Mineralisation, leaching and stabilisation of ¹³C-labelled leaf and twig litter in a beech forest soil

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Response to the comments of referee #1

Major points

(Referee comment) However, I have several issues which need clarification. In brief, the authors provide no or only minimal reflection on how the applied methods might have influenced their findings. Such as, the calculation of d13C of soil respiration by applying a Keeling plot with a simple mass balance using only two data points (most studies use at least 5!), the labeled litter originating from an CO2 enrichment experiment (several studies have shown decomposition rates of litter grown under elevated CO2 to change), the amounts of litter applied were much larger than average at the study site (probably causing reduced litter-soil contact and thereby altering moisture) & the decomposing roots in the trenching plots (increasing microbial activity and probably soil N content).

(Author reply)

1. Keeling plot with a simple mass balance:

We think the concerns of both reviewers that we used only two gas samples instead of a conventional Keeling plot (five or more gas samples) to estimate $\delta^{13}C_{resp}$ are not justified. Before the start of the experiment, we thoroughly evaluated which method we should use and finally preferred the simple mixing model because: (1) This approach requires only two gas sample to estimate $\delta^{13}C_{resp}$ in each single plot. By using five or even more samples, it would not have been possible to perform the experiment in two soil types with five replicates for each treatment (high costs for analyses). Thus, we decided us to a slight loss in the accuracy of $\delta^{13}C_{resp}$ in favour of more replicates to cover the spatial heterogeneity of $\delta^{13}C_{resp}$ and litter-derived CO₂. (2) Previous tests in our plots shortly after removing the litter layer showed that the estimate of $\delta^{13}C_{resp}$ depends primarily on the range in the CO₂ concentration and much less importantly on the number of gas samples used. This has been demonstrated for both ecosystem Keeling plots (Pataki et al., 2003) and closed soil chambers (Ohlsson et al., 2005). (3) Taking only one sample from closed soil chambers has the advantage that both concentration and δ^{13} C of chamber CO₂ are not affected by the sampling. By contrast, taking several gas samples leads to a low pressure in the chamber which might result in a slight release of ¹³C enriched soil gas (+4.4% relative to $\delta^{13}C_{resp}$). (4) We found that a similar number of ${}^{13}C$ -tracer studies have used either the simple mixing model (Subke et al., 2004; Sakata et al., 2007; Rubino et al., 2010) or Keeling plots (e.g. Ngao et al., 2005; Joos et al., 2008). We think thus that our method is state of the art.

Indeed, with a simple mixing model it is not possible to give any error estimations on $\delta^{13}C_{resp}$. However, it is also problematic to use the standard error of Keeling plot intercepts to state the uncertainty of $\delta^{13}C_{resp}$ as it reflects mainly the uncertainty of the extrapolation and only inadequately the measurement errors for instance due to soil-chamber feedbacks. Within the same litter experiment, we applied a quantum cascade laser-based spectrometer (QCLS) which measured concentration and $\delta^{13}C$ of CO₂ accumulating in soil chambers at intervals of one second (Kammer et al., 2011). The intercepts of the resulting Keeling plots (1200 data points) had standard errors of less than 0.1‰. However, analysing different sections of the Keeling plots (e.g. CO₂ accumulation 0-10 min and 10-20 min) showed that $\delta^{13}C_{resp}$ varied by up to several per mille during CO₂ accumulation. This illustrates that it is very difficult to identify the 'real' error of $\delta^{13}C_{resp}$. This issue should be a focus of future research. The QCLS experiment also demonstrated that the method used to estimate $\delta^{13}C_{resp}$ (simple mixing model or high resolution Keeling plot) barely affects the determination of the CO₂ effluxes from the ¹³C-depleted litter, which additionally confirms our decision to prefer the mixing model.

Finally, the study of Steinmann et al. (2004) cannot entirely be compared with our study, as they took gas samples from mineral soils. The CO₂ in these samples derived from both respiration and atmospheric CO₂. Thus, they had to correct for the δ^{13} C of the atmospheric CO₂. In our case, however, the CO₂ in the soil chamber is a mixture of soil-respired CO₂ and ambient CO₂ (atmospheric CO₂+ecosystem respiration). Therefore, we had to correct for the δ^{13} C of the ambient CO₂, which means for the CO₂ that was in the soil collar before it was closed with the lid. In fact, our approach is a Keeling plot of only two data points.

To justify why we used a simple mixing model to estimate $\delta^{13}C_{resp}$ we added to the method section: "We preferred this simple mixing model to conventional Keeling plots of five or more data points, as it requires only two gas samples to estimate $\delta^{13}C_{resp}$, which reduced the accuracy of the estimate only slightly but allowed the measurement of more replicates in the field. Previous tests showed that the use of two instead of five gas samples affected the values of $\delta^{13}C_{resp}$ in single plots by on average +/-0.39‰ within the same range in CO₂ concentrations. This difference was of the same magnitude as the error of the intercept extrapolation in the Keeling plots. Moreover, in the same litter experiment, Kammer et al. (2011) found very similar estimates for litter-derived CO₂ when the values of $\delta^{13}C_{resp}$ were either derived from simple mixing models or from high-resolution Keeling plots (1200 data points) using a quantum cacade laser-based spectrometer.

