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

We want to thank all referees and appreciate the comments from the scientific community. In the following, the response to all comments is given in the order of appearance. The proposed changes to the manuscript due to the respective referee comment are marked in red.

SC1	Short comment by Paul Hanson, 08.01.2020
1. Comment	The following 5 papers report on field studies of enriched background 14C isotopic tracers for multiyear controlled litter additions and the transfer of those labels into the soil. You might modify your statement on line 65 to recognize these efforts. The Kramer et al. 2010 paper is probably the most relevant, and you have already cited the related mesocosm study (Froberg et al. 2009) 1. Tipping E, Chamberlain PM, Fröberg M, Hanson PJ, Jardine PM (2012) Simulation of carbon cycling, including dissolved organic carbon transport, in forest soil locally enriched with 14C. Biogeochemistry 108:91-107, doi 10.1007/s10533-011-9575-1. 2. Parton WJ, Hanson PJ, Swanston C, Torn M, Trumbore SE, Riley W, Kelly R (2010) ForCent model development and testing using the Enriched Background Isotope Study (EBIS) experiment. JGR-Biogeosciences 115:G04001, doi:10.1029/2009JG001193 3. Kramer C, Trumbore S, Fröberg M, Cisneros-Dozal LM, Zhang D, Xu X, Santos G, Hanson PJ (2010) Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biology and Biochemistry 42:1028-1037. 4. Riley WJ, Gaudinski JB, Torn MS, Joslin JD, Hanson PJ (2009) Fine-root mortality rates in a temperate forest: estimates using radiocarbon data and numerical modeling. New Phytologist 184:387-398. 5. Fröberg M, Hanson PJ, Trumbore SE, Swanston CW, Todd DE (2009) Flux of carbon from 14C-enriched leaf litter throughout a forest soil mesocosm. Geoderma 149:181-188. [Mesocosm study in support of the larger field EBIS effort.]
Author response	We agree and appreciate the suggested references of Paul Hanson and we will add some of the literature he suggested here. We received additional suggestions from Referee #2 (RC2, comment 4) and decided to include just a selection of Paul Hansons and Referee #2s suggestions, in order to satisfy both comments and to limit to a maximum of three references per citation. We just want to note that our main focus (and also novelty of the study) is in the subsoil aspect. The suggested publications all have their relevance for discovering the fate of litter layer-C and we will include them here, but they mostly cover the topsoil or a soil depth of 0-10 cm only. We modified the sentence in lines 63 to 64 of the original manuscript as follows: “In order to quantify individual C fractions and fluxes, isotope labeling, e.g. using ^{13}C- or ^{14}C-enriched litter material, has been proven as a very powerful tool (Bird et al., 2008, Moore-Kucera and Dick, 2008, Kramer et al. 2010**).** Extensive retention of DOC in topsoil horizons has been documented for field-exposed mesocosms (Fröberg et al. 2009) or in field approaches (Kammer et al. 2012).

RC1	Comments by Referee #3, 10.01.2020
Introduction	This study investigated the impact of aboveground litter for soil organic carbon (C) sequestration and the subsequent partitioning of litter-derived C in different soil layers and OM fractions. In general, I think the data are solid and the results are valuable for understanding fates of litter C input. I have some minor comments/suggestions that could improve the manuscript.
Authors response	We want to thank the referee for his positive feedback and the helpful and constructive criticism, which helped to improve the manuscript.
1. Comment	Lines 37 and 38: This statement may be correct only for natural ecosystems. For example, OM disturbance due to tillage may be a pathway for cropping systems.
Author response	We agree with the Referees comment and modified the sentence in line 37 to 38 of the original manuscript as follows: "In forest ecosystems, major pathways of OM to enter subsoils are rhizodepositions, root exudation and dissolved organic matter (DOM) leached from the horizons above (Wilkinson et al., 2009; Rumpel and Kögel-Knabner, 2011; Kaiser and Kalbitz, 2012)."
2. Comment	Line 99: Did you also observe the amount and chemical properties of the litter? These factors could impact litter decomposition and are important for interpreting the results.
Author response	The amount of litter was about 275 g m^{-2}. This was defined according to measurements of Meier et al. (2005), who reported a litter input of a beech forest at 165 of 427 g m^{-2}. Chemical properties were not analyzed, but since the labeled leaves were harvested from the same tree type (*Fagus sylvatica*) as in the research forest, we assume that the chemical properties of the litter resemble the natural environment of our study site. We recognize this comment and modified the sentence in line 98 to 99 of the original manuscript as follows: "For the labeling, the natural litter layer was removed manually and replaced by an equivalent amount of 275 g ^{13}C enriched beech litter per m^{-2}, representing a typical input of beech litter in Germany (Meier et al., 2005). Labeled litter was prepared as …"
3. Comment	Line 271: "...both, inputs...".
Author response	We are unsure about the intention of this comment, but we assume it aimed at the comma? But manuscript text and comment are the same. No changes to the sentence in the original manuscript were made.
4. Comment	Line 301: For "DOM", did you mean DOM leached from surface soil layers?
Author response	In the first passage of this sub-chapter 4.2 (from line 290 to 314), we discuss the overall role of DOM for MAOM formation, without a specific focus on litter-derived DOM but rather relate the DOM in general, i.e. of different source. To prevent possible misunderstandings, the sentence in the original manuscript was modified in the following way: "Decomposition of roots can substantially contribute to the subsoil SOM pool as well (Rasse et al., 2005), but since root density (Heinze et al., 2018; Wordell-Dietrich et al., 2019) and root exudation (Tückmantel et al., 2017) are low in the Grinderwald subsoil, we assume that the increasing share of MAOM with soil depth rather suggests an increasing importance of DOM as a dominant source of C in this forest subsoil, irrespective of its origin."
5. Comment	Is it possible that rhizodeposition still made a considerable contribution in subsoil MAOM although root density and exudation were low, given that subsoil MAOM contents were also very low?
Author response	A considerable contribution of rhizodepositions is possible, as we also found that about 20 % of the deep subsoil SOC is present as POM (Fig. 2), most likely

	derived from roots, but we assume that it is not the dominant source of subsoil MAOM, as we discussed this in lines 298 to 301 of the original manuscript. And, yes, in absolute number, the content of subsoil MAOM is very low (Fig. 3a). In this paragraph, we wanted to highlight the shift in the importance of the different functional OM fractions from 25 % to 77 % with increasing soil depth.
6. Comment	In addition, it looks that microbial decomposition of root derived C may also increases ^{13}C values and decrease C/N ratios of MAOM; so I am wondering if the observations can fully support the conclusion that DOM leached from the surface soil layers was a dominant source.
Author response	We agree that microbial decomposition of either root-derived C or also litter-derived C may increase ^{13}C values. Together with preferential sorption of ^{13}C-depleted substances, both processes account for the ^{13}C pattern with soil depth, as we discuss in line 304 to 306. Since we do not link our observations and conclusions to recent litter-derived C alone in lines 290 to 314, we think that the response given in Referee's comment #4 and the respective modification in the text are sufficient to clarify that we relate this observation to DOM of different origin. We want to add here that the natural ^{13}C pattern with soil depth was taken into account and used to determine significant enrichments of the labeled samples. This was expressed in eq. 7 (lines 204 to 210).
7. Comment	Lines 360 to 364: Could you explain where the majority of litter-derived C goes; emitted as CO_2?
Author response	This is a good and very important comment/question, which we will definitely address. We made a detailed mass balance regarding the fate of recent litter layer-C, including DOC monitoring and surface CO_2 monitoring. Both will be subject of another publication (currently in preparation), which will focus on the budget in contrast to the present publication where focus in on the fate of litter-derived OM in soil. To answer the question: The majority of the labeled litter-C on the one hand indeed emitted as CO_2 (~ 36-40 %) and on the other hand remained in the litter layer (~ 35-40 %).
8. Comment	If so, why the older mobilizable OC did not emit as CO2?
Author response	Older OC definitely did emit as CO_2 (katabolic pathway), or is recycled by soil microorganisms and consequently used as a source to build-up biomass (anabolic pathway). Microbial decomposition is also the primary reason for the strong decrease of SOC (Fig. 1) and MAOM-C (Fig. 3) with increasing soil depth. What we wanted to highlight in the lines 360 to 364 was, that the mobilizable OC fraction contains predominantly C older than 22 months, despite showing a higher ^{13}C value compared to bulk soil or MAOM.
9. Comment	Line 366: Did you measure the amount of litter residues after 22 months?
Author response	Yes, the removed litter residues after 22 months were measured and amounted to 405 g m^{-2} per site. Considering an initial mass of 275 g m^{-2} added litter, we removed about 130 g m^{-2} more litter than applied. This difference may have resulted from freshly fallen litter material, which was smaller than the mesh size and therefore accumulated during the 22 months. The proportion of remaining labeled litter within the removed litter was about 25 %, corresponding to about 35-40 % of the initial applied labeled litter, mentioned in comment #7. We modified the sentence in line 102 to 103 in the original manuscript as follows to include this information: "In November 2016, the labeled litter was removed manually and amounted to an average of about 405 g m^{-2} per plot. We thus removed more litter than we initially applied due to incorporation of small leaf debris and beechnut shells during the 22 months. About 25 % of the removed litter were residues of the initial applied labeled litter."

10. Comment	Line 395: This statement (and may be statements in other places) is also related to the comment on root-derived C contribution to subsoil OM.
Author response	We agree that roots should always be considered in the context of soil OM. But since we already discussed the (not dominant) impact of roots at our study site in section 4.2, we prefer to end the manuscript with highlighting our main implication and not with findings of other publications (e.g. Rasse et al. 2005). Rasse, D. P., Rumpel, C. and Dignac, M.-F.: Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation, Plant Soil, 269(1–2), 341–356, doi:10.1007/s11104-545 004-0907-y, 2005.
11. Comment	Figure 1: What about the differences of bulk OC between these two sampling times; increasing, decreasing, or no detectable change?
Author response	For the majority of depth increments (9 out of 14), there were no changes between both sampling times. However, for 5 increments, including 0-5 and 5-10 cm, bulk soil OC was smaller at the second sampling compared to the first, likely due to variations in litterfall, bioturbation, and decomposition as a result of differences in precipitation. In the 22 months of litter application, about 950 mm precipitation was measured while it was only about 570 mm in the 18 months thereafter.
12. Comment	Figure 3: I would suggest deleting the grey points if they were not reliable.
Author response	We highly discussed this topic among the authors before the submission and now during the review process. We know that such low values are not realistic for SOC in any soil depth. The reason for these values is a nitrogen content close to the detection limit. Nevertheless we included the values, since all other figures show complete data sets and we wanted to be consistent and also transparent by not excluding data. To prevent misinterpretation, we decided to clearly mark them in grey. We would like to keep the data in the manuscript as presented in the original manuscript, also referring to the other four referees who accepted this presentation.

RC2	Comments by Referee #2, 15.01.2020
Introduction	This is an interesting analysis estimating the contribution of leaf litter on soil organic matter formation of each soil layers. Generally, this is a well performed field study on a relevant subject. The manuscript is quite interesting and decently written, although some descriptions and conclusions are inaccurate. I suggest revisions to address some of the issues I raise below.
Author response	We thank the referee for his positive feedback and the constructive comments.
1. Comment	This description is inaccurate. 0-10-cm soil sequestrated 0.99 g C m-2 yr-1 from labeled litter, 0.37 g C m-2 yr-1 in the 10-50-cm soil layers. It is not surprising, compared to the considerably large contribution of 0-10 cm soil C pools. 48% of the SOC stocks (0-180 cm) were sequestrated in the top 10 cm soil layer (Table 2).
Author response	We cannot follow this comment, as we do not see the inaccuracy of this statement. The recovered label in the MAOM fraction was calculated to average annual litter inputs (see RC5, comment #8) into the different soil compartments. Inputs were, as stated in the manuscript, highest in the topsoil, and lower in the subsoil compartments. We agree that this depth pattern is not surprising and can be expected, since it is known that highest DOC inputs occur in the mineral topsoil with a strong decline with soil depth (Leinemann et al. 2016). The same is true for inputs from a recent litter layer (Fröberg et al. 2007). We did not make changes to the statement in the original manuscript.

	Leinemann, T., Mikutta, R., Kalbitz, K., Schaarschmidt, F. and Guggenberger, G.: Small scale variability of vertical water and dissolved organic matter fluxes in sandy Cambisol subsoils as revealed by segmented suction plates, Biogeochemistry, 131(1–2), 1–15, doi:10.1007/s10533-016-0259-8, 2016. Fröberg, M., Jardine, P. M., Hanson, P. J., Swanston, C. W., Todd, D. E., Tarver, J. R. and Garten, C. T.: Low Dissolved Organic Carbon Input from Fresh Litter to Deep Mineral Soils, Soil Sci. Soc. Am. J., 71(2), 347, doi:10.2136/sssaj2006.0188, 2007
2. Comment	Lines 34-36: Most studies focused on SOC dynamics only in 0-10 cm soil layers? The concepts of "topsoil" and "subsoil" are confusing throughout the text. According to my understanding, the authors described the soils in the 10-to-180-cm layers as "subsoil" involving their own results. But the topsoil described here is obviously not 0-10 cm only.
Author response	It is true that the soil at our study site only has a shallow topsoil horizon, which was classified as such by using the guidelines of the International WRB and the German soil classification. Topsoil horizons are defined as surface mineral soil horizons that are either enriched in organic materials or depleted in inorganic materials (i.e. by podsolization or lessivation). In the soils under study, this refers to the genetic soil horizons AE (0-to 10 cm soil depth, see Table 1). Hence, indeed the topsoil is shallow and does not exceed 10 cm. For our study site and the soil cores, it was reasonable to define the increments 0-10 cm as the topsoil increments based on the soil horizon classification. The subsoil increments (horizons B and C in our case) were divided in 3 subsections for practical reasons. The deep mineral subsoil was defined as the soil > 100 cm soil depth, since classic soil C surveys usually draw the line at 100 cm (Jobbagy and Jackson, 2000). Reversely, we considered the increments from 10 to 50 cm as the upper subsoil. Additionally, the bulk data allowed the presentation of results from the increments in between (50 to 100 cm), which were accordingly summarized as mid subsoil. This definition was given in lines 108 to 110 of the original manuscript, and reads: "Depth increments of the soil cores taken from 0-5 and 5-10 cm are defined as "topsoil", increments between 10 and 50 cm as "upper subsoil", those between 50 to 100 cm as "mid subsoil", and increments below 100 cm as "deep subsoil". We mentioned studies on topsoil C inventories in lines 34 to 36 to introduce the reader to the topic. But of cause the topsoils in the given studies are not restricted to a soil depth of 0-10 cm, as it is a genetic criterion, dependent on the study site. Thus, it is not correct to draw the conclusion that the term "topsoil" always implies a soil depth of 0-10 cm. Jobbagy, E. G. and Jackson, R. B.: The Vertical Distribution of Soil Organic Carbon and Its Relation to Climate and Vegetation, Ecol. Appl., 10(2), 423–436, doi:10.2307/2641104, 2000.
3. Comment	Lines 38-41: This statement is not correct. Below-ground inputs may more important contribution than the litter for SOC accumulation (Nadelhoffer and Raich, 1992; Majdi, 2001; Pausch and Kuzyakov, 2018). Nadelhoffer, K. J., and Raich, J. W.: Fine root production estimates and belowground carbon allocation in forest ecosystems. Ecology, 73, 1139–1147, 1992. Majdi, H.: Changes in fine root production and longevity in relation to water and nutrient availability in a Norway spruce stand in northern Sweden. Tree Physiol., 21, 1057–1061, 2001.

