1. Sometimes you have what seems like a good idea for an investigation but in the end it turns out that the experiment did not work as expected. I'm afraid that is the case with the experiment in this manuscript. The final concluding result, Figure 7, shows that the statement in the title is not correct. What is needed to explain the observed isotopic signature of the evolved CO2 are supplementary hypotheses about time varying discrimination factors. The problem with these factors is that they are entirely arbitrary and without coupling to biological phenomena. As a result the analysis degrades to an exercise in curve fitting without explanatory power.

[*Response*] *We can see the point of the referee, but like to argue against it. To us an experiment did not work, if it was not carried out correctly, and we believe that was not the case of our experiment. A different thing is when an experiment produces results which are different from expected. These are often the most interesting experiments that contribute to formulation of opening questions and design of further experiments. Indeed, this experiment opened a few new questions that will require different experimental designs to be investigated (and we suggest in the text future research directions, as we mentioned below), however the work performed contributed to advance the current understanding of isotopic discrimination during litter decomposition. We ran 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). Starting from a conceptual model where two separated processes could explain the isotopic discrimination during litter respiration (i.e. microbial fractionation vs. preferential use of C substrate), our study moved to a different conceptual model where both processes could occur, at different levels, but the preferential use of C substrate appears to control the isotopic fractionation dynamics. Our assumptions were all very reasonable as we explain it the text: the "arbitrary" factors were anyway set and constrained in a realistic range of published values of the relevant C pool and compounds (form P15 L21 to P16 L29 of the submitted manuscript). More generally, our work was more focused on the mechanisms and not on the factors that would most likely change among different plant species and litter chemical and isotopic composition. It is also worth noticing that this is among the first experiments where both the isotope composition of the remaining litter and the* $^{13}CO_2$ *were measured over time.*

Finally, in order to better stick to the referee's comment, we can rephrase the title to "Temporal changes in stable carbon isotope discrimination during leaf litter respiration: Effects of preferential use of different carbon substrates and kinetic fractionation".

2. As a further indication of the problematic interpretation of the results is the shift in isotopic composition of a-cellulose (Figure 4). a-cellulose should be a well-defined chemical component and yet it changes strongly in isotopic composition during the experiment. It is not clear if this is a result of the extraction procedure that extracts not just a-cellulose but different compounds at different times or if it is a result of differential use of C isotopes in a-cellulose. If the latter is the case, then it might not be the selective use of substrates but the selective use of isotopes of substrates that explains the observations.

[*Response*] *We agree with the referee on the necessity to clarify this important topic, since the* α*-cellulose issue was also the object of comments from another anonymous referee. Please refer to our response to that comment for a complete discussion of possible fractionation during extraction of cellulose. We have prepared an entire paragraph to add to the discussion in order to clarify this issue (which would start at P17 L13): "Several methodology studies have been conducted recently of fractionation during cellulose extraction. The work of*

Boettger et al. (Analytical Chemistry 79 (2007), 4603-4612) showed that the method of Sohn & Reiff (1946) (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 and al. (Organic Geochemistry 39 (2008), 1545–1561) compared three different cellulose extraction 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 might 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".

3. However, even an experiment that produces results contrary to the original hypotheses may be useful if the deviations can be explained and provides help to others doing similar experiments. At the end the authors indicate some ideas of that kind but I do not think they go far enough to motivate the publication of this manuscript. I have another concern about the value of this manuscript. How could the result from this study be extrapolated to much longer time scales such that the result could be applied to the entire C pool in the soil?
[*Response*] *As we argued above, indeed we could make sense of the observed deviations (with the exception of the cellulose enrichment) and provided future direction of work. Following the referee's suggestion we have prepared a paragraph to add to the discussion to further highlight future research directions and approaches. This paragraph could be added at P17 L5, such as: "Future studies coupling ^{13}C–NMR and/or compound specific analyses of litter residues at different stages of decomposition with mass loss and respiration and ^{13}C measurements need to be performed to better clarify the relative contribution of different compounds through time to mass loss and ^{13}C-CO_2, as well as the possible formation of new macromolecular structures in decomposing litter (Preston et al., 2009) and their effects on isotopic dynamics".*

Finally, the ambition of his works was not to enable an extrapolation of the results to the entire C pool in the soil, as we clearly stated. However, we pointed out an approach, i.e. coupling multiple pool decay models with isotopic signals of pools, to start explaining isotopic dynamics, and moving further to compound specific isotope work of specific SOC pools when the first does not succeed. It's only a step but we believe this work is an important step forward.