

Interactive comment on “Relevance of aboveground litter for soil organic matter formation – a soil profile perspective” by Patrick Liebmann et al.

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RC5 Comments by Referee #5, 22.01.2020

Introduction

The article deals with a significant question that is the redistribution of fresh litter carbon into different soil C pools and the processes involved in the transfer and turnover of carbon in the whole soil profile. The use of stable isotope labeling in a field study seems really adapted. However some information is missing, sometimes with significant importance for interpretation.

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Author response

We thank the referee for the efforts and the constructive criticism which helped to improve the manuscript.

1. Comment

Main concerns: What is the percentage of remaining litter (in mass and labelling) after 22 months? This data is important to assess the percentage of litter lost by mineralization compared to the part that did not enter the soil, and to know if the incorporation of litter fits well with natural conditions (11% seems low).

Author response

After 22 months, removed litter layer amounted about 405 g m⁻² per plot with a remaining enrichment of 384.4 ‰ ¹³C. About 25 % of this removed litter originates from the initial applied labeled litter, while 75 % originates from litterfall (e.g. shells of beech-nuts), which passed through the 2 mm mesh and accumulated during the 22 months. Comparing initial and removed labeled litter, we recovered about 35-40 % as residual labeled litter, while we recovered an evolution of labeled litter-derived CO₂ of about 36-40 %. Adding the 11 % found in SOC, we know the whereabouts of roughly 85 % of the initial applied litter. The certain offset in this calculation represents the amount of label, which we were not able to recover. We consider these results as quite decent and well fitting for a labeling experiment under field conditions with a duration of nearly two years.

2. Comment

What are the properties of labeled and unlabeled litter?

Author response

We thank the referee for this comment. In fact, referee #3 (RC1, comment #2) and referee #4 (RC4, comment #2) commentated this as well, a detailed explanation can

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be found in the respective response. The labeled and unlabeled litter were the same, except of the ^{13}C enrichment. We did not go into detail about the properties since both types were comparable and no differences between the treatments can be expected. Distribution of the ^{13}C in the labeled litter was homogeneous.

3. Comment

Was the labeling realized through continuous or pulse technique? In relation to this question, what is the $\delta^{13}\text{C}$ of the remaining litter after 22 months (is it consistent with the $\delta^{13}\text{C}$ measured for initial litter or may have the incorporation process been discriminant?). The interpretation of the labeling calculation could be different if the labeling is not homogenous.

Author response

The production of the highly enriched labeled litter (at IsoLife) was realized through a continuous labeling in a $^{13}\text{CO}_2$ atmosphere and IsoLife assures homogeneous labeling of the leaf litter. The $\delta^{13}\text{C}$ ratio of the remaining litter was about 384 ‰ (mean of 5 replicate measurements at two different institutes). But related to the authors response to comment 1, there was dilution due to accumulation of unlabeled litter during the 22 months, thus we cannot determine if there was a discrimination during litter decomposition and leaching or not.

4. Comment

All the data that would allow comparing the properties of the different plots, especially labeled/unlabeled plots. For example, authors always averaged the control and the labeled plots for soil properties: the C contents and stocks (figure 1), the distribution in density fractions (in figures 2 and 6, the variability is high as mentioned in the caption: is it distributed randomly between labeled and unlabeled plots or is there significant differences?), the C/N (figure 3), WEOC (figure 4). If significant differences exist between the labeled/unlabeled plots, the interpretation of the low difference in isotopic signature

(figure 5) could be limited.

Author response

We tested for significance by using a t-Test. In total, we tested 149 sample subsets for MAOM C/N ratio and C content, WEOC data (WEOC in %, SUVA, HIX) and C distribution in the individual density fractions. All tests were done for each depth increment individually. We found that a total of 10 out of 149 tests resulted in significant differences, which were distributed randomly between several parameters and fractions and depth increments. Considering all tests, we recognize the potential differences between the labeled and unlabeled subplots as insignificant without further implications for our interpretations.

5. Comment

Table S1: add the value of the reference (C, N or $\delta^{13}\text{C}$). Moreover, was a labeled standard (in-house) used since the initial enrichment applied was high (1241-1880 permil)? What is the maximum $\delta^{13}\text{C}$ measured in the soil fractions and used for calculation?

Author response

We added all relevant values to Table S1 which were used for correction and calculation of the data. This includes the C and N data for the HOS standard and the $\delta^{13}\text{C}$ ratio for the IAEA standards. To avoid further misunderstandings, we deleted the standard substances for ^{15}N from Table S2, since these data are not included in the manuscript. There was no labeled standard included in the measurements. This was also not considered necessary, as the $\delta^{13}\text{C}$ values of the soil fractions had a maximum of -6 ‰.

6. Comment

Minor concerns: A 2-mm mesh was used to prevent new litter input during first 22 months: what about a potential leaching of additional unlabeled litter? As mentioned lines 366-368: WEOC release is possible.