	Pausch, J., and Kuzyakov, Y.: Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Glob. Change Biol., 24, 1–12, 2018.
Author response	We agree and modified the sentence from line 39 to 41 in the original manuscript as follows: "Dissolved organic matter was estimated to contribute about 19 to 50 % to the total mineral soil C stock in forest soils (Kalbitz and Kaiser, 2008, Sanderman and Amundson, 2008) and is considered as a main source of subsoil OM in temperate forest soils (Kaiser and Guggenberger, 2000), next to belowground inputs (Nadelhoffer and Raich, 1992; Majdi, 2001)." We did not include the Pausch and Kuzyakov (2018) suggestion, because they had their main focus on crop- and grasslands and not on forest soils.
4. Comment	Lines 64-66: I noticed and agreed with the comments from Paul Hanson. And: Guelland K, Esperschütz J, Bornhauser D, et al. Mineralisation and leaching of C from 13C labelled plant litter along an initial soil chronosequence of a glacier forefield. Soil Biology and Biochemistry, 2013, 57: 237-247. Kammer A, Schmidt M W I, Hagedorn F. Decomposition pathways of 13 C-depleted leaf litter in forest soils of the Swiss Jura. Biogeochemistry, 2012, 108: 395-411.
Author response	We thank the referee for the additional references. As described in our reply to Paul Hanson's comment, we included the references in the manuscripts. In line 63 to 64 of the original manuscript. The detailed response can be found in the reply to Paul Hanson's comment.
5. .Comment	Line 116: It's important to measure the mass of litter (both for initial and after 22-months) for estimating the relative contribution of the sequestrated C from litter? This is my primary concern.
Author response	We agree with the referee's opinion that the masses of the labeled litter for both time points are useful information. We already addressed this issue in RC1, comment #2 and #9.
6. Comment	Line 217: SOC content in 0-10 cm soil (8.2% here) is largely different (> 5 times) from that given in Table 1 (1.5%, the same forest plot or stand, their previous study). Is there any special on the location of the soil sampling in this study?
Author response	We agree that the discrepancy between the data in Table 1 and the soil core bulk data is confusing to the reader. To clarify this, it should be noted that the 8.2 % in our study is actually the value for the increment 0-5 cm only (visible in Fig. 1a). We recognize the misunderstanding, since we stated in line 217 " … from about 82±57 mg g^{-1} in the topsoil to …". We corrected this sentence in the following way: "Soil OC contents decreased strongly from about 82±57 mg g^{-1} in the upper topsoil increment (0-5 cm) to 3±1 mg g^{-1} in the upper subsoil at 50 cm soil depth (Fig. 1a)." When comparing the soil core bulk data with Table 1, the mean of both increments, 0-5 and 5-10 cm should be used, which would be 5.2±3.5 % SOC compared to the 1.5 % for 10 cm thick topsoil horizon from Table 1. However, this is still a 3-fold higher SOC content for the same study site, which suggests a high spatial variability and the not very well defined border between the thin organic layer and the mineral soil.

RC3	Comments by Referee #1, 19.01.2020
Introduction I	This is a nice straightforward presentation of a field study investigating the fate of surficial litter-derived carbon as it enters and travels down the soil profile. The introduction presents a good overview of the current scientific understanding and

	of the study objectives.
Author response	We thank the referee for the efforts and the positive feedback. We further appreciate the comments on alternative set-ups of the experiment.
1. Comment	As mentioned by previous reviewers, it may benefit from acknowledging past studies using radioactive carbon, as well as the few studies using stable carbon to follow the fate of surface litter
Author response	Yes, we agree with this view and modified the introduction in line 63 to 64 to acknowledge previous studies. The detailed response can be found in the reply to Paul Hanson's comment.
Introduction II	The methodological approach is described in sufficient detail, and the results are concisely presented (thank you!). This paper presents a case study-results from a specific soil. There is still value in getting the work published as is, as I agree with the authors that quantitative information on the fate of carbon inputs after they enter soils is still mostly missing.
2. Comment	Out of curiosity, why was that particular study site chosen? For convenience, or was there another more scientific reason?
Author response	The study site was chosen for several reasons. One was that the Research Unit "SUBSOM" involved 9 institutions and groups spread throughout Germany. It was relevant that the location was close to one of the central labs, the Institute of Soil Science in Hannover, where the weekly taken samples were analyzed. Another important aspect was that in a comprehensive pre-exploration of potential study sites, the Grinderwald proved to be suitable regarding water flow conditions (e.g. high sand content, not too dense, no stagnating water, in sum good water flow conditions) and C distribution (e.g. moderate C in the mineral soil) in the soil profile. Further, we looked for a site with no land-use change during the last century and for an old-growth stand > 100 yrs. And finally, we needed to get permit from the Forestry Administration to install all the equipment and conduct the experiments.
3. Comment	However, I have been trying to wrap my head around the potential broader significance of the presented study. The studied site seems to be affected (to a large extent?) by bioturbation, and a lot of recent carbon was recovered in particulate organic matter. How would the situation be different in the case of soils less affected by soil fauna? Not only in term of the topsoil carbon, but also more importantly in term of DOC leaching and redistribution lower in the profile? Would fluxes then be more important?
Author response	The bioturbation was largely restricted to the top 0-10 cm. We assume that less mixing of POM into the mineral soil would result in an initially higher sequestration of C in the organic layer, e.g. due to retention by the organic layer itself as it was shown by Fröberg et al. (2007) for a coniferous forest floor. It can be expected that if this material would have stayed on the mineral soil, is likely faster decomposed to CO_2. Concerning this effect on DOC formation and leaching we can only speculate. But in absolute means, the amount of litter translocated to the mineral soil by DOC is small (about 2 % of the applied litter after 22 months).So the effect on DOC formation and leaching should be also very minor. Fröberg, M., Berggren Kleja, D. and Hagedorn, F.: The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor, Eur. J. Soil Sci., 58(1), 108–114, doi:10.1111/j.1365 2389.2006.00812.x, 2007
4. Comment	Lastly, how would the results look like if the study had been conducted longer? Eighteen months may not be enough time to see redistribution at depth.
Author response	We agree that 18 months likely is not sufficient to detect a considerable translocation as a result of the assumed sorption-microbial processing-desorption cycles form the litter layer down to the deep subsoil.

	If we think of prolonging the experiment with the exact setting as we used it, i.e. level of ^{13}C enrichment in the labeled litter, we assume that the continuous input of new and unlabeled compounds will rather soon shift the measurable enrichments towards the natural abundance, as we already saw it in the second sampling of our experiment.

RC4	Comments by Referee #4, 20.01.2020
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.
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." - 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}C) was purchased at IsoLife, a company which is specialized on labeling plants by growing them in greenhouses under a $^{13}CO_2$-enriched atmosphere. The labeling in the $^{13}CO_2$ 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 $^{13}CO_2$-enriched atmosphere in a greenhouse, IsoLife, Wageningen, The Netherlands)…"
3. Comment	Question: how were the labelled and unlabelled litter mixed?
Author response	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
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 leave litter in autumn after the experiment started, in order to avoid a dilution of the ^{13}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

	$CaCl_2$ 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 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 13C 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 included 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 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, $\delta^{13}C$ 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}C-enriched bacteria (Kohl et al. 2015)."

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.
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 ‰ ^{13}C. About 25 % of this removed litter originates from the initial applied labeled litter, while 75 % originates from litterfall (e.g. shells of beechnuts), 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

	CO_2 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 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 _13C of the remaining litter after 22 months (is it consistent with the _13C 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 $^{13}CO_2$ atmosphere and IsoLife assures homogeneous labeling of the leaf litter. The $\delta^{13}C$ 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 _13C). Moreover, was a labeled standard (in-house) used since the initial enrichment applied was high (1241-1880 prmil)? What is the maximum _13C 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 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 ^{13}C 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 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 _13C 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 CaCl2 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 CaCl2 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 $CaCl_2$ 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 _13C 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 C_{SPT} 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 C_{SPT} (DOC in SPT) varied between 1 to 3 % but without a depth trend (Fig. 2). $\delta^{13}C$ values in the C_{SPT} 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., Urich, T., Gittel, A., 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	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	We added the information of the initial litter and its loss due to CO_2 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 CO_2 (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 CO_2 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.

Relevant changes to the manuscript besides the referee comments

- In the abstract, incorporation of recent litter-derived C into the MAOM fraction is now given in g C m^{-2} and not per year (manuscript p.1, lines 26-26).
- Abbreviations of the functional OM fractions were revised in the text, figures, tables, and the captions. Functional fractions are now consistently named MAOM, fPOM, oPOM, and WEOM. Carbon within the fractions is named C_{MAOM}, C_{fPOM}, C_{oPOM}, C_{WEOM}, and C_{SPT}.
- We added some information about correlations between surface element contents to the figure caption of Figure S5 (supplement p.9, lines 204-207).

Author comment on the marked-up version of the manuscript and the supplement

The marked-up version contains changes marked with three different colors.

Changes as a response to a referee comment are again marked in red.

Additional changes made by the authors are divided in two groups, deletion of text is marked in blue and insertion of text is marked in green.

Relevance of aboveground litter for soil organic matter formation – a soil profile perspective

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Abstract. In contrast to mineral topsoils, in subsoils the origin and processes leading to the formation and stabilization of organic matter (OM) ~~in subsoils~~ is still not well known. This study addresses the fate of litter-derived carbon (C) in whole soil profiles with regard to the conceptual cascade model, which proposes that OM formation in subsoils is linked to sorption-microbial processing-remobilization cycles during the downward migration of dissolved organic carbon (DOC). Our main objectives were to quantify the contribution of recent litter to subsoil C stocks via DOC ~~movement~~ translocation and to evaluate the stability of litter-derived OM in different functional OM fractions.

A plot-scale stable isotope labeling experiment was conducted in a temperate beech forest by replacing the natural litter layer with ^{13}C enriched litter on an area of 20 m^2 above a Dystric Cambisol. After 22 months of field exposure, the labeled litter was replaced again by natural litter and soil cores were drilled down to 180 cm soil depth. Water extraction and density fractionation were combined with stable isotope measurements in order to link the fluxes of recent litter-derived C to its allocation into different functional OM fractions. A second sampling was conducted 18 months later to further account for the stability of translocated young litter-derived C.

Almost no litter-derived particulate OM (POM) entered the subsoil, suggesting root biomass as the major source of subsoil POM. The contribution of aboveground litter to the formation of mineral-associated OM (MAOM) in topsoils (0-10 cm) was ~~0.99~~1.88 ± 0.~~45~~83 g C m^{-2}~~yr^{-1}~~, and decreased to 0.~~37~~69± 0.~~10~~19 g C m^{-2} ~~yr^{-1}~~in the upper subsoil (10-50 cm) and 0.01 ± 0.~~01~~02 g C m^{-2} ~~yr^{-1}~~in the deep subsoil > 100 cm soil depth during the 22 months. This finding suggests a subordinate importance of recent litter layer inputs via DOC translocation to subsoil C stocks, and implies that most of the OM in the subsoil is of older age. Smaller losses of litter-derived C within MAOM of about 66 % compared to POM (77–89 %) during 18 months indicate that recent carbon can be stabilized by interaction with mineral surfaces; although the overall stabilization in the sandy study soils ~~was low~~ is limited. Our isotope labeling approach supports the concept of OM undergoing a sequence of cycles of sorption, microbial processing, and desorption while migrating down a soil profile, which needs to be considered in models on soil OM formation and subsoil C cycling.

1 Introduction

The capability of soils to incorporate and preserve large quantities of organic matter (OM) is a key function in the global carbon (C) cycle (Wiesmeier et al., 2019). While in the past most studies on carbon inventories focused on topsoils, only some recent research also expands to subsoil environments (Fontaine et al., 2007;

Salomé et al., 2010; Bernal et al., 2016), considering that a significant proportion of soil OM (SOM) is stored in subsoil horizons (Batjes, 1996; Jobbagy and Jackson, 2000). **In forest ecosystems, m**ajor pathways of OM to enter subsoils are rhizodeposition, root exudation and dissolved organic matter (DOM) leached from the horizons above (Wilkinson et al., 2009; Rumpel and Kögel-Knabner, 2011; Kaiser and Kalbitz, 2012). Dissolved organic matter was estimated to contribute about 19 to 50 % to the total mineral soil C stock in forest soils (Kalbitz and Kaiser, 2008; Sanderman and Amundson, 2008) and is considered as **a** main source of subsoil OM in temperate forest soils (Kaiser and Guggenberger, 2000)**, next to belowground inputs** (Nadelhoffer and Raich, 1992; Majdi, 2001). Further, its high affinity towards reactive mineral phases, thus forming mineral-associated OM (MAOM) makes DOM an important contributor to stabilized SOM (Scheel et al., 2007; Leinemann et al., 2016).

Kaiser and Kalbitz (2012) described the interaction of OM with minerals as a sequence of processes including DOM sorption, microbial processing, and desorption, often referred to as the "cascade model". This model not only accounts for changes in dissolved organic carbon (DOC) concentration and bioavailability with depth, but also considers the depth-dependent changes in ^{14}C age of SOM (Trumbore et al., 1992) as well as in DOM and SOM composition from plant- towards microbial-derived OM, ~~likewise~~ as was found in e.g. forest soils (Guggenberger and Zech, 1994; Kaiser et al., 2004). The cascade model also points at a microbial impact on exchange reactions of OM at mineral surfaces, which has been recently confirmed in a laboratory percolation experiment (Leinemann et al., 2018). Modern ^{14}C ages of MAOM in mineral topsoil horizons, where most sorption sites are likely already occupied, also suggest such exchange of OM (Angst et al., 2018). Increasing OM degradation and transformation with soil depth often result in changes in the stable isotopic composition of SOM. In most soils, $\delta^{13}C$ 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}C-enriched bacteria (Kohl et al., 2015)**. This depth trend can also reflect a translocation of relatively $\delta^{13}C$-enriched OM to greater depth due to preferential sorption of the $\delta^{13}C$-depleted carboxylated lignin degradation products via multiple sorption-decomposition-desorption steps (Kaiser et al., 2001). On the other hand, Rumpel et al. (2012) questioned the slow turnover of subsoil OM, since DOC and root exudate fluxes can substantially increase the subsoil C pool within decades—a view which is in contrast to the frequently high ^{14}C ages of subsoil OM.

