014). Such high electro-catalytic activity has also been shown in N-doped C nanotube arrays (Gong

et al., 2009). Hence, in contrast to a promotion of microbial N_2O reduction, there is also the possibility that biochar abiotically reduces N_2O through its electrocatalytic abilities represented by a high aromaticity with low ~~H:Corg~~ $H : C_{org}$ ratios. Indeed, this is one of the various abiotic mechanisms that reduce N_2O emissions suggested by Van Zwieten et al. (2015).

4.2 Yield and nutrients

In our experiment, grain yield and plant biomass production were not increased by biochar application to soil. There is large uncertainty around the yield effect of biochar but meta-analyses reported an average increase of 10 % (Jeffery et al., 2011; Liu et al., 2013). Crane-Droesch et al. (2013) described a more detailed global response of biochar on yields. They identified a substantial and specific agroecological niche for biochar in soils with low organic C content and low cation exchange capacity, typical for highly-weathered tropical or sandy soils. Given these findings, we would not expect a large increase in productivity at our site which is rich in soil C and clay. Positive yield response could however increase with time (Crane-Droesch et al., 2013) and might not show clear effects within the first year of the biochar application yet. Our data is also in agreement with Jay et al. (2015) who showed that biochar had no effect on ~~harvest~~ the yield of different crops after a single rotational application (20 and 50 $t\ ha^{-1}$) in a sandy loam under intensive management.

Nitrogen uptake was not changed by biochar or liming. Although there was no significant difference in P uptake between the treatments, green plant material from biochar-treated plots tended to have higher uptake then the control (+100 % increase, data not shown). Vanek and Lehmann (2014) showed significant increase in P availability through enhanced interactions between biochar and arbuscular mycorrhizas.

Liu et al. (2012) reported a biochar application rate dependent decrease in emission intensity per yield, from 0.17 $kg\ N_2O\text{-}N\ t^{-1}$ in the control to 0.10 and 0.07 $kg\ N_2O\text{-}N\ t^{-1}$ with 20 and 40 $t\ ha^{-1}$ biochar applied. For an agronomic assessment of N_2O emissions it is most relevant to relate the cumulative emissions to the yield (Van Groenigen et al., 2010) . Emissions of the control per above-ground N uptake (29.6 $kg\ N_2O\text{-}N\ (kg\ N)^{-1}$) are much higher than

the reported mean of 8.4 kg N_2O-N $(kg\ N)^{-1}$ at fertilisation rates between 180 and 190 kg N ha^{-1} (Van Groenigen et al., 2010) . With biochar however, this number decreases by 60 %, whereas with lime it remains at the level of the control ($p = 0.21$). Yield based emissions are a good way to express biochars effects both on N_2O emissions and yield, but the experimental uncertainties of each dataset are also cumulated within this number.

5 Conclusions

We found a ~~53~~52 % reduction in N_2O soil emissions from biochar compared to control treatment in a maize field trial. This shows that also in temperate intensive maize cropping systems under real field conditions, N_2O emissions can be reduced substantially by biochar. There is no evidence that the reduction with biochar, relative to control, is solely induced by a higher soil pH. The pH hypothesis is thus not supported by our data.

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Table 1. Nitrate content (mg NO_3^--N kg^{-1}) in soil during the experiment. Standard error is indicated in brackets.

date	biochar	control	lime
2014-01-31	2.77 (0.41)	2.92 (0.13)	3.12 (0.25)
2014-03-31	6.26 (0.98)	8.57 (0.77)	8.40 (0.76)
2014-05-26	3.13 (0.36)	7.54 (1.18)	5.86 (1.45)
2014-06-16	9.19 (1.66)	9.38 (3.69)	11.65 (1.24)
2014-09-04	1.30 (0.15)	1.09 (0.21)	1.33 (0.26)

Table 2. Ammonium content (mg NH_4^+-N kg^{-1}) in soil during the experiment. Standard error is indicated in brackets.