2. The amounts of litter applied were much larger than average at the study site, the litter was from an CO2 enrichment experiment, no interaction between leaf and twig litter:

We are ware of that our experiment did not reproduce the natural conditions in litter layers at our research site and agree with the reviewer that this was not adequately discussed in our manuscript. Thus, we added an additional paragraph to the discussion section of the revised manuscript in which we highlight the limitations of our experiment as follows:

"The mineralisation rates of the ¹³C-depleted litter might have differed from those of native beech litter, since our experiment did not reproduce the natural conditions in litter layers at our research site. For instance, twig litter decomposed without contact to leaf litter, which probably reduced its water content due to the missing protection by the leaf layer. This in turn could have retarded the decomposition of the twigs. By contrast, the leaf layer was almost two times as thick within the plastic frames as on the surrounding soil, probably increasing the moisture in the soil-litter interface. On the one hand, this could have altered the decay rates. On the other hand, it is a common pattern that leaf litter is unevenly distributed on the forest floor. By isolating woody and non-woody litter, we also neglected any potential interaction effects between the two litter types associated with altered nutrient transport and decomposer community (Hättenschwiler et al., 2005). Apart from the moisture effect, however, it is very difficult to predict whether a mixture of leaf and twig litter would have enhanced, decreased or not affected the litter decomposition in contrast to the isolated litter (Hättenschwiler et al., 2005; Ball et al., 2008; Jonsson and Wardle, 2008). Finally, it is also uncertain if the N depletion (-14%) of the added litter resulting from the CO₂ fumigation (see Hättenschwiler and Bretscher, 2001) had any effects on the rates and the dynamic of the litter decomposition. Several litterbag studies have found slightly decreased decay rates for leaf litter produced under elevated CO_2 (e.g. Hättenschwiler et al., 1999; Parsons et al., 2008), whereas in an incubation study using leaf litter from eight tree species in Switzerland, exactly the opposite effect (+5%) was observed (Hagedorn and Machwitz, 2007). Decomposition of woody litter in mesh bags was generally not affected by the CO₂ treatment (Hättenschwiler et al., 1999; Cotrufo et al., 2000). Even though we cannot estimate degree and direction to which the sum of experimental limitations may have biased the observed mineralisation rates as compared to those of native beech litter, we think it is rather unlikely that a perfect reproduction of forest floor conditions would have changed the finding of a surprisingly fast mineralisation of twig litter C."

3. Decomposing roots in the trenched plots:

The reviewer is right that the trenching (needed to reduce autotrophic respiration) very likely altered the N and C availability in the mineral soil by reducing the plant uptake of nutrients, by decomposition of dead roots and also by eliminating root exudates. However, while this experimental artefact might have affected the C fluxes in the mineral soil, it seems very unlikely that it also had a significant influence for the C fluxes from litter, as the litter layer is commonly root free in these soils. Moreover, a fertilisation experiment (+55 kg ha⁻¹ yr⁻¹) at the same site provided evidence that an increased N availability is of marginal importance for the C fluxes in these soils at least on the time scale of one year. Finally, measuring gravimetrically the soil water content of soils within the trenched area and next to it indicated no significant effect by the trenching. Here, it should be noted that our experiment was performed on a slope, and thus lateral water fluxes probably equilibrated differences in soil moisture as the inserted plastic sheet was permeable. We think therefore that the trenching barely biased the results of our study focusing on the litter layer, but agree with the reviewer that it deserves additional discussion. In the revision of the manuscript, this was accounted for by adding to the method section. "At intervals

of two month, the soil water content was determined gravimetrically within the trenched area and next to it, showing that the trenching had negligible effects on soil moisture. The reason might have been lateral water fluxes along the slope. We cannot rule out, however, that decaying roots and a reduced plant uptake of nutrients after the trenching affected decomposition of SOM and litter. But this artefact was probably small. In an accompanying experiment at the same site, increased N additions (+55 kg ha⁻¹ yr⁻¹) had no influence on SOM mineralisation and only slightly reduced the mineralisation of litter and the leaching of DOC (F. Hagedorn, personal communication)."

Specific comments

Abstract

(1)(Referee comment) 13 soil C stocks

(Author reply) In the revised manuscript, we replaced "C stocks in soils" by "soil C stocks"

(2) *l11 delete 'only'*

We deleted 'only'.

(3) 114 centimeters not centimetres

According to my dictionary, it is 'centimetres' in British English.

(4) 121 Why don't add the findings on C twig-litter mineralization being in contrast with assumptions of most soil C models?

Following the reviewers suggestion, we added at the end of the revised abstract: "Thus, our results go against the assumption of most soil C models, which basically assume that fine woody litter mineralises much more slowly than leaf litter, but that similar proportions of the decomposed litter are transferred into more stable humus pools."

Introduction

(5) 'major' not 'mayor'

We replaced 'mayor' by 'major'

(6) 1046 l25 As I understood, only the Rendzina overlies calcareous bedrock.

We emphasised 'calcareous' since ¹³C-tracer experiments have rarely been performed on soils with calcareous bedrock. But the reviewer is right that this is true only for the Rendzina. In the revised manuscript, we therefore replaced 'calcareous' by 'base-rich' as also the Cambisol has relatively high pH values (~ 5.9).

Methods

(7) 1047 123 Can you talk about plots, meaning they are independent, when they were within a radius of 10 m?

We think it is adequate to talk about independent plots. The variability of soil CO_2 effluxes and DOC fluxes is relatively high at our research site, and was less pronounced between plots arranged in a single group (1-2m distance between plots) than between plots of different groups (10-20 m distance). This was tested before the start of the experiment. Statistical analysis of the results supported this. The random 'group' factor often contributed considerably to the variance of soil CO_2 effluxes and DOC fluxes, and thus was included in most statistical models.

(8) 1048 113 When were the soils trenched? Please provide date.

The soils were trenched 6 month before the start of the experiment. We added this information.

(9) 1047 l18 Which year?

The annual litter fall was measured in 2007. This is now mentioned in the manuscript.

(10) 1048 114 With an plastic foliar 30 cm deep you only prevent lateral but not root ingrowth from below.

We agree with the reviewer and replaced 'external root ingrowths' by 'lateral root ingrowths'.

(11) 1048 115 Could dead and decomposing roots from the trenching of the plots have influence the results by increased microbial activity due to more N and C available, as well as higher soil water content due to reduced plant water uptake?

