The study tries to reveal mechanisms of changes of $\delta^{13}C$ during long term incubation of plant residues under controlled conditions, and so to evaluate the processes of isotopic discrimination. This is very important topic as many studies are based on application of various ^{13}C natural abundance approaches, and the isotopic discrimination is commonly considered, but mechanisms remains not really known. Therefore, I think that the study may have high relevance. Despite the study climes to reveal mechanisms, the most description remains as possible mechanisms, as they are not rigorously proven by the conducted experiments. Before resubmission, the isotopic discrimination by cellulose extraction should be proven, presented and considered by calculations. Additionally, many details of the incubation experiment are lacking. There fore, I suggest major revision.

[Response] We appreciate the referee recognition of the value and relevance of our work. The referee is right that this study did not provide a certain and unique mechanism by which isotopic discrimination occurs during litter decomposition. However, we ran a sound and rigorous experimental and modelling study designed to elucidate mechanisms of discrimination during decomposition, and our conclusions do significantly advance the current knowledge, despite they also open space for further experiments ... but, is this not how science advances? As reported below, we have robust evidence that our cellulose extraction method does not discriminate. Please note that all the further page and line references are those of the submitted version of the manuscript.

General comments

1. Abstract: it is not clear how it is possible that the isotopic discrimination varied between -2 and 0 (L10) and this was described well (L11), but it was necessary to consider discrimination of -1 to -4.6. As to me this is just adjustment of the model to the data with approaches which are not proven experimentally. The Abstract should be written more clearly.

[Response] From this, as from several other comments below, it emerges that our study approach has somewhat been misunderstood by the referee. We are sorry about that. As we reported in the introduction (P15 L17) we used model simulation to test our hypothesis. In fact we run three different simulations: 1) assuming no kinetic fractionation, 2) assuming kinetic fractionation only for cellulose (i.e. the intermediate pool), 3) assuming that both the fast and the slow pool were composite and formed by group of substances of differing isotopic composition. The important novel conclusion from our work is that the latter assumption appeared to be the most realistic (i.e. results from this simulation were the closest to the measured values). Indeed, to run the model simulations we had to introduce the parameters and, for our scenarios, we used realistic fractionation factors consistent with published data, as stated in the ms. We hope to have now clarified this misconception, i.e. that we did not made "just adjustment" to the model but run model simulations on the bases of stated assumptions. To better highlight this aspect, we can modify the abstract P2 L10– L11 as: "… to the variations of δ_{RL}. The three-pool model fitted previously was combined with an isotopic linear mixing model, which included the $\delta^{13}C$ of each pool. This new three-pool model described well the dynamic of $\Delta_{(L/R)}$, in the intermediate stage of the process. This suggested etc…"

2. One important shortcoming of the study that the authors described kinetic fractionation generally, means independent on time. However, when the reaction is finished the kinetic fractionation = 0 (here I accept that it is one reactant – plant residues, and one product – CO2). So, the time course of reaction should be considered and all presentations and discussions related to kinetic fractionation should be time related.

[Response] The referee is right that in a closed system, after all the substrate has reacted, no fractionation is expressed. Indeed, our experimental unit is a closed system, since we did not add substrate with time. However, at every sampling we measured the isotopic discrimination between the "accumulated product" (i.e. CO_2 respired and trapped in the jar) and the "residual substrate" (see B. Fry, 2008 "Stable Isotope Ecology" Chap.7, for a clarification on the system and term). That discrimination actually increases with time in closed system. However, because in our system, by the end of the experiment, the reaction was less than 27% complete (i.e. only 27% of the original litter C had been mineralized), the system was still in the state when it functions as an open system, and full discrimination is expressed between product and substrate. For this reason, in our opinion, the discussion of fractionation factors in our study does not need to be time related.

We have prepared a full paragraph to add to the introduction to clarify why we discuss isotopic fractionation independent of time (a paragraph which could be added at P4 L10): "Additionally, we discuss fractionation independent on the fraction of substrate reacted. This is because, in the natural environment, litter decomposition occurs as in open system, i.e. continuous inputs, and therefore isotopic discrimination is not a function of fraction of substrate reacted. Indeed, in laboratory studies, as the one reported here, litter decomposition occurs in closed system. However, they often refer to systems where less than 50% of the substrate has reacted and therefore behaving as an open system with regards to isotopic discrimination (Fry 2008)".

