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Interactive comment on "Earthworm impact on the global warming potential of a no-tillage arable soil" *by* M. Nieminen et al.

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We thank the Referee for thoughtful comments and criticism and address each of the points below. We have done most of the changes suggested by the Referee. Where we have adhered to the original presentation, we explain the reason for doing so. The page and line numbers refer to the revised manuscript which is submitted as a supplement to this response. The Referee pointed out to the possible bias caused by the inclusion of only plant-free experiments in the recent meta-analysis of the subject. This is a worthwhile point and we discuss it shortly in the last paragraph of the paper (P17L28-33).

COMMENT: Firstly, I would like to highlight that the paper focuses on just one species, L. terrestris, and this should be clearly reflected in the title, otherwise it gives the im-

pression that it sells more than it actually does.

REPLY: We modified the title to tell that our study is about L. terrestris effects.

COMMENT: Secondly, another concern is that that the nonmidden area was not worm free, i.e. although significantly more worms were collected from the midden area, a good number of worms were also present in the reference treatment (Table 4 and P6337 L17-18). This makes the comparisons with the laboratory experiment, which included a treatment without L. terrestris, more difficult.

REPLY: The fact that L. terrestris individuals in the field increase the abundance of other fauna in the vicinity of their living sites is an important aspect in our study as it shows how short-term laboratory experiments can seldom fully mimic the situation in the field. Carrying out the laboratory experiment was aimed to test whether simplified laboratory experiments can be used to predict L. terrestris effects on GHG production. Any difference between the laboratory and field setting increases the meaningfulness and generalizability of this test. We added a sentence in the second last paragraph (P17L12-13; also in the Abstract P2L13-14) to point out that the significantly elevated faunal abundance and activity in the long-lived L. terrestris living sites in the field may be one reason for the difference in the size of L. terrestris effects on GHG emissions between the laboratory and field measurements.

COMMENT: For non-specialists readers, it would be useful to clearly state that the work by Lubbers et al. 2011 was actually based on laboratory results (P6327). This would add more value to the research presented here.

REPLY: We now better emphasize that the meta-analysis by Lubbers et al. (2013a; we assume that Referee's intention was to refer to this paper) was based on laboratory results (P5L12-14).

COMMENT: A reference is needed to support the statement given in P6328 L4-5.

REPLY : We added a reference to Whalen and Fox (2007) (P4L5).

COMMENT: If L. terrestris is the second most abundant earthworm in arable fields in Finland (P6328 L7-8), which one is the first one and how can you then justify the use of L. terrestris in this study?

REPLY: We now also mention the most common species, Aporrectodea caliginosa (P4L7-8). The reason that we focused on L. terrestris is that this species represents the anecic, deep-burrowing earthworms that are favoured by no-till agriculture and whose densities therefore increase under no-till field management. We modified the first sentence of the paragraph to better express this reasoning (P4L4-5).

COMMENT: I do not understand why two sites (A and B) were selected when the results for the site effect are only shown for CH_4 emissions. What is the relevance of this? Were these two areas different in relation to earthworm densities? What do you mean by 'they had no obvious difference in soil properties' (P6330 L1)?

REPLY: The two sites were needed to obtain a sufficient number of treatment pairs, but they also serve as a test for small-scale spatial variation in the treatment effect. For this purpose, the area was included in the statistical analyses as an explaining factor. However, as the area had no effect on CO_2 and N_2O emissions, it was removed from final models for these variables. We clarified the text to better explain this (P5L25-28). We removed the statement of "no obvious difference" as unnecessary.

COMMENT: A reference is needed to support the statement given in P6329 L25-26.

REPLY: We refer here to our experience in earthworm field studies at the local environmental conditions (P5L25).

COMMENT: One important concern regarding the gas measurements performed in the field is that they were taken immediately after the rings were inserted into the soil; this is known to decrease CO_2 fluxes due to the 'lost' autotrophic flux (root-derived) component (Heinemeyer et al. 2011). In addition, when fitting the chambers an important amount of CO_2 gets trapped inside (this also applies to the laboratory measurements).