See our reply to the third major point.

(12) 1048 l21 Calibration of gas analyzer?

The analyzer was calibrated before the start of the experiment and its accuracy was periodically checked by using three standard gases (CO2 concentration: 0, 300 and 1000 ppm). We do not feel, however, that this is important information that should be mentioned in the manuscript as it is a self-evident procedure required for the application of a LICOR gas analyzer (also described in its manual).

(13) 1048 l25 Was the lid sealed to prevent CO2 from leaking?

The lids were equipped with foamed rubber which was pressed on the soil collars with a weight. Tests in the lab showed that this prevented air leaking. To state more precisely, we write in the new manuscript: "...the soil collars were hermetically sealed for 8–40 min with a lid,..."

(14) 1048 l25 Instead of an estimate you could also give the results of [CO2] chamber -[CO2] ambient (+SD).

Following the reviewer's suggestion, we present now the exact difference of [CO2] chamber - [CO2] ambient (+SD)

(15) 1049 11 Please make clear that the glass vials are first closed with a septum and then evacuated. Have they been refilled with N2?

We agree with the reviewer that the procedure was presented in the wrong order and therefore have rewritten this sentence as follows: "glass vials (volume of 12 ml, Exetainer gas testing vials, Labco Limited, High Wycombe, UK), which had been previously closed with airtight rubber septa and evacuated with a vacuum pump to 2×10^{-2} hPa." We did not refill the vials with N₂, because previous tests at the Paul Scherrer Institute (PSI, Switzerland) showed no difference in δ^{13} C of CO₂ injected either into vials with vacuum or into vials filled with N₂ (see Joos et al., 2008).

(16) 1049 14 How many days were the samples stored before analysis?

The gas samples were analysed within 1–3 days after sampling. We added this information.

(17) 1049 I8 Keeling plot with simple mass balance equation: Most studies use a minimum of 5 samples during CO2 build-up and then apply a Keeling plot to estimate d13CSR, you need only two samples. With this approach you are assuming, d13C next to the soil collar being CO2 atmosphere (see also Steinmann et al. (2004), Oecologia), and not contaminated by SR or human breath. The slightest error in ambient samples will lead to a substantial error in your d13C-SR. If d13C ambient varies by 1 permil, respired d13C will change by ~1 permil. Moreover, with your approach you can't give any error estimations on your d13C-SR (intercept). Indeed, as your litter-labeling signal is not very strong, small variations in d13Cambient, could lead to substantial errors in estimating d13C-SR. However, as you are comparing treatments, and are probably less interested in absolute d13C-SR the implications for your study are eventually to be small. Please provide an explanation. For an error estimation you could for example use d13C of atmosphere measured at monitoring stations or apply a keeling plot overall measurements separate for each treatment and campaign.

See our reply to the first major point.

(18) 1049 114-126 How many suction plates?

There was one suction plate in each litter plot. We clarified this in the method section by adding "In each plot, we sampled...". The number of replicates is also presented in the result section.

(19) 1049 l21 What do you mean with lower side? downhill?

We replaced "lower side of the litter plots" by "downhill side of the litter plots"

(20) 1049 l24 Please add 'labeled' before litter

We added "labelled".

(21) 1049 l25 How many replicates? One litter bag per plot?

We added in brackets "one per plot".

(22) 1051 11 Sample treatment before microbial biomass extraction?

For the microbial biomass extraction, fresh litter material was used. This information was added. The only sample treatment was the removal of mineral particles before fumigation.

(23) 1051 l21 Is this also true if root respiration may be present? The difference in bare soil d13C between cold and warm season (Fig 2) suggest influence of root respiration. No differences in d13C-SOC between soil types?

We agree with the reviewer that autotrophic respiration still might have contributed to CO_2 effluxes from mineral soils due to the incomplete trenching. Nevertheless, we feel confident that with our experimental design, Eq. 2 allows the most accurate estimates of f_{litter} in soil CO_2 effluxes. A sensitivity test showed that by neglecting contributions of ¹³C depleted (-4‰ relative to δ^{13} C of SOM) autotrophic respiration in Eq. 2, we could have overestimated f_{litter} by at most 9%. This uncertainty was addressed in the revised method section as follows: "Moreover, it neglects that there still might have been CO_2 from autotrophic respiration is depleted in ¹³C by on average 4‰ relative to δ^{13} C of SOM in top soils (Bowling et al., 2008). To test the robustness of f_{litter} to this uncertainty, we assumed contributions of autotrophic respiration to mineral soil-derived CO_2 of 10–30% and varied the parameters in Eq. 2 to obtain values for f_{litter} of 0.1–0.5. This sensitivity test showed that Eq. 2 could have overestimated f_{litter} of soil CO_2 effluxes by 3–9%."

As presented in Table 1, the δ^{13} C of SOC was 0.5% higher in the Cambisol than in the Rendzina. Eq. 2 accounted for this difference as the δ^{13} C of SOC was determined in each plot individually at the end of the experiment. This is pointed out more clearly in the revised manuscript.

(24) 1051 125 How about respiration of macro soil fauna? You estimate about ~30% of leaf litter was allocated by macro soil fauna in the soil and decomposed. Might this has affect the d13C signal?

On the one hand, it is very likely that a part of litter-derived CO_2 originated from decomposition of the labelled litter by the macro fauna. On the other hand, we do not believe that there was a significant ¹³C fractionation during this process which could have affected the $\delta^{13}C$ signal, even though we have found no study addressing this issue. There was, for instance, no significant change in the $\delta^{13}C$ of the labelled litter on the surface through out the experiment (Table 2). Thus, macro fauna did not preferentially transport litter components which were enriched or depleted in ¹³C as compared to the bulk litter.

(25) 1052 l6 Would not a repeated measure anova or a linear mixed effect model be more appropriate to account for the repeated sampling design?

