

## ***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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### **Introduction**

This manuscript is well written and organised. At this stage I have comments/concerns regarding some methodological aspects which are elaborated below.

### **Author response**

We want to thank the referee for the positive feedback and the constructive criticism, which helped to improve the manuscript.

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### **1. Comment**

Also, the points touched on in the discussion are clear, but don't bring the arguments back (explicitly) to the hypotheses presented in the Introduction.

### **Author response**

We appreciate the referees comment on the discussion of the hypotheses. We agree that a direct answer to the hypothesis of the original version of the manuscript was not given. We modified the manuscript by changing hypotheses to questions from lines 70-80 of the original manuscript as follows:

“Particularly, we aim at answering the following questions:

1. Does recent aboveground litter significantly contribute to the accumulation of OM in subsoils?
2. Is OM transferred into the subsoil directly via the DOM pathway, or is subsoil OM the result of repeated sorption-microbial processing-desorption cycles?
3. To which extent is recent aboveground litter-derived C sorbed to soil minerals and does this fraction represent a source of stable SOM?

To quantify the contribution of recent litter to subsoil C stocks via DOM movement and evaluate the stability of litter-derived SOM, we... “

With the following comparison, we want to point out that we provided an answer to all three questions in our implications:

Lines 384-386: “In fact, we did not find a translocation of considerable amounts of recent litter-derived C into the deep subsoil, indicating that most translocated OM at the study site is of older age.” - This implication answers question 1.

Lines 386-387: “Our field study supports the concept that C accumulation in deeper soil involves several (re)mobilization cycles of OM during its downward migration.” -

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This implication answers question 2.

Lines 389-390: "Slowest turnover of litter-derived C was observed for MAOM compared to both POM fractions, supporting the assumption that accessibility and sorptive stabilization reduces the vulnerability of OM to microbial decomposition." - This implication answers question 3.

## 2. Comment

How was the highly labelled litter (i.e. the source of the enriched C), produced? Is it homogeneously labelled? This is important because if labelling is not homogeneous only some compound types and pools of C will be traceable, which may not represent the whole plant C well, or bias it against the movement and stabilization of certain litter-derived compounds. This could lead to substantial underestimation/over estimation (?) of the contributions or surface litter. It would also affect the overall estimation of loss. Given the type of goal, which is mainly one of quantifying contribution (vs. comparing different treatments) this is of high importance and is potentially concerning.

### Author response

Thanks for this important question. The highly labeled litter (10-14 at%  $^{13}\text{C}$ ) was purchased at IsoLife, a company which is specialized on labeling plants by growing them in greenhouses under a  $^{13}\text{CO}_2$ -enriched atmosphere. The labeling in the  $^{13}\text{CO}_2$  atmosphere was long-term and continuous, thus It can be expected that the label is homogeneously distributed in all plant compartments. We added this information to line 100 of the original manuscript as follows: "Labeled litter was prepared as a mixture of highly labeled beech litter (10 atom-% uniformly labeled due to growth under  $^{13}\text{CO}_2$ -enriched atmosphere in a greenhouse, IsoLife, Wageningen, The Netherlands). . ."

## 3. Comment

Question: how were the labelled and unlabelled litter mixed?

### Author response

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The litter types were mixed at a certain ratio as intact leaves (dried). By keeping them intact, we accepted that a 100 % homogeneous distribution on the plot at small scale was unlikely, but we wanted that the litter application resembled a fresh litterfall. To account for the potential heterogeneity on the cm scale, three cores were drilled per plot and composite samples were prepared and used for analysis and fractionation (as presented in lines 113 to 114).

## 4.1 Comment

I am assuming that the natural litter added is "fresh" litter? Or at what stage of decay is?

### Author response

Yes, we added dried undecomposed litter.

## 4.2 Comment

And what about the labelled litter? Is it senesced? Fresh? If the two litters were are different stages, this would have implications, because of the differential composition of C pools, depending on the potential scenarios. In this case, what could they be? This is also a consideration for the initial mixed litter.