Finally, air temperature was used to correct gas fluxes; but was this parameter measured inside or outside the chamber?? Finally, midden and straw samples were stored (freeze at -18oC) for 7.5 months which seems to me to be a very long period...

REPLY: Root cutting and its effects on CO_2 fluxes are not a matter of concern here since the measurements were done after harvest. We added this reasoning to the text (P6L4-7). Gases are always trapped when closing the chambers, but the inaccuracy that results from this is the same for both treatments and should not affect the conclusions about treatment effects. We only measured outside air temperature as the sun does not markedly warm the chambers late in the autumn. This is now mentioned in the text (P6L14-16). Freezing of samples for several months can sometimes not be avoided, but again it should not affect the comparisons between the treatments.

COMMENT: In relation to the laboratory incubation experiment, my main concern is that the mesocosms were filled with sieved soil which is known to increase substrate availability for microbial populations (Hartley et al. 2007) and again making the comparisons with the field experiment difficult. Additional questions regarding this experimental set-up is whether you achieved the same bulk density recorded in the field by compacting the soil in the mesocosms and whether the selected incubation temperature mimics the one recorded in the field site during the autumn period.

REPLY: Our aim was not to establish a laboratory experiment that would perfectly mimic the field situation, but to establish a typical laboratory experiment to test whether laboratory experiments in general can produce results that resemble the results in the field. Such tests are important because most earlier experiments have been carried out in the laboratory and e.g. the review by Lubbers et al. (2013a) is entirely based on laboratory studies. We added this reasoning to the end of the introduction (P5L10-14).

COMMENT: Finally, the statistical analyses of the data seem to include excessive complications. The experimental design is a full factorial layout with two factors (treatment and sampling date) in which time is a repetitive factor. Performing a Repeated Measures of ANOVA is the appropriate method to analyse the two data-sets (field and laboratory results) and therefore, I cannot understand the need for developing a model to estimate gas fluxes from the different treatments when the real data could have been presented instead.

REPLY: The core of the statistical model is the repeated measures ANOVA as suggested by the Referee . However, there were additional experimental aspects, which needed to be taken into account. First, for each midden site, a reference non-midden site was always chosen close by. Each midden - non-midden pair is therefore located in a homogenous area, which is the general motivation behind blocking. The pair effect was included into the model to reduce the experimental error and to have more precision in the estimation of the effects of actual interest (this type of blocking is in some applications known as "matched pairs"). The second experimental aspect was the area. The treatment pairs were situated in two small areas 30 m apart and although there was no obvious difference between the areas, many environmental factors are likely to vary in the background and the longer the distance the higher the variation. The area effect was therefore introduced into the model as a precaution. It turned out that for some response variables there were statistically significant area or interaction effects and taking the area effect into account helped to clarify the effects of interest. In summary, the core of the statistical model is the repeated measures ANOVA, which includes treatment and sampling date effects. However, to capture some of the "noise" (i.e. random error) we introduced the area and block effects (and their interactions) into the model as well.

COMMENT: This section is very difficult to read because the graphs and the majority of the tables are based on model estimations instead of on real data. For example, looking at Table 4 it is very surprising that the mean values with overlapping 95% CI are truly significant (see for example, soil moisture). In contrast, how can the mineral N content of the straw be so different between midden and non-midden areas?

REPLY: This is a question of some controversy, some people preferring to see the ac-

tual data and favoring – as the Referee suggests - that the arithmetic means, calculated from the data, would be presented. We think that presenting model based estimates instead of arithmetic means is more in-line with the basic purpose of performing statistical analysis on the data. Statistics is based on the idea that the data is a sample from a larger population that it represents. What we want to do is to make conclusions relating to the population, not only to the data or sample. Arithmetic means only describe the properties of the data, estimated means that are based on the statistical model are estimations (based on the data) of the population. By using the estimates we are making statistical inference, i.e. conclusions about the population. P-values and confidence intervals are also important in the statistical inference as they provide probabilistic statements regarding the uncertainty in our conclusions about the population. In more basic settings (for example one-way ANOVA) the model based means and arithmetic means are often exactly the same, which also explains why arithmetic means are often used to present results.