The statistical model used in the previous manuscript already corresponded to a repeated measure anova as the factors 'litter' and 'soil' were tested with the interaction 'litter \times time' and 'soil \times time' respectively. We agree with the reviewer, however, that a linear mixed effect model is more suitable since it is less sensitive to missing values which were an issue particularly for the DOC fluxes in the mineral soil. Thus, all analyses of variance have been revised using the nlme package from R. Even though this changed F and p values, there was no example were a significant effect has become insignificant and vice versa. The new statistical approach is described in the method section as follows: "*Statistics:* Differences in C fluxes and C pools between the litter treatments were tested with linear mixed effect models using the nlme package from R version 2.8.1 (Pinheiro et al., 2008). By including random effects for the 'plot group' and for each single 'litter plot', the models accounted for both the split unit design of the experiment and the repeated measurement structure. Beside the litter type, soil and time were used as fixed factors. In all final models, normality and homoscedasticity of the residuals were verified visually with diagnostic plots and, when necessary, the dependent variable was log transformed."

Results

(26) 1053 l 14 Give the statistical test and provide t or F-values. P-values alone are meaningless.

Here, we used the soil and litter effect of the lme model. This information was added as well as the F-values.

(27) 1053 116 'labeled litter' instead of '13C-depleted litter'?

¹³C-depleted litter' was replaced by ¹³C-labelled litter'

(28) 1053 l20 better 'litter microbial biomass', increases readability.

'microbial biomass on litter' was changed to 'litter microbial biomass'

(29) 1053 l24 add 'of the experiment'

'of the experiment' was added.

(30) 1053 120-25 Here you are not differentiating between soil types? Why? Please mention also in Table 2. Please also give the number of samples in Table 2.

Fresh litter was identical in both soils, but even after one year of decomposition the chemical parameters of litter presented in Table 2 differed negligibly between the two soils. Hence, presenting both soils separately would only marginally increase the information content of the table. Moreover, the chemical parameters are only a secondary result of our study. For the both reasons, we decided to not differentiate between the soils. This was also in favour of a better readable table. To clarify this, we added to the caption of the table: "Litter of both soil types are

combined as their chemical parameters differed only marginally. The number of replicates is ten for litter quality and six for microbial C/N."

(31) 1054 l4 add 'of SOC' to d13C

'of SOC' was added.

(32) 1054 111 Change 'CO2 release' to 'CO2 efflux'.

'CO2 release' was changed to 'CO2 efflux'

(33) 1054 112 see comment 1053 l 14

Type of test as well as F values were added.

(34) 1054 113 Please don't switch back and forth between soil CO2 efflux and soil respiration or even heterotrophic soil respiration (Fig 1 and 3). Stick to one expression, I would recommend soil CO2 efflux, as in your study the sources of soil CO2 effluxes are differing between treatments e.g., (litter, no litter) and partly trenched soils. Correspondingly, I would not use 'the soil respiration'. I also would not use heterotrophic soil respiration, with a shallow trenching, open to the bottom you will definitely have roots invading your plots.

We used different expressions for soil CO_2 effluxes, because this reads less monotonously. But we agree that the constant switching also reduced the clarity of the text. Moreover, the reviewer is right that heterotrophic soil respiration is not valid due to the incomplete trenching. Through out the revised manuscript, we use now 'soil CO_2 effluxes'

(35) 1054 116 Why are the d13C values of the two soils combined in Fig. 2? Provide reasoning.

The simple reason was to present the results in a more condensed, and thus also more readable form. Differentiating between the two soils requires an additional figure consisting of four panels. The δ^{13} C values differed much less between the two soils than between the cold and the warm season. Therefore, we focused on the later. In the revised manuscript, there are now two figures (one for CO₂ and one for DOC) in which the δ^{13} C values of both soils and both seasons are shown.

(36) 1054 118 Please give d13C values for soil CO2 efflux for both soils.

The δ^{13} C values are now presented for both soils.

(37) 1054 l21 But not significant? Also the differences in bare soil d13C-SR vs. soil+ litter d13C-SR seem to be not significant (Fig 2)!

In fact, the difference in $\delta^{13}C_{resp}$ between bare soils and soil+litter was highly significant. This information (F and p values) was added. Please note that Fig. 2 is showing primarily the range in $\delta^{13}C_{resp}$ (box plots). If only mean values (+SE) were presented, the difference would be more obvious.

(38) 1054 l24 Why? Was air temperature high or litter very wet at this day? Do you have litter temperature/moisture measurements?

At this sampling, the air temperature was clearly higher than the soil temperature. Moreover, the litter had lost only a small fraction of its C since the start of the experiment. We extended this section as follows: "The fraction of litter-derived C in the soil CO₂ effluxes (f_{litter}) peaked at 45–60% in January (Fig. 3) when the litter was still fresh and the soil temperature (1°C) lower than the air temperature (6°C). Three weeks before, however, no litter decomposition had been observed at air temperatures clearly below 0°C."

We measured the temperature also below the litter layer but found better correlations between air temperature and litter-derived CO_2 effluxes. Unfortunately, we do not have data on the litter moisture. We do not focus on the temperature effect on litter decomposition because this is a main objective of another manuscript (not published yet).

(39) Fig 1 No differences in temperature between plots? Why don't you show the temperature measurements separate for each soil type? Is 10 cm really the best depth to give when you are interested in litter decomposition?

Over the entire year, the temperature difference between plots and between soil types was negligible. However, there were indeed differences between plots on single sampling days, because soil CO2 effluxes in different plots were not measured at the same time (between 11 am and 4 pm). In the revised manuscript, we added the standard errors to the temperature curve and present the temperature for both soils separately. We used the temperature at a depth of 10 cm as it was slightly better correlated with soil CO₂ effluxes (Eq. 3) than the temperature at 5 cm.