date	biochar	control	lime
2014-01-31	1.11 (0.07)	1.00 (0.12)	0.68 (0.05)
2014-03-31	0.42 (0.24)	0.36 (0.21)	0.25 (0.21)
2014-05-26	0.11 (0.08)	0.12 (0.07)	0.47 (0.40)
2014-06-16	0.45 (0.13)	2.48 (1.80)	1.67 (0.36)
2014-09-04	0.38 (0.33)	0.39 (0.14)	0.16 (0.06)

Table 3. Cumulated N_2O emission per area and per total above-ground dry matter yield and above-ground plant N uptake for each plot.

treatment	block	N_2O per area [kg N_2O-N/ha]	N_2O per yield [kg N_2O-N/t-DM]	above-ground plant N uptake [kg-N/ha]
biochar	1	1.63	0.112	162
biochar	2	1.99	0.145	142
biochar	3	1.48	0.126	123
control	1	3.06	0.255	143
control	2	3.39	0.325	109
control	3	4.26	0.378	118
lime	1	6.76	0.591	121
lime	2	1.24	0.097	135
lime	3	2.80	0.230	131

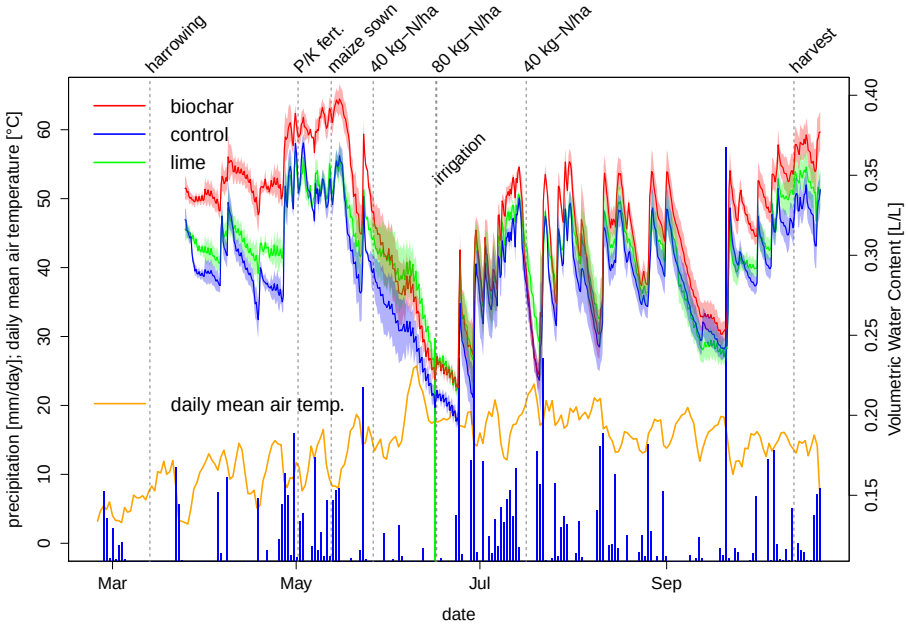

Figure 1. Soil moisture means for each treatment are shown in red, blue and green solid lines with 1 s.e. as shaded area. Blue bars show the rainfall in $\mathrm{mm\,d^{-1}}$ and the orange line is daily mean air temperature. The green bar indicates the irrigation of 33 mm with the second N fertilisation.