While the qualitative aspects of subsoil C cycling with respect to possible OM sources and processes are known, e.g. summarized by Schmidt et al. (2011) and Rumpel et al. (2012), this does not refer to the controlling mechanisms and the turnover of the different subsoil C fractions. Assessment of OM turnover in the subsoil under real conditions still remains a major challenge, as it has to involve analysis of the different C sources (plant- versus microbial-derived) and the quantification of respective in- and outputs. In order to quantify individual C fractions and fluxes, **isotope labeling**, e.g. using ^{13}C**- or ^{14}C**-enriched litter material, has been proven as a very powerful tool (Bird et al., 2008; Moore-Kucera and Dick, 2008; **Kramer et al., 2010**). **Extensive retention of DOC in topsoil horizons has been documented for field-exposed mesocosms (Fröberg et al. 2009) or in field approaches (Kammer et al. 2012)**. Yet, to the best of our knowledge, there are no field studies available that employed stable isotope tracing to estimate the contribution of recent aboveground litter to subsoil C cycling. Also the role of recent litter-derived DOM in the formation of MAOM in the soil profile has not been quantified so far, nor has ~~been~~ the biological stability of ~~the~~ this newly ~~formed C fraction against microbial decomposition~~ incorporated OM been determined.

This study therefore addresses the fate of litter-derived C in the subsoil with regard to the conceptual cascade model (Kaiser and Kalbitz, 2012) under field conditions. **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 carried out a ^{13}C-labeling experiment, where the natural litter layer on a~~n acidic~~ Dystric Cambisol underneath European beech was replaced by a ^{13}C-enriched leaf litter. The contribution of litter to subsoil OM was assessed by δ^{13}C analysis in soil cores down to 180 cm soil depth sampled 22 and 40 months after field labeling. The labeled litter was changed back to unlabeled litter before sampling of the first cores, allowing an indication of exchange processes of labeled C in the soil in the subsequent 18 months. Soil density fractionation was used to assess the contribution of young DOM to the formation of MAOM and to differentiate between particulate and dissolved pathways in the contribution of litter-derived ~~OM~~ C to subsoil OM.

2 Materials and methods

2.1 Site description

The field experiment was carried out in the Grinderwald beech forest (*Fagus sylvatica*), 40 km north of Hanover, Germany (52°34'22'' N, 9°18'49'' E) comprising a stand age of ~~ca.~~ 103 years. The common soil type in the research area is a Dystric Cambisol (IUSS Working Group WRB, 2014), which developed from periglacial fluviatile sandy deposits. The mean annual temperature is 9.7°C and the mean annual precipitation is 762 mm (Deutscher Wetterdienst, Nienburg, 1981-2010). Selected soil properties of the Grinderwald sites are given in Table 1. More detailed site descriptions can be found in Angst et al. (2016) and Bachmann et al. (2016).

2.2 Experimental set-up

The study site Grinderwald includes three soil observatories on which ^{13}C-labeled beech litter was applied (Leinemann et al., 2016; Wordell-Dietrich et al., 2019); hereafter referred to as plots 1 to 3. Each plot was divided in two compartments of 6.57 m^2 each. One compartment was labeled with ^{13}C-enriched litter and the other one remained unlabeled as control. The experiment started in January 2015. For the labeling, the natural litter layer was removed manually and replaced by an equivalent amount of **275 g** ^{13}C-enriched beech litter **per m^{-2}, representing a typical input of beech litter in Germany (Meier et al., 2005). Labeled litter was** prepared as a mixture of highly labeled beech litter (10 atom-% **uniformly labeled due to growth under $^{13}CO_2$-enriched atmosphere in a greenhouse**, IsoLife, Wageningen, The Netherlands) and ~~unlabeled~~ beech litter, which resulted in a final ^{13}C-enrichment of 1241 to 1880 ‰ (Wordell-Dietrich et al., 2019). **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.** The labeled litter stayed in the field for 22 months. In November 2016, the labeled litter was removed manually ~~from all observatories~~ **and**

amounted to an average of about 405 g m^{-2} per plot. We thus removed more litter than we initially applied due to incorporation of small leaf debris and beechnut shells during the 22 months. About 25 % of the removed litter were residues of the applied labeled litter. A total of about 36-40 % of the initially applied labeled litter-C left as CO_2 (Wordell-Dietrich, unpublished).

Following the removal of the labeled litter, three soil cores per plot and treatment (labeled versus unlabeled) were taken down to a depth of 200 cm using a machine-driven percussion coring system (Nordmeyer Geotool, Berlin, Germany). Since it was not possible for each soil core to secure the lowest increment of 180-200 cm, this depth was rejected from further processing. The cores were divided into 15 increments, starting with 5 cm increments from 0 to 10 cm, 10 cm increments from 10 to 100 cm, and 20 cm increments from 100 to 180 cm. Depth increments of the soil cores taken from 0-5 and 5-10 cm are defined as "topsoil", increments between 10 and 50 cm as "upper subsoil", those between 50 to 100 cm as "mid subsoil", and increments below 100 cm as "deep subsoil". Directly after sampling, an equivalent amount of the natural beech litter of the surrounding area was used for replacement of the litter that has been removed before. A second sampling was conducted 18 months later, in May 2018, in total 40 months after applying the labeled litter on the plots.

Soil samples were oven-dried at 60°C and sieved to < 2 mm. Three replicates per plot and treatment were combined to one composite sample per depth increment on a mass equivalent basis for further processing. Aliquots for water extractions were stored frozen (-20°C) directly after sampling.

2.3 Analysis of bulk soil

Bulk samples were analyzed for organic C (OC), total nitrogen (TN) and $^{13}C/^{12}C$ ratio, using a vario ISOPRIME cube (Elementar Analysesysteme GmbH, Hanau, Germany) elemental analyzer coupled to an IsoPrime100 (IsoPrime Ltd, Cheadle Hulme, UK) stabile isotope ratio mass spectrometer (EA-IRMS). Carbon isotope values are given in delta notation relative to the Vienna Pee Dee Belemnite standard (VPDB; Hut, 1987). Data were corrected with a variety of standards from the International Atomic Energy Agency (IAEA) and in-house standards (Supplement, Table S1). Pedogenic Fe and Al fractions were analyzed by selective extractions. Oxalate extractions were conducted according to McKeague and Day (1966) by using 0.2 M ammonium oxalate (pH 3) to dissolve poorly crystalline aluminosilicates and Fe hydroxides like ferrihydrite as well as Fe and Al from organic complexes (Fe_o, Al_o). Iron present in organic complexes, poorly crystalline as well as crystalline Fe oxides (Fe_d) was analyzed by extraction in dithionite-citrate following Mehra and Jackson (1960), modified by Sheldrick and McKeague (1975). All extraction solutions were analyzed for dissolved Fe and Al by ICP-OES (Varian 725-ES, Palo Alto, California, USA).

Water-extractable ~~OC~~ OM (WEO~~C~~M) was used as surrogate of DOM migrating in the soil profile (Corvasce et al., 2006). **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), 25 g of fresh, field-moist soil were extracted with 1 mM $CaCl_2$ solution at a soil/solution ratio of 1/3. Samples were shaken horizontally for one hour at a frequency of 180 rpm at 4°C. After centrifugation for 30 min at 3,500 *g* at 4°C, extracts were filtered through 0.45-µm cellulose-nitrate membranes (Sartorius Stedim Biotech GmbH, Göttingen, Germany). **Prior to the filtration, filters were pre-rinsed with 250 mL of the 1 mM $CaCl_2$ solution.** ~~Total OC (TOC)~~ Organic carbon concentrations in the extracts (C_{WEOM}) were measured by high-temperature combustion with a varioTOC elemental analyzer (Elementar, Hanau, Germany). The $\delta^{13}C$ values of WEO~~C~~M were measured with an isoTOC cube coupled to an IRMS vision (Elementar, Hanau, Germany; ~~(~~Leinemann et al., 2018)~~)~~. The ultra

violet (UV) absorbance at 280 nm of WEOC was measured with the Specord 200 UV-vis spectrometer (Analytic Jena AG, Jena, Germany). Specific ultra violet absorbance at 280 nm (SUVA) was calculated according to Chin et al. (1994) as the ratio of UV absorbance at 280 nm and DOC concentration. Prior to fluorescence measurements, samples, if necessary, were diluted to absorbance values < 0.1 at 280 nm. Thereafter emission spectra from 300 nm to 500 nm were measured at an excitation wavelength of 254 nm (Zsolnay et al., 1999) at a Perkin Elmer LS 50 luminescence spectrometer (Perkin Elmer, Waltham, MA, USA). For all measurements the scan rate was 100 nm min^{-1} and the ~~Ex-slit : Em-slit~~ Ex-slit/Em-slit was ~~15 : 10~~ 15/10. The stability of the instrument was checked with the Raman peak of deionized water at 350 nm. The fluorescence emission index (HIX) was calculated as the ratio of the area between 435-480 nm and the area between 300-345 nm of the emission spectrum (Zsolnay et al., 1999) using FL Winlab Software.

2.4 Density fractionation

Samples for density fractionation were selected in order to represent the topsoil (0-5; 5-10 cm), the upper subsoil (10-20; 20-30; 30-40; 40-50 cm), and the deeper subsoil (100-120; 120-140 cm). Density fractionation was conducted according to Golchin et al. (1994a, 1994b), with the following adjustments based on pre-tests. Aliquots of 25 ± 0.05 g bulk soil were separated into two light fractions (LF), free and occluded particulate OM (fPOM and oPOM), as well as one heavy fraction (HF) containing MAOM. After adding 125 mL sodium polytungstate (SPT) solution (SPT 0, TC-Tungsten Compounds, Grub am Forst, Germany) with a density of 1.6 g cm^{-3} (Kaiser and Guggenberger, 2007; Cerli et al., 2012), the suspensions were manually stirred and allowed to rest for one hour. Afterwards, the samples were centrifuged at 4,000 *g* and 17°C for 30 min (Cryofuge 6000, Heraeus Holding GmbH, Hanau, Germany) and the supernatant, containing fPOM material, was filtered through 0.45-µm polyethersulfone filters (PALL Life Sciences, Ann Arbor, Michigan, USA). The fractionation of the fPOM was repeated once. In a second step, aggregates were destroyed to release oPOM by ultrasonic treatment (Sonopuls HD2200, Bandelin electronic GmbH & Co KG, Berlin, Germany) with an energy input of 60 J mL^{-1} (Gentsch et al., 2015; Schiedung et al., 2016). Prior to the treatment, ultrasonic power of the sonotrode was assessed calorimetrically and ultrasound durations were calculated according to North (1976). After centrifugation at 6,000 *g* for 30 min, the supernatant with oPOM material was filtered as well. Both fPOM and oPOM were washed with ultra-pure water (18.2 MΩ) until the electrical conductivity of the eluate was < 5 µS cm^{-1} (Angst et al., 2016). The HF was washed three to four times with 200 mL ultra-pure water until the conductivity was < 50 µS cm^{-1}. The water used for washing the HF was collected and measured for dissolved OC (C_W). We also measured dissolved OC in all post-treatment SPT solutions. This SPT-mobilized C (C_{SPT}) was taken to represent mobilizable and potentially labile soil OC (Gentsch et al., 2018), derived from POM and MAOM. The dissolved OC concentrations were measured within two days after the fractionation by high temperature combustion with a limit of quantification of 1 mg C L^{-1} (Leinemann et al., 2016), using a Vario TOC cube (Elementar, Hauau, Germany). Aliquots of both liquid phases were freeze-dried similar to the soil fractions for analysis of OC, TN, and $^{13}C/^{12}C$ ratios by EA-IRMS. Due to negligible amounts of POM material in the deeper subsoil samples (100-140 cm), ~~no further differentiation between~~ fPOM and oPOM ~~was done~~ were no longer differentiated. The mean mass recovery in fPOM, oPOM, and HF after fractionation was 99.1 ± 0.9 %. The mean C recovery after fractionation was 98.3 ± 26.5 %, including data for the mobilized C_W and C_{SPT}. On average, 2.0 ± 2.2 % of the ~~recovered~~ C was mobilized by the fractionation procedure. Nitrate and ammonium were extracted from bulk and HF samples to analyze inorganic N contents (N_{min}) ~~in order to obtain organic N~~

~~(ON) contents by substraction of N_{min} from TN~~. Extraction was carried out according to Blume et al. (2010) by mixing 4 ± 0.01 g soil with 16 mL 0.0125 M $CaCl_2$ solution and shaking the mixture for 1 h on an over-head shaker. After sedimentation, the supernatant was filtered through 0.45-µm cellulose acetate filters (BerryTec GmbH, Grünwald, Germany) and measured by a segmented flow analyzer (San++ analyzer, Breda, The Netherlands) with a limit of quantification of 0.1 mg N L^{-1}. Organic N contents were calculated by subtraction of N_{min} from TN. 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. M**ethod description and data are presented in the ~~s~~Supplement.

2.5 Calculations and statistics

Soil OC stocks (kg m^{-2}) were calculated according to Eq. (1):

$$\text{OC stock} = \text{OC} \times \text{density} \times \text{increment thickness} \times 0.01, \quad (1)$$

with the OC content (mg g^{-1}) and bulk density of the fine earth fraction (g cm^{-3}) of each soil increment multiplied by the increment thickness (cm). The proportion of each SOM fraction (OC_{frac}, in %) in percent of the total recovered OC was calculated based on the sum of all fractions (ΣOC):

$$OC_{frac} = \frac{OC_{frac.}}{\sum OC\ (C_{fPOM}, C_{oPOM}, C_{MAOM}, C_{SPT})} \times 100\ \%. \quad (2)$$

Water-extractable OC (C_{WEOM}) was calculated as the percentage proportion relative to OC in the respective bulk soil sample, according to Eq. (3):

$$C_{WEOM} = \frac{OC_{extracted}}{\text{Bulk OC}} \times 100\ \%. \quad (3)$$

As mentioned earlier, all soil fractions released C to the C_{SPT} pool, whereas the C_W fraction solely originated from the MAOM in the HF fraction. Thus, the C_W fraction was added to the MAOM. Further, the $\delta^{13}C$ values of the MAOM (C_{MAOM}, in ‰) were corrected for the $\delta^{13}C$ values of C_W by using Eq. (4):

$$\delta^{13}C_{MAOM} = \frac{M_{MAOM} \times \delta^{13}C_{MAOM} + M_{Cw} \times \delta^{13}C_{w}}{M_{MAOM} + M_{Cw}}, \quad (4)$$

with M_{MAOM} as the C mass (mg) of the HF fraction, M_{Cw} as the C mass (mg) in the total washing solution, and the $\delta^{13}C$ values (‰) of both fractions ($\delta^{13}C_{MAOM}$ and $\delta^{13}C_W$, respectively).