(40) Fig 2 The d13C values of bare soil CO2 efflux (cold season) are with about -24.5 permil quite different from d13C of SOC (-26.7-27.8 permil). Does this reflect a measurement error caused by your simplified form of the keeling plot?

As discussed in our response to major point one, there is no reason why our 'simplified form of the keeling plot' biased the estimates of $\delta^{13}C_{resp}$ more than would have an ordinary Keeling plot. Pronounced temporal variations of $\delta^{13}C$ of heterotrophic soil respiration are common even for a single day (e.g. Betson et al., 2007; Moyes et al., 2010).

(41) 1055 19 Are these estimations of litter loss influenced by the amount you gave? Recalling from the Methods, you gave about 2 x more leaf litter and about 7 x more twig litter than average for the study site.

Microbial decomposition commonly increases linearly with the amount of available litter C, and thus it is very unlikely that the amount of litter did affect the litter loss directly. However, there might have been indirect effects through altered conditions in the litter layer (see major point three).

(42) 1055 114 How was litter-derived DOC calculated? Did you know the d13C of through-fall?

Litter-derived DOC was calculated with Eq. 2 but using the δ^{13} C of throughfall DOC instead of the δ^{13} C of DOC in the bare soil. We added to the definition of Eq.2: " δ^{13} C_{control} is the δ^{13} C in the adjacent 'bare soil' plot or of the throughfall DOC (for DOC leaching from the litter layer)". Moreover, we added the δ^{13} C values of throughfall DOC to Fig. 2.

(43) 1056 112 I can't see the spring effect on DOC in Figure 3.

This point of the reviewer, we do not understand as the spring effect on DOC leached from the litter layer is obvious in Fig. 3: About 80% of litter-derived DOC between January and the start of April, about 40% of litter-derived DOC between the start of April and June due to increased contributions of throughfall DOC.

(44) 1056 112 please change sentence to 'This is indicated by the large difference of d13C in DOC (litter layer) between the cold and the warm season.'

We changed this sentence as suggested.

Discussion

(45) Any effect of elevated CO2 on litter quality and decomposition? Several studies report slower decomposition of leaves grown under elevated CO2. -Would your results have changed if litter and twigs would have been combined? -As you gave larger than usual amounts of litter, the contact of the litter with the soil might have been reduced, altering oxygen availability, moisture and decomposition. -Also, I am wondering how the framing of the plots might has affected decomposition rates by increasing temperature and moisture? Any control measurements?

See our response to the second major point. The effect of the framing on soil moisture and soil temperature was negligible. This was indicated by very small differences between our plots and measurements of soil temperature and moisture at a nearby meteorological station.

(46) 1058 l12 'cm' not 'mm'

'mm' was replaced by 'cm'

(47) 1058 119-26 This should be shortened and more consistent. First you state leaf litter contributes <20% to SR, then you give an actual number (10-12% for leaves and 4-6% for twigs).

This section was really not very consistent and thus rewritten in the revised manuscript as follows:

"In the investigated beech forest ecosystem, litter-derived CO_2 appears to be a major component (~ 50%) of soil CO_2 effluxes mainly on warm winter days when the leaf litter is still fresh (Fig. 4). On an annual scale, however, decaying leaf litter accounted only for 10–12% of the annual C losses from soils through CO_2 and twig litter for 4–6%. This was estimated by combining the rates of the C mineralisation (220–340 mg CO_2 -C g litter C⁻¹ yr⁻¹) with the amounts of litterfall at

our site. The fraction of leaf litter is roughly half of that found in a ¹³C-tracer study in a French beech forest. In contrast to our study, they estimated twice as large C losses through CO_2 from leaf litter (62% of initial C during one year). It must be noted, however, that they linearly interpolated between litter-derived CO_2 effluxes and did not account for the temperature dependency of litter decomposition."

(48) 1058 l26 Please explore how decomposing fine roots might have affected the contribution of leave litter mineralization to SR.

We added to the discussion section: "In our study, we might have underestimated the contribution of litter decomposition to soil CO_2 effluxes, because the CO_2 effluxes from mineral soils were probably increased after the trenching of soils due to mineralisation of dead roots."

(49) 1061 120 Could the faunal community be adapted to the average amount of litter (you gave ~2 times more), and thereby can't increase their activity linearly with increasing amounts of litter? This might explain the with other studies comparable lower removal of leaf litter by soil fauna.

We think the reviewer has a point here. In contrast to the microbial activity, the activity of the macro fauna will probably not increase linearly with the amount of litter. In the revised manuscript, we therefore write: "We assume that the macro fauna was less important for the transport of litter within the plots than in the native forest floor, since the soil fauna's activity might not have increased linearly with the larger availability of litter material. While about 25% of the added leaf C was recovered on the soil surface within the plots after one year of decomposition (Fig. 1), there was no leaf layer left on soils next to the plots."

Response to the comments of referee #2

Major points

(Referee comment 1) The authors use 13C depleted leaf and twig litter derived from a 4 years FACE experiment. Given the FACE experimental design (branch release of CO2) but most importantly the time of fumigation (4 years) and the fact that mature deciduous trees have very high C-reserves, it is most likely that the litter 13C signal was not homogeneous, especially for the twig litter (which would also explain why the 13C in the twigs was less depleted than in the leaves. Result that the authors do not comment at all!). Additionally, most of the C fluxes investigated refer to only about 30%, or less, of the litter C, and to the easily decomposable and soluble fraction. The likely lack of homogenous labeling and the fact that fluxes refer to a preferential group of C molecules, make not acceptable the mixing model applied (Eq. 2), which is based on the assumption that both litter types and SOC would behave equally with regards to

discrimination during C mineralization (i.e. CO2 efflux) and DOC leaching. To me the only way for the authors to solve this problem is to: i) clearly acknowledge the likelihood of non homogeneous labeling; ii) measure the delta13C-CO2 evolved in laboratory incubation from leaves and twigs litter and use those as the end members in the mixing model to quantify flitter in respiration fluxes; iii) similarly, measure the delta13C-DOC from leaves and twig litter as extracted in the laboratory and use those in the mixing model for flitter in DOC fluxes.