Author response

The 20-mm mesh was used to reduce mixing of the labeled litter with fresh litterfall in autumn and to be able to remove that fresh litter in late autumn to prevent a massive interference of unlabeled litter in the following 12 months of the experiment. Hence, there was just limited leaching from the material lying on the mesh until it was removed in a weekly interval. But as was replied to a similar question raised in RC1, comment #9, litter <20 mm (e.g. fruits) could have passed the mesh. This also led to an amount of removed litter (after 22 months) which was larger than the initial application.

7. Comment

Was the WEOC extracted on frozen samples (line 115) or on field-moist samples (line 129)?

Author response

Water extractable OC was extracted on field-moist samples after thawing, as samples were frozen directly after sampling for storage and comparability reasons. We responded in detail to a similar question in RC4, comment #6. We added the missing information about the thawing process to line 129 of the original manuscript. It reads: “Prior to the extraction, the frozen samples were thawed for 24 hours at 4°C and thereafter sieved to < 2 mm.”

8. Comment

Line 322-325: the sentence is not clear for me. Which recalculation was done? Is it to correct the input of litter to the soil of the experiment that was not representative of typical “annual” litter input? If it is the case: what about this difference (line 98-99, authors mentioned a “equivalent amount of litter” added to the plots)?

Author response

In lines 322 to 325 we wanted to express that the amount of labeled litter-derived C in

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the MAOM fraction of different depths after 22 months was used to estimate an incorporation rate per year. The data basis is the litter-derived C in g m⁻² at the first sampling, as it is given in Fig. 6a. The data of each individual increment were cumulated with respect to our 3 main soil compartments topsoil, upper subsoil and deeper subsoil. Data for the 3 compartments were then recalculated to a yearly basis (12 months/22 months). We are aware of the implications of this recalculation, which is that a linear incorporation of litter-derived C over the 22 months is assumed. This is likely not the case, as the initial translocation and incorporation (first weeks) may be higher than after 20 or 22 months, but in the end we are stating an estimate. We added this assumption to line 325 of the original manuscript as follows: “For the Dystric Cambisol under European beech, the observed recoveries of ¹³C in MAOM in the 22 months of labeled litter application were recalculated to average annual litter inputs from the recent litter layer into the HF of about were estimated as 0.99 ± 0.45 g C m⁻² yr⁻¹ in the topsoil, 0.37 ± 0.10 g C m⁻² yr⁻¹ in the upper subsoil, and 0.01 ± 0.01 g C m⁻² yr⁻¹ in the deeper subsoil. This estimation follows the assumption of a constant input of labeled litter-derived OM during the 22 months, which is a sufficient approximation for this estimate but may not reflect the actual conditions in the field.

9. Comment

The XPS part does not seem to be related to the study. Is it necessary? Additional Table S3, Figure S1, figure S3 and figure S6 are not cited in the text for example. At least Table S3 and Figure S5 seem redundant. I would delete the Table S3 (or simplify it? The replicates should be averaged).

Author response

We appreciate the referee's notice about relevance and citations of the supplementary material. We agree that a presentation of the detailed XPS results in the form of a table (Table S3) may not be necessary, since the most relevant part of the data is also given as a more concisely figure (Fig. S5). But we see a benefit in showing these

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data, because sorption of C to minerals and the formation of MAOM is taking place on mineral surfaces. Consequently, investigations of the surface composition of the HF is a helpful tool. The data given in Fig. S5 for example show and also validate that surface C and N contents are decreasing with soil depth as seen in the EA-IRMS analysis. Vice versa, higher contents of Fe and Al were found with increasing soil depth, resembling a higher proportion of uncovered mineral surfaces and, in theory, potentially available sorption sites. Due to their value as additional information, we suggest to keep the XPS analysis included. However, we agree to reduce its presence in the supplement by deleting Table S3 and Fig. S6. We added a citation of Fig. S1 to line 226 of the original manuscript: "...5 in the deep subsoil (Fig. 3b), similarly to the bulk soil C/N (Fig. S1)." We added a citation of Fig. S3 to line 241 of the original manuscript: "... , whereby the contribution of the deeper depth increments was very minor (Fig. 6a, Fig. S3)."

10. Comment

Line 131: Were the filters pre-rinsed? Was the effect of cellulose pollution on $\delta^{13}\text{C}$ of WEOC assessed? Is it negligible (depending on the DOC content of the WEOC extract)? If this pollution is equivalent for the labeled/unlabeled WEOC (and if WEOC is equivalent for labeled/unlabeled plots), it may not impact the isotopic calculation but should be mentioned.

Author response

Yes the filters were pre-rinsed with 250 ml of the 1mM CaCl_2 solution. In a pre-test we tested for the pollution by the filters, but the effect was negligible. However, for each extraction we also run 2 blank samples (only the CaCl_2 solution) with all steps (shaking, centrifuging, extraction). The TOC of the blanks were used for the correction of TOC from the water extracts. And we also determined the isotope ratio of the blanks, but they had a similar signature as WEOC (control). We added the missing information to line 132 of the original manuscript as follows: "Prior to the filtration, filters were

pre-rinsed with 250 mL of the 1 mM CaCl₂ solution”.