The ^{13}C-labeled samples were used to calculate the proportion of native SOC (SOC_{nat}, in %) and label-derived SOC (SOC_L, in %) by Eq. (5) and Eq. (6):

$$SOC_{nat} = \frac{^{13}C_L - {}^{13}C_{in}}{^{13}C_{uL} - {}^{13}C_{in}} \times 100\ \%, \quad (5)$$

$$SOC_L = 100 - SOC_{nat}, \quad (6)$$

with $^{13}C_L$ as the $\delta^{13}C$ value of the labeled sample, $^{13}C_{uL}$ as the $\delta^{13}C$ value of the unlabeled control in the same soil depth, and $^{13}C_{in}$ as the $\delta^{13}C$ value of the initial labeled litter.

The recovered label-derived SOC was further quantified by ~~estimating~~ calculating the SOC stocks in each respective depth, further calculating the proportion of label-derived SOC, and finally relating the label-derived SOC to the amount of the labeled C in the litter input. The total recovered label was calculated as the sum of label recovered in all OM fractions and respective soil depth increments, and given in g C m^{-2}. The potential loss over time was calculated as the relative decrease of recovered label in the 18-month~~s~~ interval between both sampling times.

If not stated differently, data are given as the mean of three replicates ± the standard deviation (SD). Depths refer to the mean depth per depth increment. $\delta^{13}C$ values (‰) of the labeled samples and fractions ($^{13}C_L$) were tested for significant enrichments compared to the natural variations of the control with the upper 90 % quantile limit of the frequency distribution (Nielsen and Wendroth, 2003), using Eq. (7):

$$^{13}C_L > \bar{X}_{uL} + \left(SD_{uL} \times t_{\Phi;p}\right), \quad (7)$$

with $\bar{X}_{uL}$ as the mean and SD_{uL} as the standard deviation of the unlabeled control samples of the respective soil increment (n = 3). The t-value originated from the Student's t-distribution (Φ = n-1, p = 0.9). Only values passing this comparison were used for recovery calculations. Data were tested for normal distribution by using Shapiro-Wilk normality test, prior to linear correlation analyses. Analyses were performed with SigmaPlot 14 (Systat Software GmbH, San Jose, USA) by using Pearson correlations (for normal distributed data, $p < 0.05$) or Spearman Rank Order correlations (for not normal distributed data, $p < 0.05$). Label recoveries in density fractions and WEO~~C~~M were tested for significant changes with depth and between both sampling times by analysis of variance (ANOVA, $p < 0.05$) with the Tukey test as post-hoc analysis.

3 Results

3.1 Depth distribution and properties of SOC

Soil OC contents decreased strongly from about 82 ± 57 mg g^{-1} in **the upper topsoil increment (0-5 cm)** to ~~3~~4 ± 1 mg g^{-1} in the upper subsoil at 40-50 cm soil depth (Fig. 1a). Within the deeper subsoil, OC content further decreased to about 0.2 mg g^{-1} in the deepest increment at 160-180 cm. Organic C stocks in the topsoil (0-10 cm depth) ~~amounted to~~ averaged about 5.5 kg C m^{-2} ~~as the mean of~~ at both sampling dates, representing 48 % of the OC stock down to a soil depth of 180 cm (Table 2). Deeper subsoil only accounted for 5 % of the SOC stock (Table 2).

Directly underneath the litter layer, the majority of SOC was present as POM (Fig. 2). With increasing soil depth, the relative contribution of ~~POM C~~ C_{POM} to SOC decreased to < 25 %, whereas the contribution of ~~MAOM C~~ C_{MAOM} increased. As for SOC, also the ~~MAOM C~~ C_{MAOM} content declined from about 10 to 22 mg C g^{-1} HF in the topsoil to 0.3 to 0.4 mg C g^{-1} HF in the deeper subsoil of 100-140 cm soil depth (Fig. 3a). The C/N ratio of the MAOM decreased with depth from about 20 in the topsoil to ~ 5 in the deep subsoil (Fig. 3b),

similarly to the bulk soil C/N (Fig. S1). Mean ~~values~~ ratios from the first sampling in November 2016 ~~showed minor~~ were insignificantly, but consistently higher ~~ratios~~ compared to the second sampling in May 2018. The C_{SPT} fraction amounted to 1 to 3 % of the SOC for all soil depths without a consistent trend **(Fig. 2)**. The contribution of ~~WEOC~~ C_{WEOM} showed an increase with soil depth from 0.2 % of SOC in the topsoil to 0.7 to 1.3 % in the deeper subsoil (Fig. 4a). In addition, water extracts showed a compositional change with increasing soil depth, as SUVA values decreased below 10 cm soil depth until reaching the minimum in the deep subsoil (Fig. 4b). The ~~so called~~ humification index derived from fluorescence spectra first increased from the topsoil to its maximum in the heavily rooted upper subsoil (Heinze et al., 2018; Wordell-Dietrich et al., 2019). Below, a constant decrease with increasing soil depth was observed (Fig. 4c).

3.2 Labeled litter-derived C in functional soil OM fractions

Based on $\delta^{13}C$ values, bulk OM was more enriched in ^{13}C from labeled litter than MAOM (Fig. 5a, b). Enrichments in MAOM were significant down to 20 cm soil depth compared to the control. After 40 months, the ^{13}C-enrichment of MAOM was still significant down to 20 cm, but $\delta^{13}C$ values ~~moved~~ shifted closer towards the background (Fig. 5b). Water-extractable OC showed a significant ~~δ~~^{13}C-enrichment ~~to~~ at greater soil depth (60 cm) compared to the bulk soil and MAOM at both sampling dates (Fig. 5a-c). Below this depth, there was still a noticeable ~~δ~~^{13}C-enrichment of ~~WEOC~~ C_{WEOC} in the labeled plots, albeit not significant.

After 22 months, about 11.2 % of the ^{13}C-labeled litter exposed at the soil surface was recovered in the selected depth increments (0-50, 100-140 cm), ~~whereby the contribution~~ with a minor contribution of the deeper depth increments ~~was very minor~~ (Fig. 6a**, Fig. S3**). Considering the ^{13}C of litter origin at 50-100 cm soil depth by linear interpolation between the increments 40-50 cm and 100-120 cm, this value would increase by 0.03 % only. The majority of 87 % of the ~~recovered~~ ^{13}C label was ~~found~~ recovered in the first 5 cm of the topsoil~~. Below~~, while below 40 cm~~, only minor enrichments in ^{13}C were found for individual fractions~~ the recovery was negligible (< 0.2 % of total ~~recovered~~ labeled litter). Eighteen months later, the recovered labeled ^{13}C was lower in all depths compared to the first sampling, albeit not significant due to large variations between the plots, with a total recovery of 1.8 %. In the soil increments below 40 cm, ~~no~~ the label ~~was recovered at all~~ vanished completely in the density fractions at the second sampling ~~in the density fractions~~, while minor proportions of label were still recovered within ~~WEOC~~ C_{WEOM} **(Fig. 5c)**.

In total, we found that within 22 months about 8.7 ± 5.6 % of the applied labeled litter was incorporated ~~into~~ as POM in the mineral topsoil ~~within 22 months~~ (Fig. 6a). This corresponds to 9.9 ± 6.1 g C m^{-2} fPOM and 1.0 ± 0.9 g C m^{-2} oPOM, most of it located in the 0-5 cm topsoil increment. Below, the contribution of labeled litter-derived POM decreased strongly. Nevertheless, recovered labeled litter in the oPOM fraction was detected at even greater depth (30-40 cm) after 40 months. Litter-derived ^{13}C in the MAOM fraction represented 0.7 to 2.0 % of the ~~summed up~~ recovered label in the top 20 cm ~~of the soil profile~~ at both sampling dates (Fig. 6), representing a contribution of litter-derived C to the total ~~MAOM C~~ C_{MAOM} of only about ~ 0.2 % ~~in the top 20 cm~~. Below, ~~only smaller~~ contributions were ~~found~~ even less. Also the C_{SPT} fraction, particularly that of the topsoil and upper subsoil of the first sampling date, showed a ^{13}C-enrichment (Fig. 6a).

However, 18 months after replacing the labeled by unlabeled litter, the proportion of labeled litter-derived C in the SPT solution decreased by 84 % on average (Table 3) and the label was only detectable down to 20 cm soil depth **(Fig. 6b)**.

Proportions of labeled litter-derived C in WEO~~C~~M illustrated clear depth and temporal trends (Fig. 7). The ~~WEOC~~ C_{WEOM} fraction in the topsoil contained more than 1 % of C originally derived from the litter layer at the end of the labeling period in November 2016, with a strong decrease with depth. Below 40 cm, proportions were consistently ~~smaller than~~ < 0.2 %. Eighteen months after litter replacement, the contribution of labeled litter-derived C in WEO~~C~~M decreased to < 0.3 % in the whole soil profile.

Mean loss of the recovered litter-derived ^{13}C over the time period of 18 months between the two samplings was 79 %, and all fractions showed a considerable loss of > 65 % (Table 3). The losses followed the sequence: fPOM (89 %) > WEO~~C~~M (80%) > oPOM (77 %) > MAOM (66 %), respectively. **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).**

4 Discussion

4.1 Particulate OM in the soil profile and contribution of litter-derived POM

Particulate OC contributed 59 ± 16 % to SOC in the Grinderwald topsoil. This high contribution of POM likely is a consequence of translocation by the meso- and macrofauna, as bioturbation can drive both, inputs and mineralization of SOC (Wilkinson et al., 2009). Results are somewhat higher than findings of Schrumpf et al. (2013) who reported 25 ± 16 % POM contribution to the SOC for several European study sites. Below the topsoil, amounts of POM were only minor (Supplement, Fig. S2). The proportional decrease of POM with soil depth confirms findings of Kaiser et al. (2002), who reported a similar decrease in the contribution of POM to SOM from about 65 % in the topsoil to 5 % in the subsoil C horizons, illustrating a decreasing role of root input and bioturbation in subsoil horizons (Heinze et al., 2018). Our results suggest that the majority of POM in the topsoil is not ~~strongly~~ directly connected to annual litter inputs as these are very small compared to the total POM pool. Similar to our observations, Lajtha et al. (2014b) reported ~~for a long term litter manipulation experiment~~ that a 2-fold increase of litter input ~~does~~ did not affect the C concentrations in either the bulk soil, POM, or the ~~MAOM~~ HF fraction of the mineral topsoil and upper subsoil within 20 years. They concluded that forest ~~soil C~~ SOC pools are not tightly coupled to changes in aboveground litter inputs on the short-term. In the upper and deeper subsoil, recent litter-derived POM was barely present after 22 months, and completely vanished after 40 months, suggesting that most POM in the subsoil rather derives from root biomass.

In the 18 months between both samplings, we found that 89 % of recent litter-derived fPOM and 77 % of the oPOM material were lost in the soil profile. Consequently, new POM inputs are unstable and prone to decomposition, in line with reported turnover times of < 10 years (Gaudinski et al., 2000; Baisden et al., 2002). Along with that, Crow et al. (2009) described the aboveground litter as the source of the most actively cycling soil C. The smaller C loss from oPOM compared to fPOM within 18 months (77 and 89 %) reflects a better protection of occluded POM ~~material~~ compared to free POM—even in this loamy sand soil (Table 1).

4.2 Mineral-associated OM and incorporation of litter-derived C via the DOC pathway

Beside bioturbation and rhizodeposition, translocation and sorption of DOM to the soil matrix are the other prominent processes transferring C to the subsoil (Kaiser and Kalbitz, 2012; Mikutta et al., 2019). The observed strong decrease in the contents of mineral-associated OC with soil depth (Fig. 3a) is in line with smaller root exudation rates (Tückmantel et al., 2017) and DOC fluxes (Leinemann et al., 2016) with increasing soil depth at

the Grinderwald site. This also reflects a decrease of available sorption sites ~~as~~ with depth due to increasing sand contents (Table 1) and decreasing amounts of poorly crystalline Fe phases ~~(Fe_o contents) are decreasing~~ (Fe_o contents; Supplement, Table S2). Leinemann et al. (2016) observed a decrease in SUVA values of DOM with increasing soil depth, indicating a preferential sorption of plant-derived compounds in the upper parts of the soil profile. Specific UV absorbance and the fluorescence indices (HIX) of our water extracts showed a similar decline with soil depth, thus underpinning sorption as a relevant process. Decomposition of roots can substantially contribute to the subsoil SOM pool as well (Rasse et al., 2005). But since root density (Heinze et al., 2018; Wordell-Dietrich et al., 2019) and root exudation (Tückmantel et al., 2017) are low in the Grinderwald subsoil, we assume that the increasing share of MAOM with soil depth rather suggests an increasing importance of DOM as a dominant source of C in this forest subsoil**, irrespective of its origin**. This depth trend was accompanied by a compositional change of MAOM as indicated by decreasing C/N ratios and increasing $\delta^{13}C$ values. Fresh litter-derived MAOM in the topsoil had typically wide C/N ratios of about 19 to 22 and low natural abundance $\delta^{13}C$ values of about -27 to -28 ‰ (Figs. 3b, 5b). Microbial processing (Six et al., 2001; Schmidt et al., 2011) and preferential sorption of ^{13}C-depleted plant-derived phenols in the topsoil (Guggenberger and Zech, 1994; Kaiser et al., 2001) alter the SOM characteristics with increasing soil depth by narrowing the C/N ratio and increasing the ^{13}C content. In line with this view, the $\delta^{13}C$ of MAOM in the unlabeled control soil showed a consistent increase with decreasing C/N ratio with depth (Supplement, Fig. S4), thus pointing towards an increasing contribution of microbially processed MAOM with soil depth, as proposed in the "dynamic exchange" or "cascade model" (Kaiser and Kalbitz, 2012). Gleixner (2005) likewise attributed this trend to a higher contribution of plant and root litter in topsoil horizons, whereas the deeper subsoil horizons are dominated by microbial-derived OM. A change towards microbial-derived OM is further supported by decreasing SUVA and HIX values ~~within the WEOC~~ of WEOM from the upper subsoil downwards, suggesting more aromatic and complex plant-derived OM components like phenols being retained in the topsoil, while more microbial-derived components like carbohydrates are present in the subsoil.