This concern of the reviewer is based on a misunderstanding. We used ¹³C-labelled litter originating from six-year-old beech trees and not from mature trees. Thus, most of the organic C was assimilated in a ¹³C-depleted CO₂ atmosphere and the ¹³C label was very likely homogenously distributed. In the revised manuscript, we clarified this as follows: "The labelled litter originated from six-year-old beech trees of a CO₂ enrichment experiment in Switzerland, in which trees had been exposed to ¹³C-depleted CO₂ for four years (Hagedorn et al., 2005)." We also have no evidence that the C that was lost from the litter through CO₂ or DOC leaching differed significantly in its ¹³C signature as compared to the remaining C. Over one year of decomposition, the δ^{13} C of the litter changed only slightly and not significantly (Table 2). Moreover, the δ^{13} C of the initial DOC flush (data not presented) with only very small fractions of throughfall DOC was equal to the δ^{13} C of the bulk litter. Therefore, we feel confident that our mixing model is the best approach with our experimental design to estimate fractions of litter-derived C in C fluxes and pools.

(2) Unfortunately, the authors did not use highly labeled (i.e. 13C enriched) litter and, therefore, their partitioning may be questionable. The raw isotope data are rarely given, but, as for example in the case of 13C in SOC, the authors mention an isotopic shift within the order of magnitude of the range of natural variation in delta13C SOC at the site (0.2 - 0.5%) which is certainly too small to justify any attempt of C partitioning. Additionally, while the authors provide statistics (i.e. means and s.e.) on the results of mixing models, it is not described in the data analyses section how these were calculated. Once the authors have all the needed end members for their mixing models (see the comment above), they should apply the Phillips and Greg's (2001), spreadsheet(it is free from download http://www.epa.gov/wed/pages/models/stableIsotopes/ isotopes.htm) to calculate the uncertainty on their f values. This would significantly strengthen the results presented and show when isotope data are adequate to trust source partitioning.

In our manuscript, we have already mentioned that the contribution of labelled litter C was calculated for each plot individually. This means that we applied the mixing model for each single plot with specific values for the end members instead of using average values of the end members as in the spreadsheet of Phillips and Greg. Means and their standard errors of litter contribution and litter-derived C were then calculated from the five replicates of each treatment. In the revised manuscript, this information was added. Hence, no error propagation was needed! For example, to determine f_{litter} of CO₂ in a single plot we used in Eq. 2 (1) the $\delta^{13}C_{\text{resp}}$ in the 'soil+litter' plot, (2) the $\delta^{13}C_{\text{resp}}$ in the 'bare soil' of the same plot group (distance 1-2m), (3) the $\delta^{13}C$ of the bulk litter (measured before the start of the experiment) and (4) the $\delta^{13}C$ of the SOC measured in the top soil of the same litter plot at the end of the experiment. This approach takes advantage from spatial autocorrelations of $\delta^{13}C$ of mineral soil-derived CO₂. Tests previous to the

start of the experiment had shown that the variance of $\delta^{13}C_{resp}$ as well as of $\delta^{13}C$ -SOC was considerably smaller within plot groups than between them. For CO₂ and DOC, there were very few examples in which the $\delta^{13}C$ value in the 'soil+litter' plot was not decreased relative to the $\delta^{13}C$ value in the adjacent bare soil. Therefore, we feel confident that our source partitioning is reliable! Even though the shift in the $\delta^{13}C$ of SOC (0-2cm) was only 0.2-0.5‰, it was significant (p<0.01) and could be used to estimate litter-derived C. Only three out of twenty litter plots had $\delta^{13}C$ values of SOC 0-2cm which were not lower than those in the adjacent bare soils. Finally, it should be noted that highly labelled litter might improve source partitioning but has also drawbacks. Such litter has been exposed to ¹³C-enriched CO₂ often only for a limited time period (hours to few weeks), and thus the label is probably much less homogenously distributed than the label in the litter we used. It is also quite a challenge to achieve highly labelled twig litter (time and cost intensive).

(3) Because of the weak label, the authors had to add lots of litter material to the extent that it reached most unrealistic litter C input values, in particular for the twigs input. I understand that the authors had to do it, to see an isotopic signal, yet they cannot use their mechanistic study to extrapolate results at the ecosystem level and quantitatively discuss litter contribution to C fluxes at the site. The best they can do with this experimental design is to discuss the effect of litter quality (i.e. twigs vs leaves) on C mineralization, DOC leaching and eventually fragmentation. Thus, all the sections on up scaling should be deleted (see specific points below).

We agree with the reviewer that our results must be interpreted with caution due to the large amounts of litter used. We have therefore added an additional paragraph, in which we discuss how the difference between experimental and native litter layers could have affected the estimates of litter-derived CO_2 (see our reply to major point 2 of referee # 1). We do not agree, however, that our experimental design allows no extrapolation at the ecosystem level. First of all, we think that our litter amounts are not as unrealistic as criticised by the reviewer. On the one hand we added indeed larger amounts than the annual litter fall at our site. On the other hand, it is a common pattern that litter is unevenly distributed on the forest floor. There are often areas where litter accumulates for instance due to dislocation caused by wind or due to the micro topography (tree trunks, rocks, small depressions etc.). At first glance, the amount of twig litter added (2 kg m^{-2}) appears to be very large. But in fact, this corresponds to a litter layer of about two centimetres. Such concentration of twig litter on the forest floor is not uncommon at our study site. Second, how many studies have investigated litter decomposition and particularly pathways of litter-derived C under completely natural conditions? Of course, there are the wonderful tracer experiments in the Oak Ridge Reservation (USA), which took advantage of a unique standlevel ¹⁴C-labelling originating from a local industrial release (e.g. Swanston et al., 2005). But most other experiments have not reproduced natural conditions: Several studies have doubled litter inputs (e.g. DIRT experiment), tracer studies have often used only one litter type, and finally the conditions in litterbags and lab incubations might differ more from reality than those in our tracer experiment. Decay rates from the later have commonly been used to model soil C amounts, which is also an extrapolation at the ecosystem level. There is a hugh number of studies now, which have looked at the mechanistic of litter decay (effect of quality on C loss etc.), but hardly any