### Author response

We agree that different decomposition stages would massively influence the intended homogenous leaching of DOM from both litter components (labeled and natural). We considered that and have chosen litter in the same stage, meaning leaves after senescence but before shedding for both types of litter, and from very young trees in the field.

## 5. Comment

I don't understand how the 20mm (do you mean 2 cm mesh?) could prevent the leaching of the naturally fallen litter to reach the soil

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#### Author response

The mesh, with its mesh size of 2 cm, was not installed to prevent leaching (this would imply a complete water blockage of the area, destroying the natural conditions). The mesh had two main functions. First, it prevented translocation of the labeled litter by wind, potentially onto the control sites. Second, it allowed us the removal of freshly fallen leaf litter in autumn after the experiment started, in order to avoid a dilution of the  $^{13}\text{C}$  signal in the following year. Since the former explanation was not given in the submitted version of the manuscript, we now added this to line 101 of the original manuscript as follows: "A net (2 cm mesh size) was installed on top of the litter layer to, first, prevent surface translocation by wind, and second, to avoid dilution of the labeled litter over time by the seasonally fallen litter."

#### 6. Comment

I am confused by the handling of the samples for water extractions. Line 115 says they were soil subsamples were frozen directly after sampling for water extractions, but later one it says field-fresh samples were extracted. The freezing and thawing will have an impact on the C composition of the soil solution from the breaking of the microbial cells, putting cellular contents into solution, potentially. Then also, if the soils were not extracted soon after field collection the C composition of the soil solution and its isotopic composition would potentially change too. With such the low levels of enrichment that reach the sub-soil, these unintended impacts of the handling could alter the results.

#### Author response

We agree with the reviewer, that the term "field fresh" is confusing and not correct. In fact, the samples were kept frozen and after storage thawed for 24 h at 4°C. Thereafter the samples were sieved (< 2 mm) and then extracted with 1 mM  $\text{CaCl}_2$  solution. We see the point that freezing and thawing might have an impact on the C composition of the water extracts. However, we decided to freeze the samples to treat them equally. The assumption behind this was, if all samples were stored in the fridge at

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4°C, microbial turnover would be still active. Furthermore, due to the large amounts of samples ( $n=90$ ) we were not able to extract them all after the same time of storage. In consequence, there would also be a bias due to the different storing time in the fridge. Therefore, we decided to freeze all samples. We added this information to the original manuscript at line 129 as follows: "Prior to the extraction, the frozen samples were thawed for 24 hours at 4°C and thereafter sieved to < 2 mm. Following the procedure of Chantigny et al. (2006), [ . . . ]."

#### 7. Comment

It would be good to explain the general purpose of the investigation of HF surfaces in the methods.

#### Author response

We agree that a short description of the general purpose of the HF surface investigations is helpful for the reader to make use of the data in the supplement. We added the following sentence to line 175 of the original manuscript: "Surfaces of the HF were further investigated by X-ray photoelectron spectroscopy (XPS) with respect to the elemental composition as a function of soil depth. Method description and data are presented in the Supplement."

#### 8. Comment

Not methodological: In the Introduction, some potential reasons for the  $^{13}\text{C}$  enrichment with depth are mentioned; there are some new developments about this gradient such as evidence also suggesting there is a contribution also of the microbial composition of the necromass (e.g. Biogeochemistry 2015, 124: 13-26)

#### Author response

We thank the referee for this additional view, which we did not include so far in our manuscript. The aspect of a compositional change within the microbial community and its necromass may not influence the implications from our study, but we agree

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that it should be mentioned in the introduction to this topic. We therefore modified the sentence in lines 51 to 53 of the original manuscript as follows: “In most soils,  $\delta^{13}\text{C}$  values increase with soil depth, which is related to the isotopic discrimination of the heavier C isotopes during microbial respiration (Nadelhoffer and Fry, 1988, Balesdent et al. 1993, van Dam et al. 1997) or a shift in the fungal to bacterial ratio in favor of the more  $^{13}\text{C}$ -enriched bacteria (Kohl et al. 2015).”

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