On average 1.46 ± 0.67 % of the fresh litter layer C was associated with minerals in the topsoil, 0.57 ± 0.12 % in the upper subsoil, and only 0.01 ± 0.02 % in deeper subsoil compartments 22 months after adding the labeled beech litter, emphasizing the subordinate importance of recent aboveground litter inputs to soil C stocks in all depths, especially the deeper subsoil. Also Lajtha et al. (2014a) showed that 50 years of doubled litter inputs in a deciduous forest stand did not result in a net accumulation of OC in the topsoil HF, likely as sorption sites in topsoils are already largely occupied by OM (Mikutta et al., 2019). The ~~element~~ chemical composition ~~on the mineral surfaces of the HF~~ of the HF particle surface layer supports this assumption, as the C and N contents decreased ~~on the mineral surfaces~~ with increasing soil depth (Supplement, Fig. S5). Additionally, a higher content of mineral-borne Al and Fe within the ~~surface layer of soil particles~~ HF surface layer with increasing depth suggests a higher proportion of uncovered mineral surfaces (Supplement, Fig. S5).

For the Dystric Cambisol under European beech, the ~~observed recoveries of ^{13}C 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.** Fröberg et al. (2007a) reported annual DOC fluxes of about 4-14 g C m^{-2} yr^{-1} in 15 cm soil depth and 1.5 to 4.5 g C m^{-2} yr^{-1} in 70 cm soil depth,

whereof on average 14 % ~~were~~ derived from recent litter. This corresponds to fluxes of 0.5 to 2 g C m^{-2} yr^{-1} and 0.2 to 0.6 g C m^{-2} yr^{-1}, respectively, which is similar in magnitude as the observed ^{13}C fluxes from the labeled litter into the HF at our study site. Given this similarity, it is reasonable to assume that recent litter-derived C contributes to the MAOM pool in different soil depths mainly by the DOC pathway. The decreasing input and contribution ~~and input rates~~ of recent litter-derived C with depth further implies that there is an increasing contribution of older OC to DOC with increasing soil depth, as likewise found when dating ^{14}C ages of DOC (Don and Schulze, 2008).

There was a substantial decrease of the recovered ^{13}C label in the MAOM fraction within the 18 months between the first and second sampling. This can be explained either by desorption of litter-derived compounds (either due to microbial degradation or abiotic exchange processes) and/or sorption of fresh unlabeled DOM~~, which diluted the $^{13}C/^{12}C$ ratio to values close to the background and thus regarded as not significantly enriched.~~ We assume that sorption of DOM from the soil solution and the accompanied replacement of litter-derived C from mineral surfaces is the most plausible reason for the observed ^{13}C loss. ~~Considering that~~ This is because the C content of the ~~MAOM fraction~~ HF at both samplings was rather constant ~~at both samplings~~ (Fig. 3a) and ~~taking into account~~ the considerable DOC fluxes of 0.7 to 2.1 g m^{-2} yr^{-1} in the deep subsoil ~~at 150 cm soil depth at this study site~~ (Leinemann et al., 2016)~~, we assume that the dominant processes involved were the sorption of DOC from the soil solution and the accompanied replacement of litter-derived C from mineral surfaces~~ ensure sufficient probability for sorption and displacements reactions. In total, 1.69 g m^{-2} of initially 2.54 g m^{-2} recent litter-derived MAOM were lost throughout the soil profile (66 %) within 18 months. ~~most of it located in the upper 20 cm~~. This indicates that young OM associated with minerals, especially in the upper soil, is not effectively stabilized by mineral surfaces (Schrumpf et al., 2013). The minor retention of ^{13}C by soil minerals and the subsequent remobilization of mineral-bound C in the topsoil ~~at the Grinderwald site~~ are both facilitated by the generally low contents of clay (< 3 %) and pedogenic Fe and Al oxides (Supplement, Table S2)~~, and the likely dominance of illite in the clay fraction~~ In addition, the clay fraction might be dominated by illite—being a rather less sorptive phyllosilicate under acidic conditions (Kaiser et al., 1997).

Despite the fast transformation of recently formed MAOM in the topsoil, this is not resulting in a ~~detectable~~ significant downward translocation of C within the timeframe of 18 months. This hints to intense microbial processing as desorbed or exchanged recent litter-derived C has a higher bioavailability (Marschner and Kalbitz, 2003). ~~Thus, a partly~~ Another reason for explaining the minor ^{13}C transfer to the subsoil would be the downward translocation of ~~recent~~ unlabeled litter-derived C ~~(not labeled)~~ (after litter displacement), ~~within the soil solution will thus cause a continual dilution of~~ which could have diluted the tracer with increasing soil depth. ~~However~~ On the other hand, at the second sampling, part of the translocated DOM was likely already originating from horizons (O layers and upper mineral soil horizons) already enriched in ^{13}C, thus potentially counteracting the dilution by new unlabeled DOM to a certain extent. ~~Microbial processing may further contribute to the observed losses, as desorbed or exchanged recent litter-derived C has a higher bioavailability (Marschner and Kalbitz, 2003).~~

4.3 Mobilizable OM – linking litter inputs and MAOM formation

The concept of C translocation from topsoil into the subsoil assumes continuous exchange processes at mineral surfaces, leading to partly desorption of microbially altered OM and thus its downward transport (Kaiser and Kalbitz, 2012). Here, WEO~~C~~M was considered to represent such mobilizable OM, being most susceptible to

translocation and, hence, a source for subsoil ~~OC stocks~~ OM. Accordingly, we found an increasing importance of WEO~~C~~M with increasing soil depth, as ~~it constitutes a higher proportion to the SOC~~ its proportion to SOC was higher in the subsoil ~~compared to~~ than in the topsoil~~,~~ ~~implying that a higher proportion of soluble OM is present in the deeper soil compartments~~. This implies that the deeper soil compartments comprised relatively more soluble OM. A similar depth trend was detected for the mobilization of C during density fractionation, supporting the findings for WEO~~C~~M. In accordance with Chantigny (2003), WEO~~C~~M represent~~ed~~s only a small part of SOC, but was more enriched in litter-derived ^{13}C than bulk SOC or MAOM (Fig. 5). Despite the higher enrichment, this accounted only for < 1.7 % of ~~the~~ total WEO~~C~~M ~~pool~~, suggesting that the majority of mobilizable OC is older than 22 months (for sampling in November 2016) or 40 months (for sampling in May 2018). In line with this, Fröberg et al. (2007b) and Hagedorn et al. (2003) reported that recent litter-derived DOC contributes only minor to the total DOC leached from the organic layer into the mineral soil.

The high $\delta^{13}C$ values of ~~WEOC~~ C_{WEOM} (Fig. 5c) and the strong decline of litter-derived C in ~~WEOC~~ C_{WEOM} within the upper 20 cm of the soil profile (Fig. 7) suggest that litter-derived POM is a considerable source of WEO~~C~~M. For example, the beech litter residues that were removed after 22 months and sieved < 5 mm still contained up to 2 % C_{WEOM} (data not shown), which might become liberated ~~during infiltration of soil solution~~ in soil. In the subsoil, WEO~~C~~M likely derives ~~to a higher proportion from MAOM next to roots~~ from MAOM and root-derived POM, the latter representing a negligible fraction in the deeper subsoil at the Grinderwald site. In a recent soil column experiment, Leinemann et al. (2018) showed that 20 % of the MAOM can be replaced by percolating DOM in samples collected from three depths down to 100 cm soil depth. Most intriguing, we did not observe a downward migration of the ^{13}C label within WEOC 18 months later, again pointing to losses of litter-derived C in all soil increments by microbial decomposition. This assumption is supported by findings from Tipping et al. (2012) who showed that the majority of DOM released from the mineral matrix can be lost by mineralization. This also matches well to the fact that subsoil MAOM is only to a minor extent ~~derived~~ fed by recent litter-derived C sources. In summary, topsoil WEOC at least partly derives from the recent litter layer, whereas this is not the case in the deeper soil. This finding thus supports the view, as proposed in the cascade model, that the downward migration of C involves the mobilization of older SOM components.

5 Implications

A prominent concept for the build-up of soil OC stocks not only considers the input of plant residues into soil but also the subsequent fate of OM inputs ~~in the soil~~, where C is assumed to undergo a sequence of cycles including sorptive retention, microbial processing, and desorption on its way down the soil profile (Kaiser and Kalbitz, 2012). This study thus investigated the impact of recent aboveground litter for OC sequestration and the subsequent partitioning of litter-derived C in different soil layers and OM fractions. Annual C inputs from the recent litter layer into the mineral soil were ~~relatively~~ low compared to the C already stored in soil. Most of new litter-derived C is retained in the topsoil, mainly as POM. 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. Our field study supports the concept that C accumulation in deeper soil involves several (re)mobilization cycles of OM during its downward migration. The large C losses in the topsoil during a period of 18 months without concomitant increase in subsoil C indicate that the young SOC, especially in the form of POM, represents an actively cycling C pool. ~~Slowest~~ Slower 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. The loss of about 66 % of the C from the HF within 18 months, however, confirms earlier findings (Schrumpf et al., 2013) that part of the MAOM is rather labile, especially in the presence of less reactive minerals such as quartz or illite at our study site.

In summary, given the highly active C cycling in the topsoil and upper subsoil at the Grinderwald site, only marginall~~y~~ C from a recent litter layer enters the deep mineral subsoil. The build-up of subsoil C stocks is thus not connected to a direct transfer from the litter layer but goes along with repeated sorption and remobilization cycles of OM during downward migration over a much longer period than 3.5 years.

Author contribution. AD, KK, RM, and GG designed the experiment and PL, FK, and PWD carried it out in the field. PWD, LRD, FK, and PL processed the samples and did the analyses. SKW conducted the XPS measurements. PL took the lead in preparing the manuscript, with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the research unit SUBSOM (FOR1806) – "The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils". The authors would like to thank Timo Leinemann for all his work in the first SUBSOM phase, including the labeling and sampling for this experiment. We thank Frank Hegewald for support in the field, Manuela Unger for carrying out the ^{13}C analysis of the water extracts, and all lab teams for assistance in the lab. We also want to thank Leopold Sauheitl for helpful discussions about density fractionation and detection limits and Jürgen Böttcher for helpful discussions about data analysis. We further thank Markus Koch and Moritz Rahlfs for valuable comments on earlier versions of this manuscript.
We gratefully acknowledge comments from 5 anonymous reviewers and Paul Hanson, which helped to improve the manuscript. We acknowledge support from the Open Access Publishing Fund of Leibniz Universität Hannover.

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Table 1. Selected soil properties given as the mean of all three sites (n = 3) and standard deviation in brackets (data adopted from Leinemann et al. (2016)).

Horizon WRB[1]/ KA5[2]	Depth [cm]	OC[3] [mg g^{-1}]	TN[4] [mg g^{-1}]	pH [$CaCl_2$]	Clay ----------	Silt [mg g^{-1}]	Sand ----------
AE/Ahe	0-10	15.18	0.59	3.2	19	282	699
		(1.72)	(0.06)	(0.2)	(3)	(56)	(57)
Bsw/Bsv	10-23	9.59	0.41	3.5	27	307	666
		(2.52)	(0.09)	(0.4)	(11)	(81)	(90)
Bw/Bv	23-67	4.65	0.26	3.9	26	332	642
		(1.96)	(0.04)	(0.1)	(4)	(99)	(103)
C/Cv	67-99	1.07	0.08	3.9	29	255	716
		(0.46)	(0.02)	(0.2)	(8)	(41)	(47)
2C/IICv	99-138	0.34	0.07	4.1	21	87	891
		(0.11)	(0.09)	(0.1)	(14)	(55)	(66)
3C/IIICv	138-175	1.05	0.10	4.0	32	268	700
		(0.11)	(0.11)	(0.3)	(44)	(422)	(466)
4C/IVCv	175+	0.29	0.03	3.9	19	58	923
		(0.14)	(0.04)	(0.2)	(6)	(8)	(14)

[1] according to IUSS Working Group WRB (2014)

[2] according ~~Ad-hoc-Arbeitsgruppe Boden, (2005)~~ **, i.e. according to** ~~German soil classification t~~o German soil classification (Ad-hoc-Arbeitsgruppe Boden, 2005)

[3] Organic carbon (OC)

[4] Total nitrogen (TN)

Table 2. Mean OC stocks in bulk soil of different soil compartments down to 180 cm presented as absolute values and as percent of total soil OC stock (n = 12 and standard deviation is given in brackets).

Soil compartment	Depth [cm]	Mean OC stock [kg m^{-2}]	% of total OC stock
Topsoil	0-10	5.51 (3.67)	48 (13)
Upper subsoil	10-50	3.91 (0.67)	40 (10)
Mid subsoil	50-100	0.76 (0.35)	7 (3)
Deeper subsoil	100-180	0.50 (0.33)	5 (3)

Table 3. Mean contents of labeled litter-derived OM in different soil fractions of all depth increments used for density fractionation (0-50 cm; 100-140 cm) ~~for the sampling after~~ 22 months ~~after labeled litter application in November 2016~~ (November 2016) and ~~the sampling~~ 40 months (May 2018) after labeled litter application (n = 3 and standard deviation is given in brackets) ~~and the calculated loss of litter-derived OM between both samplings (18 months) in percent.~~ The percentage loss over 18 months was calculated based on differences of C contents in OM fractions at both samplings. **Overall, 36-40 % of the initially applied litter was lost by respiration during 22 months of field exposure (Wordell-Dietrich, unpublished).**

	Recovered November 2016 [g m^{-2}]	Recovered May 2018 [g m^{-2}]	Loss over time [%]
C_{MAOM} [1]	2.54 (0.92)	0.85 (0.52)	66
C_{fPOM} [2]	9.89 (6.14)	1.11 (0.96)	89
C_{oPOM} [3]	0.98 (0.91)	0.23 (0.24)	77
C_{SPT} [4]	0.54 (0.35)	0.08 (0.08)	84
C_{WEOM} [5]	0.15 (0.06)	0.03 (0.01)	80

[1] Carbon in mineral-associated ~~organic matter~~ OM (C_{MAOM})

[2] Carbon in free particulate ~~organic matter~~ OM (C_{fPOM})

[3] Carbon in occluded particulate ~~organic matter~~ OM (C_{oPOM})

[4] Sodium polytungstate-mobilizable C (C_{SPT})

[5] Carbon in water-extractable organic ~~C~~ matter (C_{WEOM})

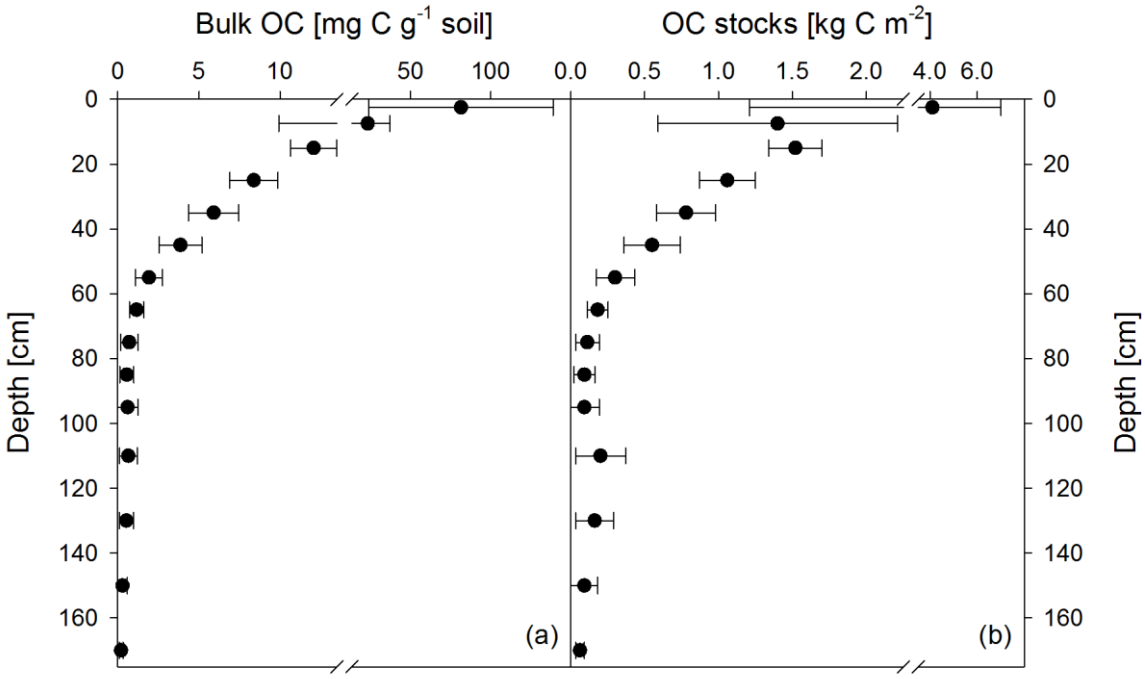

Figure 1. Mean bulk OC contents of both sampling times (November 2016, May 2018) (a) and calculated ~~carbon~~ OC stocks as the mean of both sampling times (b). Apparent re-increasing OC stocks below 100 cm are the result of doubling the thickness of the analyzed depth increments (i.e. 5 cm increments from 0 to 10 cm, 10 cm increments from 10 to 100 cm, and 20 cm increments from 100 to 180 cm). Data show the mean of 12 samples and error bars ~~show~~ depict the standard deviation.