study has combined different pathways of C loss from specific litter types to estimate its importance for C accumulation and contribution to CO₂ and DOC fluxes, which are some of the relevant issues regarding prediction of future C storage in forests. We think our results are suitable enough to make an attempt in this direction, even though we agree that this should be made in a more speculative way than we did it. In the revised manuscript, we discuss several times potential artefacts of our experimental design and emphasise the uncertainty of extrapolations. At the beginning of the last section, for instance, we write: "The results of our ¹³C-tracer experiment cannot directly be extrapolated to the investigated beech forest ecosystem as the litter layer differed from the native litter layer both quantitatively and qualitatively. Nevertheless, our study provides evidence that decomposing twigs could be less important for the C storage in these soils than leaves because:.."

(4) A part from the very high input of twig litter (see above), the other high artifact of this study is that twigs were left to decompose in the absence of leaf litter. There is now a clear understanding that synergistic effects occur when litter decompose in mixture. This is always the case for twig litter which, as the authors state, at the site makes only 30% of the standing litter, the remaining being leaves. While this study provide interesting information on the decay patters of twigs, it does not tell us if the same would happen in the real world, where twigs decomposition occur within the standing leaf-litter layer. At best the authors need to acknowledge this important artifact of their study, justify it and discuss results accordingly.

The reviewer is right that the separation of leaf and twig litter from each other might have influenced decay rates as compared to the native litter layer. The assessment of mixture effects on decomposition of ¹³C-labelled litter, however, would have gone beyond the scope of this study. Results from studies on mixture effects also do not show a clear picture. Synergistic effects are more frequent than antagonistic effects, but both can occur in low and high quality litter. And sometimes decay rates from single litter types could simply be added (additive effects). We discussed this in the revised manuscript as follows: "By isolating woody and non-woody litter, we also neglected any potential interaction effects between the two litter types associated with altered nutrient transport and decomposer community (Hättenschwiler et al., 2005). Apart from the moisture effect, however, it is very difficult to predict whether a mixture of leaf and twig litter would have enhanced, decreased or not affected the litter decomposition in contrast to the isolated litter (Hättenschwiler et al., 2005; Ball et al., 2008; Jonsson and Wardle, 2008)."

(5) Modeling of soil respiration is done on the sole basis of temperature. Is soil moisture at the site never below the threshold where it controls soil respiration (around 50% WC)? The authors either have to demonstrate that soil moisture never plays a role at the site, or apply a soil model that accounts for both temperature and soil moisture, as generally done when soil respiration from discrete measures is scaled up to annual fluxes.

Previous studies have shown that soil respiration (SR) at our site is strongly controlled by soil temperature and only in specific years additionally by soil moisture (Ruehr et al., 2010). While no water limitation on SR was detected in 2007, SR was reduced in summer 2006 at relative soil water contents of about 40%. For the time period of our experiment (Nov 07-Nov 08), the relative

soil water content rarely dropped below 55% as it frequently rained in the warm season. This explains why no correlation between soil CO_2 effluxes and soil moisture was observed in our study. Thus, it was not necessary to include soil moisture to model cumulative C losses from soils through CO_2 . We added this information at the beginning of the result section as follows: "Here, it is important to note that the litter layer was mostly wet throughout summer 2008 with frequent rain and the soil moisture at a depth of 10 cm rarely dropped below 15 vol-% (about 55% relative soil water content), which is the threshold for water limitation on soil respiration at our site (Ruehr et al., 2010). Thus, no correlation between soil CO_2 effluxes and soil moisture was observed."

Additional minor points

(1) P1044L4 Use the term "depleted' rather than "labeled".

'labelled' has been replaced by 'depleted'

(2) P1048L12 "Root" should be better defined as "autotrophic" respiration. What is the root depth, were 30 cm enough to discard roots?

'root respiration' has been replaced by 'autotrophic respiration' through out the manuscript. We are aware of that the trenching might have removed the autotrophic respiration incompletely (see our reply to specific comment 23 of referee # 1)

(3) PL1048L27 A side for the other referee's comment on the keeling plot approach which, I agree, done on only 2 points, and with small air samples (20 ml), may cause lack of accuracy in the estimation of the source d13C, I also find surprising that the authors did not use reference vials (i.e. filled with reference gas at the time of sampling) to estimate issues related to vials leaking of CO2 and CO2 adsorption/desorption from the septa, which are always an issue when using vials at atmospheric CO2 concentration and for isotope work. The authors should provide the made and type of vials and more details on vials testing (I assume they did test them prior to use!).

The two-point Keeling plot is discussed in our reply to the first major comment of referee # 1. The glass vials (incl. septa) used in our study have been employed in various other studies to sample gas for ¹³C analyses of CO₂ (e.g. Steinmann et al. 2004; Joos et al., 2008, Hagedorn et al., 2010). They had been thoroughly tested at the Paul Scherrer institute (see also Joos et al., 2008). These tests showed that concentration and δ^{13} C of CO₂ (three standard gases: 320ppm, 480ppm, 1030ppm) added to vials did not change for one month of storage in the lab (even without the use of a desiccator with N₂ atmosphere!). The septa were an issue only for the δ^{18} O of CO₂. In the revised manuscript, we have added the type of vial (volume of 12 ml, Exetainer gas testing vials, Labco Limited, High Wycombe, UK) and also mention that the vials had been tested by Joos et al. (2008).