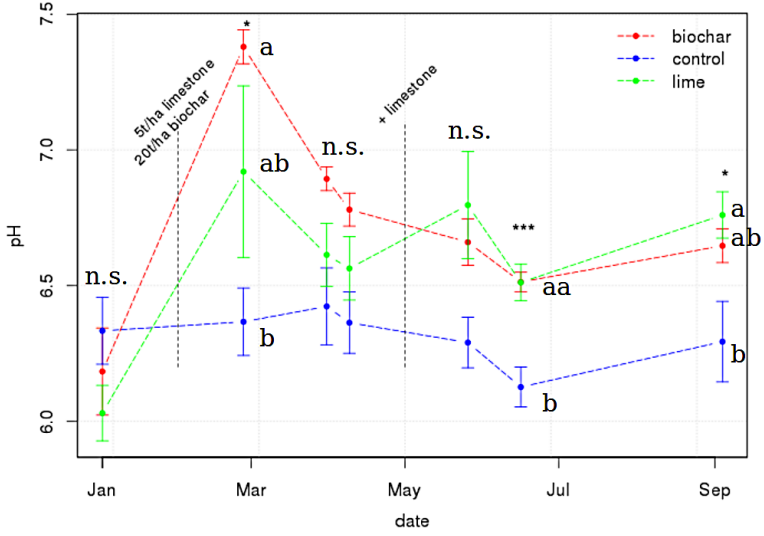

Figure 2. Soil pH (mean with 1 s.e. bars) during the time of the experiment. Significant differences ($p < 0.05$) are indicated with stars according ANOVA test and Tukey Honest Significant Differences (TukeyHSD) are indicated by different letters, n.s. = not significant.

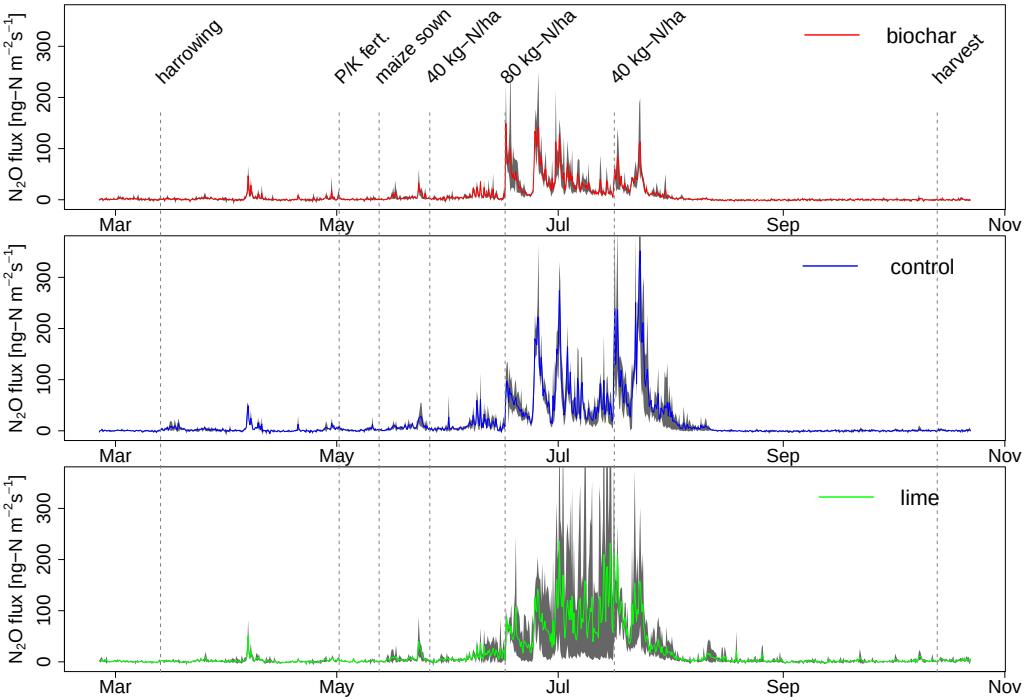

Figure 3. Mean N_2O emissions for each treatment (coloured line) with highest and lowest replicate in grey.