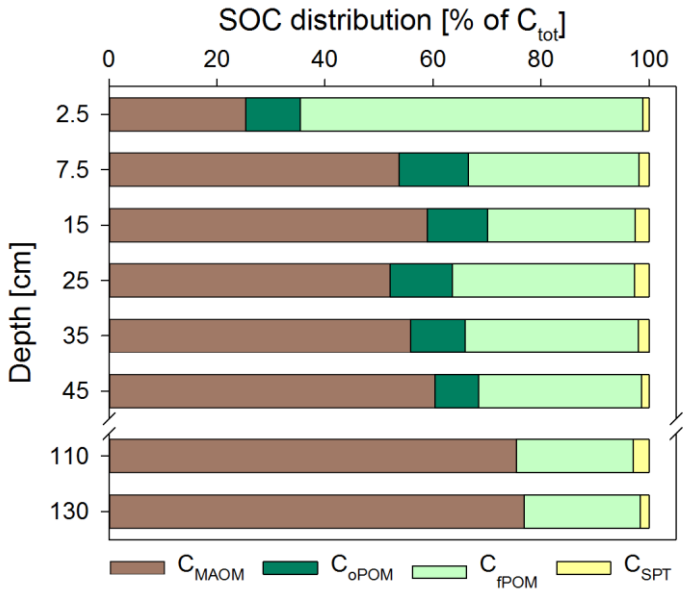

Figure 2. Soil ~~organic carbon~~ OC (SOC) distribution ~~separated into the mineral-associated organic matter (MAOM) within the heavy fraction corrected for the loss during washing by the C_W contents (C_{MAOM}), the occluded particulate organic matter (oPOM) fraction (C_{oPOM}), the free particulate organic matter (fPOM) fraction (C_{fPOM}), and the carbon found in the density solution (C_{SPT}) as the mean of both samplings (n = 12, standard deviation varied for C_{MAOM} between 7-19 %, for C_{oPOM} between 2-5 %, for C_{fPOM} between 7-19 %, and for C_{SPT} between 0.3-5 %).~~ in the Dystric Cambisol at the Grinderwald site as a function of soil depth: C in mineral-associated OM (C_{MAOM}), occluded particulate OM (C_{oPOM}), and free particulate OM (C_{fPOM}); C mobilized by sodium polytungstate during density fractionation (C_{SPT}). All data are given as mean of both samplings (n = 12, standard deviation varied for C_{MAOM} between 7-19 %, for C_{oPOM} between 2-5 %, for C_{fPOM} between 7-19 %, and for C_{SPT} between 0.3-5 %). Note, the C_{MAOM} fraction was corrected for the C loss during washing (see material and method section).

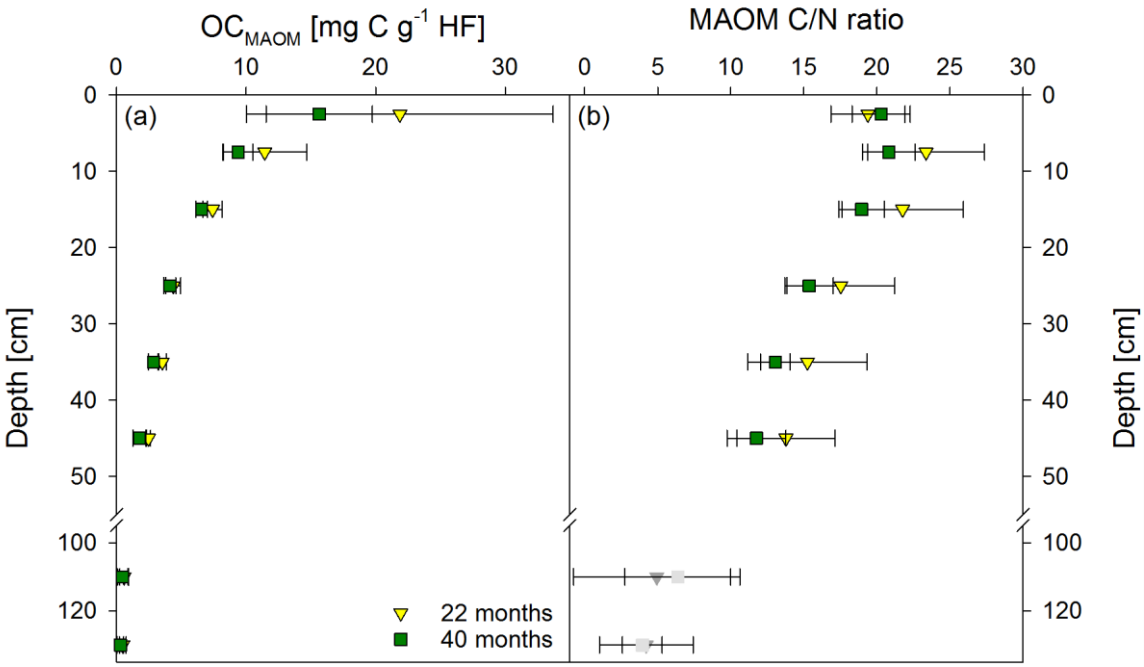

Figure 3. Mean ~~organic~~ OC contents in the heavy fraction (HF) (a) and mean C/N ratios (b) of the mineral-associated organic matter fraction (MAOM) from both sampling times, 22 months and 40 months after labeled litter application (n = 6, error bars represent the standard deviation). **Nitrogen contents in the HF were corrected for extractable nitrate and ammonium contents;** ~~Nitrogen~~ **N** contents in samples below 100 cm were ~~not~~ unreliable and C/N ratios are, therefore, marked in grey.

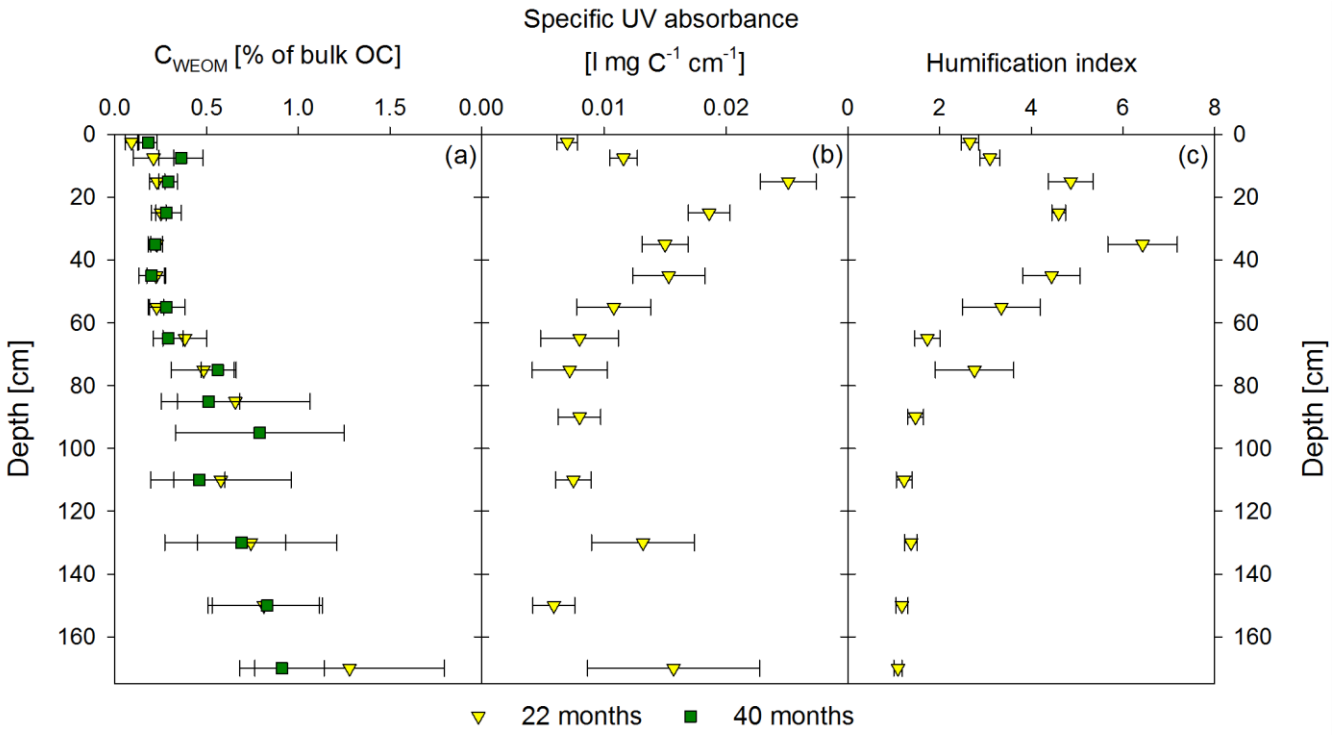

Figure 4. Mean proportion of water-extractable ~~organic~~ OC (C_{WEOM}) per depth increment in % of the total soil ~~organic~~ OC in bulk soil for both sampling times, 22 months and 40 months after labeled litter application (n = 6, error bars represent the standard deviation) is shown in (a). Specific UV absorbance at 280 nm (b) and humification index deduced from fluorescence spectra (c) of the water extracts are given as the mean (n = 6) of the first sampling in November 2016. Error bars represent the standard error.

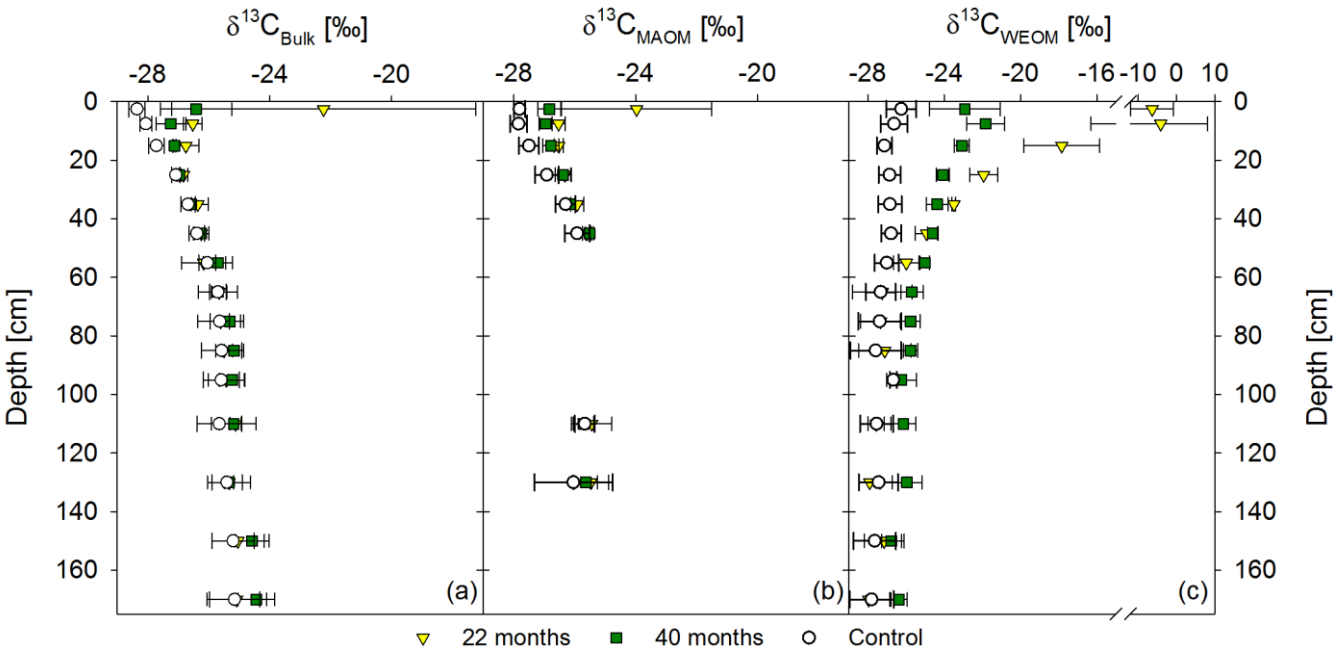

Figure 5. Mean $\delta^{13}C$ values ~~in~~ of the bulk soil (a), mineral-associated OM (~~MAOM~~ C_{MAOM}) (b), and water-extractable ~~organic C~~ OM (~~WEOC~~ C_{WEOM}) (c). The graphs show labeled samples of both sampling times, 22 months and 40 months after labeled litter application in colored symbols, compared to the respective unlabeled background distribution in white symbols. Labeled samples represent the mean of three replicates per sampling time, while the control represents the mean of both sampling times (n = 6). Please note that the X-axis in (c) has a different scale.