3. The experimental design is not clear from Sections 2.1. and 2.2. How many treatments were used? How frequently CO2 was sampled? Which controls/references were used?
[Response] We are not sure what the referee refers to here. The experimental design was set to monitor decomposition of fresh litter under controlled conditions (both in temperature and moisture). We followed the changes in litter respired CO_2 (rates and amounts), litter mass loss and their C isotopic compositions at natural abundance. Our set up has no treatments and therefore no controls. As clearly stated is Material & Methods Section (parts 2.1 and 2.2) we only worked with A. unedo leaf litter. With regards to frequency of sampling, we report in the first line of the material and method section on litter respired CO_2 measurements that we sampled at intervals that varied between 1 and 60 days. Additionally, Figure 5 reports each sampling event with respect to the day of incubation.

4. How was the fractionation by chemical extraction of cellulose tested? During the extraction of cellulose from litter and its preparation for d13C measurement 13C discrimination is very probable. This is common by the most extraction and fractionation approaches. It is not presented anywhere, how strong was this discrimination.
[Response] Indeed, the referee is right and ^{13}C discrimination may occur during cellulose extraction. Different cellulose extraction protocols are commonly used in dendroecology studies (Rinne et al. Chemical Geology 222 (2005) 75– 8; Endurlat et al., Tree Physiology 30 (2010), 1515–1527, among others), for which the actual value of $\delta^{13}C$ of α-cellulose is very important with regards to photosynthetic discrimination and for past climate reconstruction. In the majority of these studies, however, the assessment of the isotopic discrimination of cellulose due to the extraction protocol is not performed (for example by using commercial/reference cellulose material), as most of these studies purified the samples until reaching α-cellulose. This is a well know problem, and to overcome it, several methodology studies have been conducted recently. The work of Boettger et al. (Analytical Chemistry 79 (2007), 4603-4612) has showed that the method of Sohn & Reiff (method #1: 7% $NaClO_2$ at 60°C, 5% NaOH at 50°C), which was similar to ours, did not induce significant biases in $\delta^{13}C$ of commercial, reference cellulose material. Likely, Wissel et al. (Organic Geochemistry

39 (2008), 1545–1561) compared three different cellulose isolation methods on aquatic plants and freshwater sediments. One of these methods (JUEL) was very close to ours. The authors applied also the three methods on reference cellulose powders (Fluka, Avicel and IAEA-C3). They fund small but insignificant changes in $\delta^{13}C$ of the reference powders, whatever the three methods which were shown also to give very similar results. And finally, we would like to highlight a specific point: under the hypothesis that our extraction method did not purify completely the α-cellulose, ours samples should have a significant amount of either lignin or various carbohydrates inducing both heterogeneity among replicates and a $\delta^{13}C$ shift in accordance with the "polluting" compound. Hence, this hypothesis would not explain the evolution of the $\delta^{13}C$ of the extracted cellulose, as it increased with time, while the more decomposed samples should be relatively lignin-enriched (which is generally ^{13}C-depleted with regard to cellulose, as indicated in P16 L20-29). Moreover, the standard errors values of $\delta^{13}C$ of extracted cellulose were always under 1% of the mean (P12 L5-8), showing their homogeneity among five replicates.

We are sorry that we did not report before this consideration in our ms, and we are thankful to the referee for spotting it. To clarify this important point we have prepared a full paragraph to add (which would start at P17 L13) to the discussion, and which presents this response (see also the response to the other referee).

5. The study was done with litter alone, means without soil. I guess that many processes including litter decomposition would be different (rates and directions of fluxes) in the presence of soil. As well as I understood, no control with soil was considered in experimental design.