(4) P1051L6 Wasn't Jenkinson who first provided the MB k factors? Anyway, even if the reference is correct, in both those studies k factors were calculated for soil extracts and not for litter. I would assume that extraction efficiency my significantly differ in litter (should it not be higher given the lack of mineral adsorption?) and the authors should not use these factors. At the top of my head, I do not recall studies on k factors for litters, but the authors should look for those and eventually use more appropriate k factors. But my suggestion, given that they never use the microbial data for C budgeting, is to simply present flush data. (i.e. fumigated – non fumigated, without multiplying for the extraction factor).

We agree with the reviewer that the extraction efficiency of microbial biomass is probably higher for litter than for soil, even though we have found no study which has assessed k factors for litter. Following the suggestion of the reviewer, we therefore use now directly the C/N ratios of the flush data without taking into account that the extraction efficiency for C and N might have differed.

(5) P1051L20 Please refer to the general comment above and apply a more correct mixing model to this study. Also refrain from using delta13C in this setting, this is a symbol used for isotopic discrimination between a source and a product which is not the case here, and calculated with a different formula. Thus, it is misleading here.

As discussed in our response to major comment one, we feel confident that in our case, Eq. 2 is the most adequate mixing model to estimate fractions of the ¹³C-depleted litter. We agree, however, that the delta symbol is not correct and misleading. Thus, we replaced it in the denominator of Eq. 2 by $(\delta^{13}C_{litter} - \delta^{13}C_{SOM})$.

(6) Fig.6 First of all should be Fig.1 since it is the first to be discussed. Also, as it is I believe is misleading. I suggest the authors to present for the litter bags and isotopic approach, the C losses vs C remaining, on a 100% bases. This way it is made evident the difference between the two methods and the fact that CO2 losses measured by the isotopic approach equal C losses in litter bags, i.e. bags only measure C mineralization fluxes and limit C fluxes belowground. Also, for the isotope approach, the fraction not accounted for can be clearly stated.

In the revised manuscript, previous Fig. 6 is now Fig. 1. However, we would like to keep the figure's focus on the recovery of the labelled litter to highlight the gap in the ¹³C-mass balance. The fact that CO_2 -C losses measured by the isotopic approach equal C losses in litter bags is less important for our discussion (this is discussed more deeply in another manuscript).

(7) P1054L12-15; P1058-16-20 Remove (see general comment #3)

Sorry, but we do not understand why these sentences should be removed?

(8) P1055L26-27 I do not understand this sentence.

We agree that this sentence was difficult to understand. Since it contained no relevant information, we deleted it.

(9) P1060L20. Highly speculative. Rephrase with "On the basis of our results, we may hypothesis that ..."

We rephrased this sentence following the suggestion of the reviewer.

(10) P1061#4.3 Despite I believe that the authors are right here, this entire section is based on speculation, what if the isotope data were not accurate enough to close the budget, have you looked at the errors on f? Given that bioturbation was not measured, it seems to me too much to give it a full section in the discussion. The authors may keep their discussion but in a much more speculative framework and providing clear reference to the limit of their approach.

As criticized by the reviewer, we cannot rule out that the gap in the ¹³C-mass balance resulted partly from uncertainties in the measurement and modelling of litter-derived C fluxes. These uncertainties, however, hardly explain while the missing amount in the budget was much larger for leaf litter (~30% of initial C) than for twig litter (~10%), given that the same methods were used. We think this difference provides nice evidence that soil fauna preferred leaf litter more than twig litter. To date, information on the downward transports of litter C via soil fauna is very sparse. Therefore, we would like to keep this section in our discussion. But following the suggestion of the reviewer, we present our results and conclusions in a much more speculative framework. In the revised manuscript, we write: "To date, very few studies have made attempts to quantify downward transports of litter C via soil fauna which is a driving process for C storage especially in base-rich forest soils (Scheu, 1997). In our study, this pathway of C loss could not explicitly be measured as the isotopic label of the added litter was too small. Nevertheless, by adding the measured fluxes of litter-derived C, we can speculate on differences in the biologically mediated transport of woody and non-woody litter.

In both soils, the sum of C fluxes from the ¹³C-depleted litter and the litter recovered on the soil surface and at a depth of 0–2 cm amounted to about 90% of the added twig litter C, but only to 70% of the initial leaf litter C (Fig. 6). We cannot rule out that the gap in the ¹³C-mass balance resulted partly from uncertainties in the measurement and modelling of litter-derived C fluxes. However, the fact that much more of the twig-derived C was recovered as of the leaf-derived C, despite identical methods used, provides evidence that the export of litter via soil fauna to the mineral soil was much larger for leaf litter (~30% of initial C) than for twig litter (~10%). This assumption is supported by the mass losses from the litterbags with a mesh size of 1 mm, which excludes macro fauna. After one year, about twice as much leaf litter remained in the litterbags as in the unconfined litter on the forest floor (Fig. 6). In contrast, litterbags only slightly affected the mass loss from the twig litter. This finding is in accordance with that of Hättenschwiler et al. (1999) that restricting access of soil fauna to decomposing litter affected mass losses from beech leaves but not from spruce branchlets.

We assume that the macro fauna was less important for the transport of litter within the plots than in the native forest floor, since the soil fauna's activity might not have increased linearly with the larger availability of litter material. While about 25% of the added leaf C was recovered on the soil surface within the plots after one year of decomposition (Fig. 1), there was no leaf layer left on soils next to the plots.

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