11. Comment

The WEOC extraction and the density fractionation were done in parallel (not sequential). So is there a relation between the DOC collected in SPT fraction and the WEOC? Line 227, authors mentioned that the “no consistent trend” was observed for DOC of SPT. What about the $\delta^{13}\text{C}$ of SPT?

Author response

In previous studies, e.g., Gentsch et al. (2018) we could show that SPT solution is one relevant pool of C, which should be considered and measured after density fractionation. Especially if the aim is to set up a carbon balance, neglecting the carbon which dissolves in the SPT solution during fractionation will automatically lead to a loss of several % C, one of the reasons why we list the SPT fraction next to the soil fractions. From a methodological point of view, we consider a comparison and relation to WEOC as possible but not useful, since the functionality behind the WEOC fraction (e.g. potential to be mobilized and translocated by the soil solution, bioavailability) cannot be transferred to the CSPT fraction. The main reason is that the soil is under extreme conditions during fractionation (high salt contents, ultrasonic dispersion, and a higher soil to water ratio), likely mobilizing C, which would not be mobilizable under natural conditions. With regards to our results, we also do not see a close relation, as WEOC proportions consistently increased with soil depth (Fig. 4a), while proportions of CSPT (DOC in SPT) varied between 1 to 3 % but without a depth trend (Fig. 2). $\delta^{13}\text{C}$ values in the CSPT fraction were lower than those in the WEOC, the same is true for the recovered labeled litter-derived C. This may likely imply that the density fractionation treatment mobilized a higher amount of older and better stabilized C compared to the water extraction.

Gentsch, N., Wild, B., Mikutta, R., Čapek, P., Diáková, K., Schrumpf, M., Turner, S., Minnich, C., Schaarschmidt, F., Shibistova, O., Schnecker, J., Urlich, T., Gittel, A.,

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Šantrůčková, H., Bárta, J., Lashchinskiy, N., Fuß, R., Richter, A. and Guggenberger, G.: Temperature response of permafrost soil carbon is attenuated by mineral protection, Glob. Change Biol., 24(8), 3401–3415, doi:10.1111/gcb.14316, 2018.

12. Comment

Line 169-174: Measurement of nitrate and ammonium, for calculation of organic nitrogen were mentioned, but never used in result. Delete?

Author response

Nitrate and ammonium data were used for correcting the N-contents of the bulk data and MAOM before calculating the C/N ratios (Fig. 3b, Fig. S1). But we see the potential misunderstanding and modified the respective figure captions by adding this information to line 641 of the original manuscript and line 113 of the supplementary material. It now reads as “Nitrogen contents in the HF were corrected for extractable nitrate and ammonium contents.”.

13. Comment

Line 174-175: Is the XPS really useful for this study?

Author response

This question is related to Referee comment 9 and the reader is referred to the authors' response given there.

14. Comment

Line 228: cite Figure 2

Author response

We added a citation of Fig. 2.

15. Comment

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Line 248: cite Figure 5c

Author response

We added a citation of Fig. 5c.

16. Comment

Line 258: cite Figure 6b

Author response

We added a citation of Fig. 6b.

17. Comment

Line 267: Sentence is not clear: what means “similar loss for recovered material”, is it 77% of mass or of carbon?

Author response

The 77 % represent the loss of labeled litter-derived C in the bulk soil samples when comparing the first with the second sampling, similar to our statement of losses in the different functional fractions of 66 to 89 % in the sentence before. We were mentioning the bulk soil losses in this context in order to give the reader an impression about the decent consistency of soil fractions and bulk data. For clarification, we modified the statement in line 266 to 267 of the original manuscript as follows: “The decline of label from mass-weighted individual OM fractions was similar in magnitude to the loss of labeled litter-derived C in the bulk samples (77 %; data not shown).”

18. Comment

Table 3: add the % of initial litter that was lost by mineralization compared to the material remaining after 22 months. Express the values in % of C “entering” the soil.

Author response

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We added the information of the initial litter and its loss due to CO₂ respiration in the Table 3 caption (it now reads as: “Overall, 36-40 % of the initially applied litter was lost by respiration during 22 months of field exposure (Wordell-Dietrich, unpublished).”) as well as in the text in lines 102 to 103 in % of the initial label (it now reads as: “A total of about 36-40 % of the initially applied labeled litter-C left as CO₂ (Wordell-Dietrich, unpublished).”). By comparison with the amounts of recovered labeled litter-derived C in the soil profile, it will be evident for the reader that CO₂ respiration is more than 3-times as high as incorporation in the soil.

19. Comment

Figure 1, 3, 4: show the mean and SD of labeled and unlabeled plot, of 22 and 40 months.

Author response

We checked labeled and unlabeled plots for significant differences (by use of a t-Test) as it was mentioned in the Authors response to comment no. 4. Since differences were insignificant for the vast majority of tests and the only difference between labeled and unlabeled plots (in treatment and sample processing) was the label application, we rather consider labeled and unlabeled samples as field replicates for all non-isotopic parameters.

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