[Response] We currently lack an understanding of isotope discrimination during decomposition of plant litter, and this is largely due to the complexity and composite nature of the substrate. We believe that we first need to improve our mechanistic understanding of isotope dynamics during the leaf litter mineralization process, before we can move to the next level, i.e. study it in the field. In fact, we agree with the referee that adding soil to our experimental units would have introduced a much larger range of C compound complexity. We chose incubating fresh litter only, according to Fernandez et al. (2003, see references in the text), in order to prevent this over-complication (that we would not have been able to investigate with only one tracer) in C compounds. This complexity needs to be investigated apart, as did Blagodatskaya et al. (2010). For this reason, we considered our Arbutus litter as a model litter substrate, which should not be compared with soil organic matter, as we indicated in the Abstract (L3).

6. In section 2.6. Statistical Analyses it should be mentioned how many variables were fitted in total, and how many measurements were done. As to me it looks suspicious if a three pool model will be fitted – means 6 parameters and if each pool has own isotopic fractionation – additionally at least 3 parameters of isotopic discrimination (may be some other; depending on the controls and references) are fitted. So, what is the interdependence of the many parameters by the fitting?

[Response] The Eq. 2 comprised indeed 6 parameters (two per each C pool). However, only two f_i parameters were fitted, the third one being deduced following f1+f2+f3=1. This has now been clearly stated in the Material & Method section. As for all fitted models, the parameters are never completely independent from one another. The AIC criterion (P5 L212) was calculated, and used to indicate the usefulness of adding one C pool, i.e. adding virtually two parameters. In a revised version of the ms, to clarify this point, we added the number of fitted parameters.

On the other hand, as explained above and below, the three isotopic fractionation parameters were not "fitted" but set accordingly to either measured values (e.g. respired CO_2, α-cellulose, remaining litter) or values derived from the literature, to make different simulation scenarios. And we amply discussed the hypotheses on which such scenarios were based.

7. I think that the authors are not aware about the different meaning between 'simulation' and 'fitting'. I have not find any simulations in the ms. All the lines calculated are based on a simple fitting of exponential curves on the measured points. This means fitting.
[Response] We do are aware of the difference between fitting and simulation. Indeed, the litter decomposition dynamics investigation part is based on curve fitting (Eq. 4). However, in section 3.3.2 'Model simulation' (P12 L9-14, See Figure 7) we investigated the possibility to explain the temporal change in measured $\delta^{13}C$ of respired CO_2. For doing this, we used a model of which some parameters were already fitted (from Eq. 4) and others (=the isotopic parameters of Eq. 5 and 7) were set (and no fitted) according to measurements (i.e. of intermediate pool) or derived from literature. These different model outputs (simulations), presented in Fig. 7, correspond to different fractionation scenarios and we use the correspondence between them and the measured dynamics as a way to test our hypotheses.

8. When the authors try to separate two processes, it should be clarified in the whole text, where they really measured discrimination, where apparent discrimination and where different utilization.
[Response] We agree with the referee that this is confusing and therefore in the text we always refer to the specific process involved (e.g. microbial fractionation or preferential substrate use). As we stated in the text (P4 L8-9) in this work, 'discrimination' refers to any difference between the C substrate (leaf litter) and the product of its mineralization (CO_2).

9. 3.3.2. 'Model simulations' It is obvious that fitting the 3 component curve on such a dynamics will lead to very good correspondence between measured and fitted data. However, the disagreement between measured and fitter d13C values clearly shows that the suggested 3 pool model does not well properly.
[Response] As explained in response to the comment #6, we compared model outputs (Eq. 7) with measurements. The $\delta^{13}C$ of respired CO_2 were not modelled by only fitting, but by combining curve fitting and linear isotopic mixing. The different simulations (P12 L9-14) showed the successive assumptions we needed to add for matching the measurements (Simulation #3, Fig. 7).

10. It was necessary to explain the isotopic composition of CO2 by addition of isotopic discrimination to the fast and slow pools. Is it possible to get the same results by addition of isotopic discrimination to the intermediate pool only? If the intermediate pool contribute at the start and at the end to the CO2, then it should be possible.
[Response] What the referee points out (i.e. adding isotopic discrimination of intermediate pool only) actually corresponded to the simulation #2 (δ_{RLSim2}, P13 L2-3, Fig.7). We used the change in $\delta^{13}C$ of measured α-cellulose (which we identify as to be consistent with the intermediate pool) and lead to the greatest discrepancy with regard to the measured $\delta^{13}C$ of respired CO_2. This result suggested us to perform the simulation #3 as presented at P13 and hereafter.