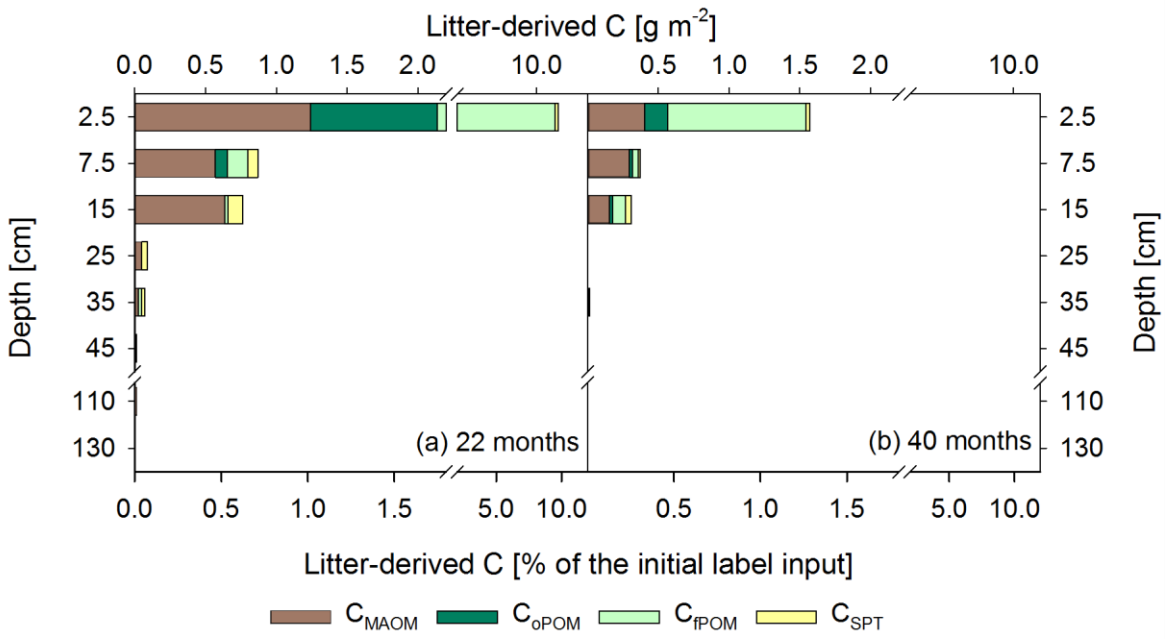

Figure 6. Mean labeled litter-derived ^{13}C recovered in different ~~soil fractions (mineral-associated organic matter (MAOM), occluded particulate organic matter (oPOM), free particulate organic matter (fPOM), and SPT mobilizable (C_{SPT})) in g m^{-2} on the upper X-axis and in % of the label added with the replaced litter on the lower X-axis at sampling in November 2016, 22 months after labeled litter application (a), and May 2018, 40 months after labeled litter application (b).~~OM fractions: C in mineral-associated OM (C_{MAOM}), occluded particulate OM (C_{oPOM}), and free particulate OM (C_{fPOM}); C mobilized by sodium polytungstate during density fractionation (C_{SPT}). Upper X axis shows the recovered ^{13}C in g m^{-2} and lower X axis shows the % recovery of initially added labeled litter after 22 months (a) and 40 months following labeled litter application (b). Bars show the sum of all fractions per depth increment, while the different colors represent the respective contribution of each fraction to the total recovery (n = 3). According to ANOVA tests there were no significant changes in ^{13}C recovery for each fraction with depth per sampling, due to high standard deviations in the range of 0.02–0.53 for C_{MAOM}, 0.01–0.75 for C_{oPOM}, 0.02–4.9 for C_{fPOM}, and 0.01–0.13 for C_{SPT}.

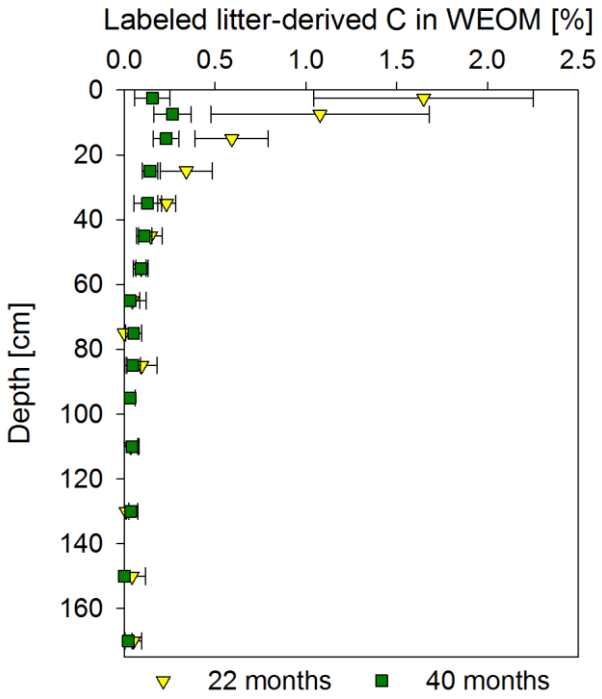

Figure 7. Mean proportion of litter-derived C in water ~~extracts~~ extractable organic OM (WEO~~C~~M) in % of the initial label input for both sampling times, 22 months and 40 months after labeled litter application, with soil depth (n = 3; error bars represent the standard deviation). According to ANOVA tests significant changes between both samplings were only present in the 0-5 cm and 10-20 cm increments ($p < 0.05$). Significant differences between soil increments were only present for the topsoil increments compared to all subsoil increments for each sampling time.

Supplementary material

Table of contents

S1 Methods

S1.1 Surface element analysis

X-ray photoelectron spectroscopy (XPS) analysis was performed with an Axis Ultra DLD instrument (Kratos Analytical, Manchester, UK), using monochromatic AlKα radiation (1486.6 eV), operated at 20 mA and 10 kV. Survey spectra were recorded with a pass energy of 160 eV, a dwell time of 500 ms, and a resolution of 1 eV, while C 1s detail scans were obtained with a pass energy of 20 eV, a dwell time of 259.7 ms, and a resolution of 0.1 eV, with three sweeps per measurement cycle. The take-off angle was 0° and ultra-high vacuum during measurement was 4×10^{-7}Pa. For measurement, the ~~MAOM~~ ~~fraction~~ HF was fixed on a sample bar with carbon conductive tape (Agar Scientific Elektron Technology UK Ltd., Stansted, UK) with an area of about 15 mm^2. Per sample, three spots were measured, comprising an area of 300 × 700 µm each in the slot modus. For charge compensation the neutralizer was active during measurement, however, complete compensation was not possible and the survey spectra were corrected relative to the Si 2p peak at a binding energy of 103 eV (Si-O bond, Okada et al., 1998; Woche et al., 2017). Survey spectra were quantified with the software Vision 2 (Kratos Analytical, Manchester, UK), using a linear baseline and the implemented relative sensitivity factors. ~~Carbon speciation was performed with the software CasaXPS (Version 2.318PR1.0, Casa Software Ltd., UK) by defining four peaks with respect to the C oxidation state, (C1) O=C-O, O=C-N at 289.3 eV; (C2) C=O, O-C-O at 287.9 eV; (C3) C-O, C-N at 286.4 eV; and (C4) C-C, C-H at 284.8 eV (Gerin et al., 2003). Carbon species were further assigned to the following groups: (C1) carbon with three bonds to oxygen and/or nitrogen as in carboxyl and amides (O=C-O, O=C-N), (C2) carbon with two bonds to oxygen as in aldehydes and ketones (C=O, O-C-O), (C3) carbon with a single bond to oxygen or nitrogen as in carbohydrates and amines (C-O, C-N), and (C4) carbon with bonds to carbon or hydrogen as in aliphatic and aromatic compounds (C=C, C-C, C-H) (Kögel-Knabner, 2002; Gerin et al., 2003; Poggenburg et al., 2018).~~ ~~The peak shapes were symmetric with a Gauss/Lorentz ratio of 85/15, using a linear baseline. The full width at half maximum (FWHM) was constrained between 1.4 and 1.8 eV (Gerin et al., 2003) and the peak position was allowed to vary by ± 0.5 eV.~~ The content of all detected elements is given in atomic ~~%~~ percent (atom-%). ~~and fitting results are given as percentage of total peak area~~.

S2 Tables

Table S1. Relevant sStandard substances for included in the EA-IRMS measurements for calibration, correction, and quality control.

Substance	Company	Characteristics*
Quartz sand** (Blank)	In-house standard	
High organic sediment (HOS)	IVA Analysetechnik, Meerbusch, Germany (In-house)	**7.17 % C; 0.57 % N**
USGS 25	IAEA***	
Cellulose	IAEA***	**-24.72 δ^{13}C [‰]**
Caffeine	IAEA***	**-27.77 δ^{13}C [‰]**
N1	IAEA***	
N2	IAEA***	
$CaCO_3$	In-house standard	**-8.17 δ^{13}C [‰]**
Needle litter	In-house standard	

*Please note that all data presented in this study were corrected by using the given values only. Excluded properties are not part of this publication.
**Washed with HCl and glowed at 1040°C
***International Atomic Energy Agency, Seibersdorf Laboratory, Vienna, Austria

Table S2. Contents of dithionite- and oxalate-extractable Fe (Fe_d resp. Fe_o) and oxalate-extractable Al (Al_o) and Mn (Mn_o). Extractions were conducted for the samples from the first sampling in November 2016. Data show the mean (n = 6) with the standard deviation in brackets.

Depth increment [cm]	**Fe_d [mg g^{-1}]**	**Fe_o [mg g^{-1}]**	**Al_o [mg g^{-1}]**	**Mn_o [mg g^{-1}]**
0-5	2.38 (0.21)	1.03 (0.22)	0.56 (0.16)	0.29 (0.31)
5-10	2.42 (0.66)	1.13 (0.53)	0.50 (0.10)	0.05 (0.04)
10-20	2.71 (0.33)	1.51 (0.33)	0.64 (0.18)	0.14 (0.20)
20-30	2.42 (0.36)	1.22 (0.22)	1.05 (0.21)	0.38 (0.43)
30-40	2.11 (0.25)	0.95 (0.14)	1.31 (0.23)	0.58 (0.43)
40-50	1.87 (0.21)	0.75 (0.09)	1.08 (0.13)	0.51 (0.37)
50-60	1.70 (0.16)	0.60 (0.11)	0.94 (0.11)	0.53 (0.18)
60-70	1.65 (0.33)	0.51 (0.14)	0.64 (0.11)	0.61 (0.17)
70-80	1.84 (0.89)	0.45 (0.18)	0.48 (0.16)	0.54 (0.16)
80-90	1.68 (0.62)	0.40 (0.20)	0.38 (0.13)	0.70 (0.24)
90-100	1.65 (0.70)	0.40 (0.22)	0.35 (0.13)	0.58 (0.18)
100-120	1.99 (1.14)	0.49 (0.36)	0.40 (0.20)	0.68 (0.33)
120-140	2.47 (1.88)	0.60 (0.51)	0.41 (0.25)	0.57 (0.37)
140-160	2.04 (2.12)	0.42 (0.49)	0.29 (0.26)	0.57 (0.25)
160-180	1.15 (0.83)	0.23 (0.18)	0.16 (0.08)	0.89 (0.24)

Table S3. Surface element composition of the MAOM fraction, including a set of the most common elements in soil in at%, derived from quantification of XPS survey spectra. Traces of W were remnants of density fractionation, using sodium polytungstate (SPT). Data show the mean of three replicate measurements per sample, SD in brackets.