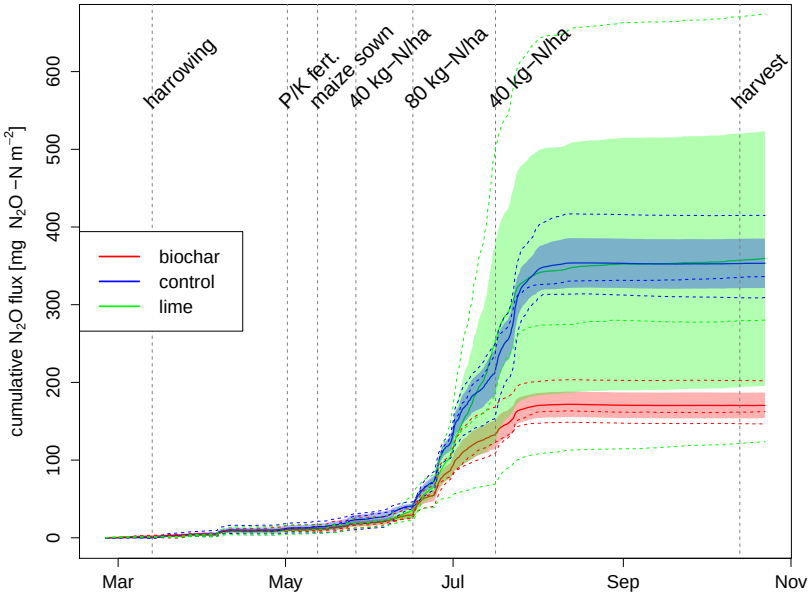

Figure 4. Mean cumulative N_2O fluxes as solid lines. Shaded areas represent the standard error of the mean from the 3 replicates (dashed lines) per treatment.

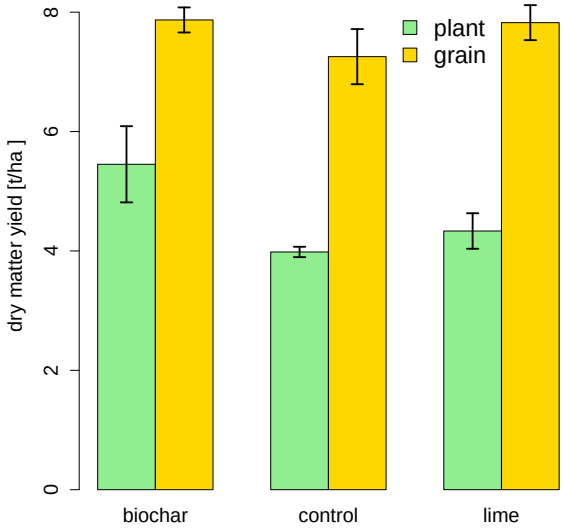

Figure 5. Yield and above-ground plant biomass production. Error bars show one standard error ($n = 3$).

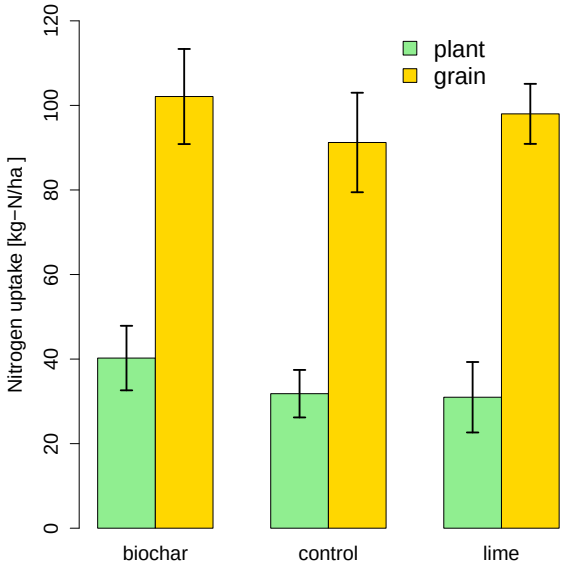

Figure 6. N uptake by ~~plant~~ above-ground biomass (stem, leaves) and ~~grain~~grains. Error bars show one standard error ($n = 3$).

~~P uptake by plant and grain. Error bars show one standard error ($n = 3$).~~