11. The background of the paper is based on the model 3 pools decomposing exponentially; and the authors assume that this is the only possibility. However, it is possible that

decomposition don't follow the classical 1st order kinetics. Microorganisms decomposing the litter are completely disregarded by the 3 pool approach. It is obvious that the described isotopic discrimination between the litter and CO2 occurs not in one step, but with the 'help' of microorganisms. Therefore, the isotopic discrimination may follow other rules as suggested by the 3 pools model.

[Response] We agree with the referee, the micro-organism effect may certainly need to be more explicitly formulated with regards to the time scale and the considered process. It depends on which degree of complexity we want to describe our system and for which purpose. In our study, we wanted to test one of the two most frequently invoked hypotheses explaining isotopic discrimination between litter and respired CO2, i.e. preferential use of C substrate. Our study led to the conclusion that temporal variations of $\delta^{13}C$ of respired CO_2 could be better explained by this preferential use. Despite this result, our data do not completely exclude microbial fractionation: We showed and discussed that some C isotopic fractionation during cellulose decomposition probably occurred and this fractionation can potentially be attributed to microbial fractionation (P18 L1-18). However, this fractionation (simulation 2) cannot explain the observed dynamics. Moreover, we indicated at P15 L21 that the change in the discrimination factor a_i of the labile pool could reflect a change in the use of a variety of labile compounds by the micro-organisms or being the value of $\delta^{13}C$ of microbial biomass itself. The magnitude and impact of direct microbial fractionation on a given substrate is still under debate (see the references in the manuscript).

12. There are too many assumptions for the model (number of pools, their d13C values, etc.). As the authors have not proven it experimentally, the relevance of these assumptions is very weak; with consequences for conclusions of the study.

[Response] We agree that we had to formulate several assumptions for reaching the model outputs (Simulation #3, Fig. 7). However, the number of C pools is not outstanding, nor are the $\delta^{13}C$ values used. In fact, a 3–pool litter decomposition dynamics was recently supported by LIDET, a word wide litter decomposition study (Adair EC et al. (2008) Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Global Change Biology 14:2636-2660), and the values of our model parameters were either measured or derived from the literature. The only arbitrary assumption is with regards to the fractionations factors (linear effect) applied in the model simulations, and anyway they were simulating very likely scenarios of isotopic fractionations.

Our aim was not to build a novel model, but simply by an empirical approach to constrain temporal variations of isotopic data by the $\delta^{13}C$ of the potential C pools. On the other hand, we clearly stated several times (P15 L19-20; P16L18-19; P18 L19-21) that the C isotopic compositions (and their discrimination) of the different potential pools at natural abundance should be more documented (which is still limited), in order to build an isotopic-based decomposition model, in the future.

13. The Discussion should be split in 2-3 sections each with clear focus. May be these sections should be focuses on the 3 fractionation possibilities suggested in the last section.

[Response] We thank the referee for this suggestion and we intend to separate the discussion in three sections, with them being related to the three objectives of our study, as mentioned at the end of the introduction.

14. Generally I agree with the three possibilities for d13C changes during incubation (P18). However, in my view not any of these possibilities were rigorously proven in the study.

[Response] We agree with the referee that the mechanism(s) responsible of the isotopic fractionation of cellulose was not proven in our study. This topic requires heavy microbial,

biochemical and molecular biology tools (medium growth, PLFA, DNA-SIP, etc…). But we acknowledged that this topic represents anyway a challenge for future works (P18 L19-21). However, our work did prove, by a combination of rigorous experimental and modelling work: 1) that there was a discrimination during litter decomposition, 2) that microbial fractionation of individual compounds alone cannot explain it, 3) that selective substrate use is the process most heavily involved, but that specific substrates consumption have to be followed, and lumping them in three pools (on the basis of their decay rates) is not enough to account for isotopic discrimination.