Plot	Depth [cm]	O	C	N	Na	K	Ca	Mg	W	Fe	Al	Si
1	0-5	51.36 (1.59)	27.32 (2.74)	1.07 (0.08)	0.56 (0.05)	0.05 (0.02)	0.01 (0.02)	0.03 (0.03)	0.07 (0.03)	0.29 (0.14)	2.00 (0.08)	17.24 (1.31)
	5-10	55.84 (0.41)	21.03 (1.02)	0.75 (0.06)	0.61 (0.08)	0.13 (0.04)	0.01 (0.02)	0.07 (0.01)	0.03 (0.01)	0.54 (0.11)	2.34 (0.15)	18.66 (0.43)
	10-20	58.50 (0.85)	16.72 (1.38)	0.63 (0.08)	0.92 (0.08)	0.23 (0.04)	0.02 (0.03)	0.00 (0.01)	0.05 (0.03)	0.91 (0.09)	3.57 (0.15)	18.45 (0.90)
	20-30	59.43 (0.66)	15.82 (0.78)	0.62 (0.14)	0.81 (0.10)	0.12 (0.04)	0.07 (0.02)	0.11 (0.12)	0.07 (0.02)	1.22 (0.06)	4.82 (0.25)	16.91 (0.35)
	30-40	57.13 (0.60)	20.04 (0.22)	0.92 (0.22)	0.45 (0.12)	0.06 (0.05)	0.03 (0.05)	0.15 (0.10)	0.28 (0.04)	1.34 (0.25)	5.96 (0.70)	13.65 (0.52)
	40-50	56.51 (2.84)	20.64 (3.99)	0.83 (0.06)	0.46 (0.02)	0.06 (0.03)	0.08 (0.05)	0.00 (0.00)	0.34 (0.03)	1.53 (0.13)	6.43 (0.34)	13.13 (0.91)
	100-120	64.78 (0.56)	7.17 (0.53)	0.17 (0.10)	1.11 (0.04)	0.48 (0.10)	0.14 (0.03)	0.34 (0.11)	0.19 (0.01)	1.94 (0.27)	7.41 (0.35)	16.27 (0.36)
	120-140	64.82 (0.51)	7.37 (0.64)	0.02 (0.04)	0.90 (0.07)	0.28 (0.02)	0.12 (0.04)	0.32 (0.03)	0.11 (0.01)	1.97 (0.19)	6.02 (0.50)	18.06 (0.47)
2	0-5	51.45 (4.38)	29.20 (7.09)	1.06 (0.04)	0.50 (0.20)	0.06 (0.06)	0.03 (0.03)	0.02 (0.02)	0.00 (0.00)	0.26 (0.10)	1.77 (0.28)	15.65 (2.71)
	5-10	48.53 (3.45)	32.42 (1.86)	0.85 (0.39)	0.90 (0.21)	0.16 (0.06)	0.00 (0.00)	1.13 (1.95)	0.00 (0.00)	0.54 (0.32)	2.89 (0.33)	12.58 (0.49)
	10-20	57.44 (1.99)	18.68 (1.67)	0.75 (0.28)	0.79 (0.14)	0.10 (0.06)	0.00 (0.00)	0.19 (0.29)	0.00 (0.00)	0.74 (0.16)	2.85 (0.43)	18.46 (0.90)
	20-30	58.98 (0.75)	18.14 (0.89)	0.82 (0.25)	0.77 (0.08)	0.04 (0.01)	0.01 (0.01)	0.00 (0.00)	0.05 (0.05)	1.20 (0.13)	4.50 (0.15)	15.49 (0.11)
	30-40	55.30 (0.72)	24.03 (1.18)	1.05 (0.18)	0.45 (0.08)	0.02 (0.02)	0.03 (0.05)	0.00 (0.00)	0.26 (0.03)	1.22 (0.09)	5.72 (0.09)	11.92 (0.64)
	40-50	58.47 (0.59)	18.35 (1.80)	0.86 (0.04)	0.45 (0.11)	0.02 (0.03)	0.00 (0.00)	0.01 (0.01)	0.27 (0.05)	1.33 (0.28)	5.84 (1.00)	14.39 (3.01)
	100-120	64.65 (0.38)	8.98 (0.25)	0.47 (0.09)	1.20 (0.06)	0.30 (0.05)	0.18 (0.04)	0.04 (0.08)	0.17 (0.05)	1.72 (0.11)	7.30 (0.47)	15.00 (0.28)
	120-140	64.55 (0.20)	9.23 (0.32)	0.19 (0.18)	0.93 (0.10)	0.26 (0.04)	0.11 (0.04)	0.00 (0.00)	0.09 (0.01)	1.85 (0.21)	5.72 (0.22)	17.08 (0.10)
3	0-5	55.02 (3.32)	24.17 (4.44)	1.04 (0.33)	0.65 (0.25)	0.05 (0.03)	0.00 (0.00)	0.00 (0.00)	0.03 (0.02)	0.19 (0.18)	1.62 (0.66)	17.24 (2.20)
	5-10	55.95 (4.68)	22.67 (5.77)	0.85 (0.20)	0.72 (0.15)	0.04 (0.03)	0.06 (0.06)	0.37 (0.37)	0.03 (0.03)	0.72 (0.15)	2.49 (0.23)	16.10 (2.17)
	10-20	55.70 (1.71)	22.24 (2.27)	0.96 (0.14)	0.69 (0.03)	0.02 (0.02)	0.06 (0.06)	0.00 (0.00)	0.12 (0.03)	0.95 (0.23)	3.71 (0.09)	15.56 (1.07)
	20-30	52.21 (3.75)	27.61 (6.43)	1.06 (0.16)	0.45 (0.15)	0.00 (0.00)	0.03 (0.05)	0.03 (0.03)	0.17 (0.03)	1.05 (0.14)	4.93 (0.58)	12.47 (1.91)
	30-40	54.39 (2.70)	25.55 (3.79)	0.97 (0.25)	0.47 (0.06)	0.04 (0.03)	0.00 (0.00)	0.00 (0.00)	0.24 (0.02)	1.20 (0.23)	5.46 (0.36)	11.69 (0.63)
	40-50	59.54 (0.62)	17.00 (1.23)	0.90 (0.05)	0.61 (0.10)	0.07 (0.05)	0.06 (0.05)	0.00 (0.00)	0.24 (0.03)	1.28 (0.07)	6.71 (0.40)	13.59 (0.59)
	100-120	64.05 (1.24)	9.96 (1.08)	0.26 (0.14)	1.03 (0.09)	0.21 (0.04)	0.11 (0.10)	0.00 (0.00)	0.16 (0.04)	1.60 (0.31)	6.75 (0.13)	15.85 (0.66)
	120-140	64.93 (2.26)	7.82 (3.49)	0.28 (0.25)	0.99 (0.21)	0.21 (0.05)	0.11 (0.10)	0.00 (0.00)	0.10 (0.02)	2.18 (0.08)	6.13 (0.43)	17.25 (1.52)

S3 Figures

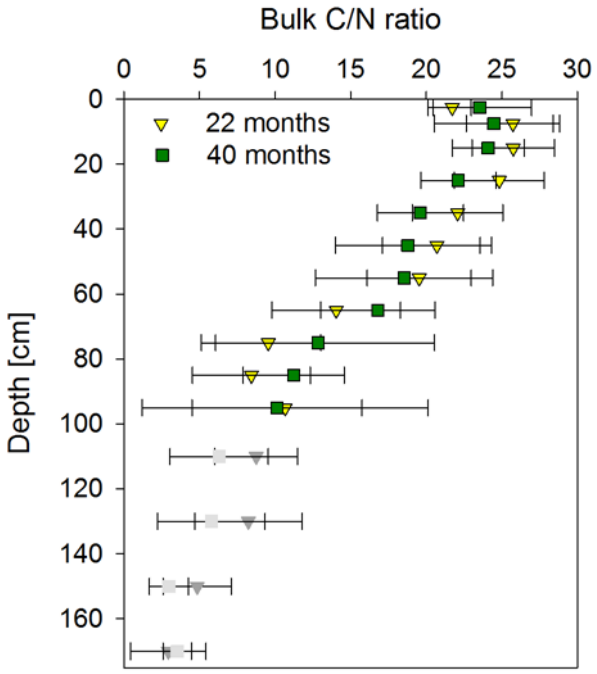

Figure S1. Mean C/N ratio of the bulk soil from both sampling times, 22 months and 40 months after labeled litter application. Data show the mean of 6 samples and error bars show the standard deviation. The y-axis shows the mean depth of each soil increment. **Nitrogen contents of the MAOM fraction were corrected for extractable nitrate and ammonium contents.** Nitrogen contents in samples below 100 cm were increasingly below the detection limit and not reliable, therefore C/N ratios are marked in grey.

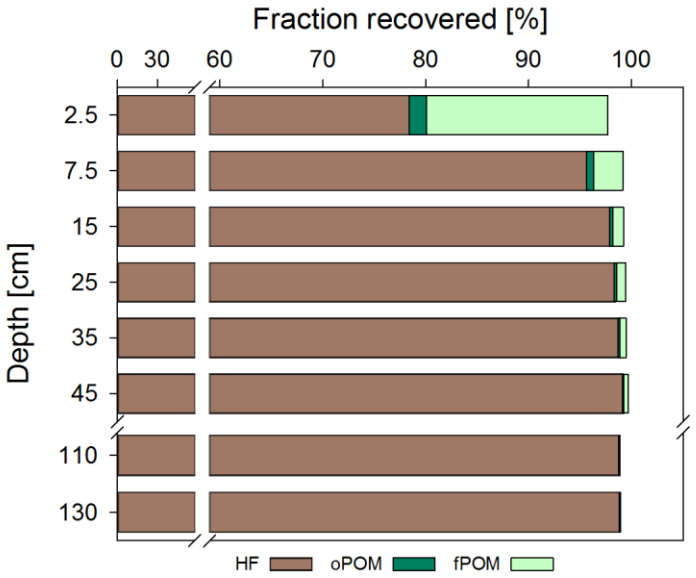

Figure S2. Mean mass recovery and fraction distribution of the soil density fractions heavy fraction (HF), occluded particulate organic matter (oPOM), and free particulate organic matter (fPOM) as the mean of both sampling times (November 2016 and May 2018). The y-axis shows the mean depth of each soil increment. Bars show the mean of 12 samples, the standard deviation varied for HF between 0.3-15 %, for oPOM between 0.1-1.6 %, and for fPOM between 0.1-18 %. Please note that for better visibility, both axes have breaks.

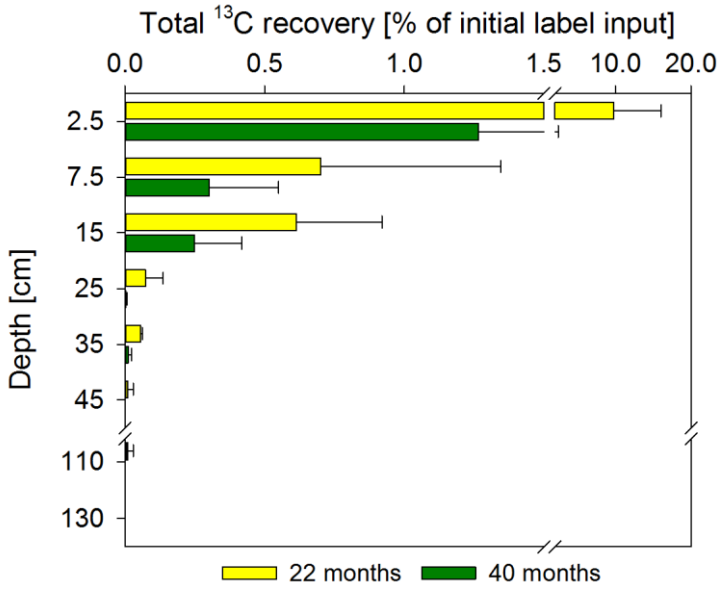

Figure S3. Mean ^{13}C recovered at each sampling time, 22 months and 40 months after labeled litter application, in % of the initial label input (n = 3). Bars show the sum of all fractions per depth increments, error bars depict the standard deviation. According to ANOVA analysis, there were no significant differences ($p > 0.05$) in the total recovered ^{13}C per depth increment between both sampling times, except of the depth 30-40 cm ($p = 0.004$). Please note that for better visibility, both axes have breaks.

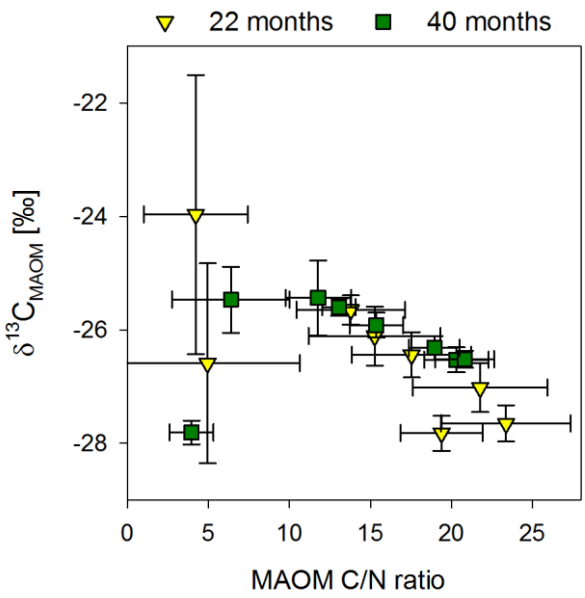

Figure S4. Correlation of the ^{13}C abundance of the mineral-associated organic matter (~~MAOM~~ C_{MAOM}) in the unlabeled control samples on the Y-axis and the corresponding C/N ratio on the X-axis from both sampling times, 22 months and 40 months after labeled litter application. Data show the mean of three replicates, error bars depict the standard deviation. Spearman correlation resulted in a significant negative correlation for both variables for the first sampling in November 2016 ($r = -0.677$, $p < 0.05$) and the second sampling in May 2018 ($r = -0.883$, $p < 0.05$).

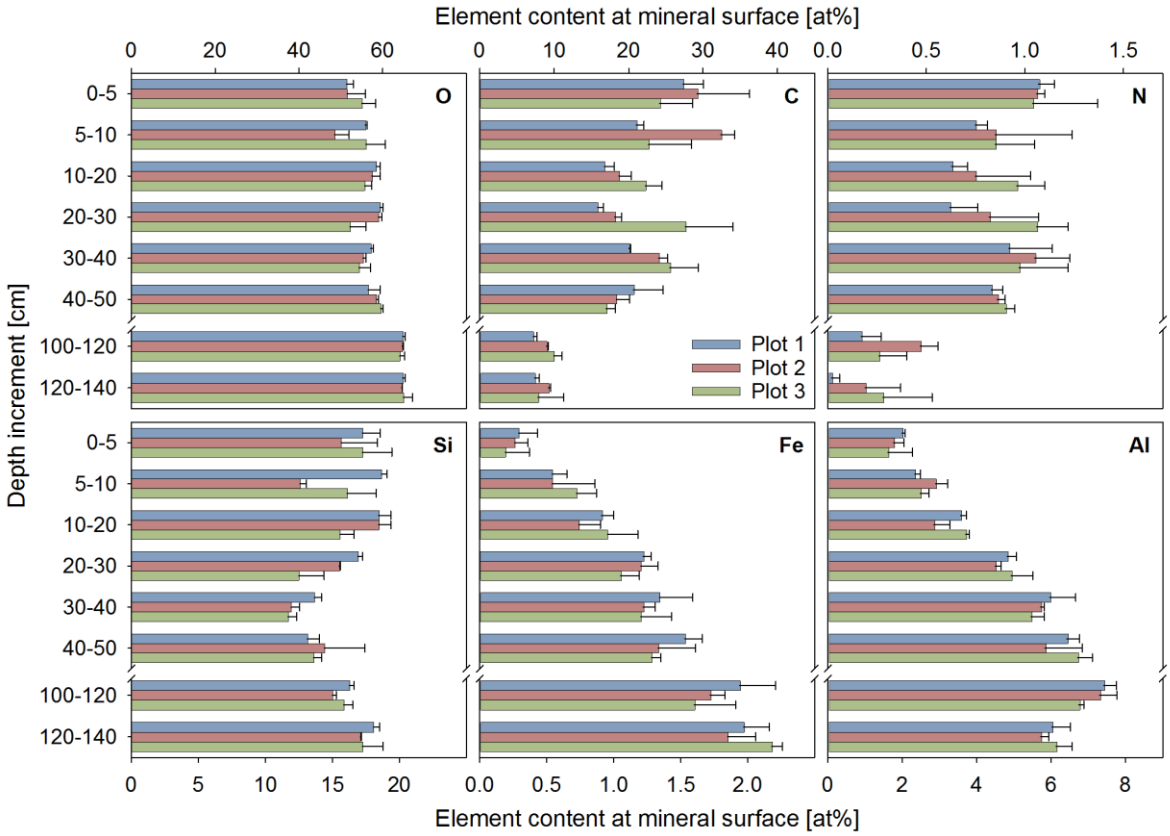

Figure S5. Contents of selected elements on the heavy fraction (HF) mineral surface layer according to XPS analysis. Bars show the mean of three spots measured per sample per plot and depth increment, error bars represent the standard deviation. Please note that the X-axis have different scales. Please note, element contents were highly correlated as a function of soil depth. Negative correlations were observed for example for Fe-C (r^2 = 0.82, p = 0.0021) and Al-C (r^2 = 0.58, p = 0.0295). Positive correlations were observed for example for Fe-Al (r^2 = 0.85, p = 0.0012) and C-N (r^2 = 0.90, p = 0.0004).

Figure S6. Distribution of carbon species of the mineral-associated organic matter (MAOM) fraction with depth of plot 1 (a), plot 2 (b), and plot 3 (c), derived from XPS C1s detail scans in percent of the total peak area. Carbon species were divided according to the C oxidation state and further assigned to the following groups: carboxyl and amides (O=C–O, O=C–N), aldehydes and ketones (C=O, O–C–O), carbohydrates and amines (C–O, C–N), and aliphatic and aromatic compounds (C=C, C–C, C–H).

S4 References

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