The Referee raises a question how the estimated mean values with overlapping 95% CI:s can be statistically significant in Table 4. In statistics, if the CI:s of means do not overlap, the means are necessarily significantly different. However, especially in more complicated experimental arrangements, the means can also be statistically significantly different even when the CI:s overlap. For instance, in our study, all experimental units are not independent and so the "rule of thumb" for deducing statistical significance from CI overlap is not as exact as in situations with independent observations and e.g. a one-way ANOVA model.

COMMENT: Another important concern is the number of slugs in the midden treatments; this aspect is totally ignored in the discussion and although, I am not an expert on the role of these molluscs in SOM decomposition, it makes me wonder whether, for example, the mucus they secrete could also promote microbial activities and be also responsible for the ‘worm’ effect.

REPLY: We added a few lines of discussion about the potential effects of slugs on CO2 and N2O emissions (P15L22-26) and also refer to possible effect of midden macro-fauna in the Abstract (P2L13-14).

COMMENT: The fact that no information is provided about microbial populations in these treatments makes it difficult to establish whether the treatment differences are due to the worms or to their indirect effects on microorganisms. One way to tackle this would have been to subtract the control fluxes from those derived from the worm treatments to calculate the contribution of the worms.

REPLY: In the laboratory experiment, we measured the emissions that were produced by the earthworm and were thus able to calculate the proportion of total emissions produced by the earthworm. These proportions are presented in the text for each gas and for instance, in the case of CO2 we discuss how earthworm respiration can fully explain the increased CO2 production in the presence of earthworms. Subtracting control fluxes from treatment fluxes would hide the difference in the level of gas fluxes between the field and laboratory and even the subtracted values would not reliably tell the direct earthworm contribution. This is because part of the difference between control and treatment is most likely due to the effects of the earthworm on microbial activities.

COMMENT: In the case of the laboratory experiment, the main aspect I would like to highlight is the higher flux values; especially those for CO2, when compared to the results obtained in the field (see Table 2). I think this can be explained by the fact that the soil was sieved which facilitated microbial access to C and N substrates. On the other hand, the observed decreases of these fluxes over time (P6338 L9-10) could be explained as a result of microbial acclimation or substrate depletion (another topic hotly debated in the literature). All these aspects should be considered in the discussion section.

REPLY: We added discussion of these topics to the paragraph, where we discuss the differences between the field and laboratory measurements (P17L3-10).

COMMENT: Discussion: Overall, I found this section too long and difficult to follow. I think it can be greatly shortened by summarising and combining paragraphs. For example, the first 25 lines in P6342 can easily be summarised and integrated in previous paragraphs. I would advice to follow a logic structure and discuss all those aspects regarding a particular flux together and not scattered throughout this section.

REPLY: As suggested we removed the paragraph that discussed the direct and indirect earthworm effects and integrated the text into the paragraphs of the three gas fluxes. Now the discussion goes through the three gases one by one and ends up with a discussion of the GWP estimates of no-till fields and the laboratory-field comparison.

COMMENT: I would also like to clarify that the changes that earthworms produce in their environment are actually ‘direct effects’ (P6342 L1-3). The fact that when you remove the worms from the system, the effects still persist proves that they are direct. Indirect effects are, for example, when their impact on gas fluxes is mediated by a third (e.g. microorganisms), which I think is what happens here, that microbial effects are more important than the direct effects of the worms.

REPLY: In the paper we define direct and indirect emissions as follows: "Direct emissions originate from earthworm metabolism and indirect from the changes earthworms induce in their environment." (P14L13-15). In our opinion, this is in agreement with the reviewer’s reasoning (gases produced by the animal itself are direct environmental effects and the changes in gas production by soil microorganisms due to earthworm activity are indirect environmental effects).

COMMENT: In conclusion, I think this paper could make a good contribution to the journal if the fact that the whole study focuses on just one earthworm species is made clearer both in the title and discussion and consequently, the description of the relevance of the results is toned down. In my opinion, this study does not resolve the issue but highlights the importance of performing field measurements and that laboratory incubations tend to magnify the results.

REPLY: We have addressed the concerns presented here in our replies above.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C4561/2015/bgd-12-C4561-2015-supplement.pdf

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