Other remarks

15. P2 L2 Decomposition in soil or without soil?
L7 during which period?
L8 probably fitted instead of simulated
L8 the isotopic composition cannot be higher; the d13C value can be higher
[Response] L2: Without soil, we clarified now by saying "leaf litter"
L7: Over a 1-year period (added in abstract)
L8: The referee is right, in that case "modelled" should be used
L8: we changed to "The ^{13}C relative abundance"

16. P3 L1-5 these 3 options are only a small part of the possible isotopic applications in soil C studies. So, it should be deleted or the really whole range should be presented.
[Response] We agree that these examples are a small part of the possible applications in soil C studies. But we intended to give only example of uses as tracer in particular for investigating the processes implied in soil CO_2 fluxes at the seasonal and year scale. We added "for example" to the text.

17. P3 Usually isotopic discrimination is considered by linear mixing models by correction of values of the end members. This will be done in the most studies.
[Response] We are not sure what the referee's point is here. Indeed, when fractionation factors are known, as for example in food webs, the trophic enrichment is added to the end members (or subtracted from the mixture) in food sourcing mixing models. We do not yet have a fractionation factor for litter decomposition, or even for C mineralization, and this study actually aims to clarify if we need one to account for when applying mixing models using litter isotopes.

18. P4 top It should be considered however, that if the processes are complete (decomposition is finished) there is no discrimination. So, the further conclusions are relevant only for partial decomposition.
[Response] Yes the referee is right. See our response to general comments #2.

19. P4 L11 and L13 : : : delete heterotrophic. The respiration is all times heterotrophic; therefore, it is not correct to link respiration and heterotrophic in one phrase. Correct at all places in text
[Response] Done

20. P5 L8 It cannot be different at any time: If the mass balance low is correct (and this is a fundamental rule) then it should be at least one time period during decomposition when d13C of litter is = d13C of CO2.

[Response] We are not actually sure what the referee refers to here. In systems where fractionation occurs, at any given time during the reaction the product has a different isotopic composition from the substrate, but when all the substrate reacted the accumulated product has the same isotopic signal of the initial substrate. Since we refer to the difference between the litter substrate and the CO_2 at any given time, we believe that our sentence is correct and does not violate the law of the mass balance.

21. P5 L18 further P6 L8 this is not clear: if the moisture is > 100% of gravimetric content, then it will be a puddle of water at the bottom of the jar.
[Response] This would be correct if considering volumetric water content. Gravimetric water content is defined as the ratio in a given sample between its mass of water divided by its dry mass. A porous medium such as strongly organic soil (low density) or organic leaf litter may content a heavier amount (and not higher volume) of water than its dry mass.

22. P8 Section 2.4. P8 L21 what are C6 and C8?
[Response] Those are IAEA references materials (P8 L23-24): C6 (sucrose) and C8 (oxalic acid) were chosen in addition to the internal standard (P8 L23) in order to have a 3-point calibration for our IRMS. We added theses characteristics of the standards in the Material & Methods section.

23. P9 L11 not clear what are the 10 variables? This was not mentioned before.
[Response] See L12: please note that "ten" referred to "replicates" and not "variables". We specified at this point of the manuscript the number of replicates used for calculating daily means: ten replicates for the CO_2 variables (respiration rate, CO_2 concentration, $\delta^{13}C$), five replicates for litter material (mass, $\delta^{13}C$, cellulose).

24. P10 L17 ANOVA analyses sounds not well
[Response] We tested the date effect as independent variable on $\delta^{13}C$ (of respired CO_2, of remaining litter, of cellulose), our dependent variable. There are no other independent variables nor missing points, so one-way ANOVA, to our knowledge is the appropriate test to use.

25. P11 L4 How the incubation days under controlled conditions were recalculated for years?
[Response] The decomposition experiment was conducted in closed mode: the CO_2 concentration calculated each date represents the total respired CO_2 since the previous measurement date. We just cumulated the CO_2 amount over the experiment duration (371 days and re-ajusted the mean value for a duration of 365 days.

26. P11 L19 As well as I understood, the incubation was in closed jars. How leaching can occur is not clear.
[Response] Fractionation and leaching are two important processes that contribute to above ground litter mass loss in nature. Here we simply say that in our experiment mass loss was well accounted for by the respiration measurements, as expected since none of the other two processes could have occurred. In fact, we sprinkled the litter sample with deionised water very carefully and gently at the beginning in order to not provoke a water flow (and thus leaching).When re-ajusting the gravimetric water during the experiment, a very small amount of water was added (several µL). The jars were, of course, closed at the bottom to avoid any losses of litter fragments or DOC.

27. P11 L19 What kind of two sets? It was nothing written in M&M about.

[Response] See the Material & Methods section, P6, L10-13.

28. P14 L4 here and at other places: please use depleted (or enriched) when the product is compared to the substrate. Not on the reverse way.
[Response] Done

29. P17 L24 This is surely not correct. There were studies reported d13C changes of CO2 during incubation of plant residues, and this by incubation with soil. Also the other studies already referred in the ms, should be analyzed more carefully.
[Response] We checked the bibliography we cited in our manuscript and none of them showed a ^{13}C change for cellulose isotopic composition during decomposition. We changed the sentence by referring only to "changes of $\delta^{13}C$ of litter cellulose during in vitro incubation". If the referee could provide us a reference, we will be glad to add it and correct our statement.

30. Fig 3 About one third of the points show the deviation from the 1:1 line more than 10%. It means that the measurement error is comparatively high. How to explain it? Are the points means of 5 replicates or individual measurements?
[Response] We stated in the "2.6 Calculation and statistical analysis" section that all variables were expressed as daily means (we found a typo error, thanks!) with either ten (CO_2 variables) or five (bulk litter material) replicates (P9 L12-13). But for better clarity, we now specify in the Results (Section 3.2) and in the figure legend that values refer to means.

31. Fig 4 Cellulose content where? The temporal variation of isotopic content is not presented here, as there are only 2 points.
[Response] The cellulose content is in open circles, as indicated in the figure legend. Concerning the temporal variation of $\delta^{13}C$ of cellulose there are indeed 2 mean values in this figure. It is for this reason, as clearly stated, that we assumed a linear interpolation between these two measurements (i.e. constant kinetic fractionation, P12 L28) for incorporating this variation into Eq. (5). We are aware that this is not the ideal way but we could not do any other way since we had not extracted the cellulose at intermediate samplings (lack of funding).

32. Fig 4 The change of d13C of cellulose is about 2 %o when about 25-30% of cellulose was decomposed. It means that the d13C of endmembers in the cellulose (I am not sure what it can be), is about 6-8%o. Is it possible? I think it is impossible.
[Response] We are sorry but we are not sure what the referee refers to here. What would be the end-members, in which system? The final cellulose and the d13C emitted from the cellulose, with the initial cellulose representing the mixture? Still we do not see the point the referee is making. We did simulate $\delta^{13}C$-CO_2 evolution from litter assuming a 2–‰– fractionation for the cellulose pool (simulation 2), and the simulation showed that values would have been more enriched than observed, by only 1‰ to 3‰ (see figure 7).

32. Fig 5 Start with the upper panel. The error bars of the difference should be calculated and presented.
[Response] Done

34. Fig 6 The regression line here depends on two points only. All other points are clumped together and no trend in obvious there. How the R2 was calculated: based on means or on individual replications?

[Response] We agree with the referee that the 2 lower points strengthen the correlation. But, these two points are not outliers thus not an artefact of the relationship (as they represent the measurements of two latest dates, which were done at a longer time interval with respect to the other points). Thus there is no reason to discard these two points. However, when removing these two points, the cloud displays a trend for a positive relationship (R^2 = 0.23, P = 0.08). We added in the legend that the points represent mean values (see response to comment #30).

35. Fig 7 It is not clear how the discrimination factors were estimated.
[Response] We did specify that these discrimination factors were found through a sensitivity analysis (P13 L9-14).We added in the figure legend a reference to the text. The sensibility of the model was simply assessed by attributing different values of either a_1 or a_2 for all dates (the other parameters being fixed). By cross-comparing the model outputs, we fund these a_1 and a_2 values which allow the best match with the measurements.