

Response to Anonymous Referee #1

Comment: “the conclusion about isotopic difference, which is the main focus of this manuscript is based on a difference <0.5 per mil. – This is within the instrumental error!”

Response:

We highly appreciate the referee’s evaluation of our manuscript and his/her comments helped a lot in improving the paper by describing some methodological details more precisely. Most of all, we thank the referee for his/her critical questioning regarding the results because this triggered the inclusion of new sentences in the manuscript and changes in the original text to make clear that the value of the instrumental error of the isotopic mass spectrometer used in our study is significantly lower than the isotopic differences described in the paper between both eucalyptus clones. In fact, the precision of the Deltaplus (FinniganMat, Bremen, Germany) isotopic mass spectrometer used for these $\delta^{13}\text{C}$ analyses, i.e., the value of one standard deviation “ $1\sigma_{10}$ ” for the analysis of $\delta^{13}\text{C}$ of the laboratory standard “acetanilide” based on 10 measurements “ $n=10$ ” was $\pm 0.102\text{‰}$, which is quite similar to the instrument precision reported by other authors for $\delta^{13}\text{C}$ values using comparable equipments in recent times (King et al., 2012: $1\sigma=\pm 0.09\text{‰}$; Brodie et al, 2011: $1\sigma=\pm 0.1\text{‰}$; Gavrichkova et al., 2011: $1\sigma=\pm 0.05\text{‰}$) or even back to the nineties (Barrie and Prosser, 1996: $1\sigma=0.05\text{--}0.1\text{‰}$). Therefore, from the above cited precision value of our study it can be straightforwardly recognized that the isotopic difference of 0.4‰ found between the litter from Anselmo and Odiel clones clearly surpassed by more than three times the value of our instrumental error ($1\sigma_{10}=\pm 0.102\text{‰}$).

Moreover, the previously mentioned precision of our isotopic measurements ($1\sigma_{10}=\pm 0.102\text{‰}$), combined with the small variability found between samples collected from the diverse ecosystems with the same eucalyptus clone (the 3 forest plantations of the same type exhibited standard deviation values between 0.1 and 0.3, Table 1), resulted in statistically significant differences between the isotopic composition of both types of litter, as it can be verified by analyzing the variance of the $\delta^{13}\text{C}$ data given in the right column of Table 1 for Anselmo or Odiel clones growing over granitic bedrocks (ANOVA, $P<0.046$). As a result of the referee’s concern on the true extent of the instrumental error, all these detailed precision data were included in the new version of the manuscript to explicitly explain the fact that the isotopic difference found between the litter collected from both types of clone plantations surpasses by far the instrument precision, defined as the standard deviation of replicate analysis of standard reference materials.

Former text (Material and methods, section “2.3.3 Isotopic analysis (^{13}C)”, page 2829, line 8): “Litter samples were oven-dried (40°C) and finely ground ($<100\ \mu\text{m}$) for isotopic analysis. To show possible isotopic changes during the biodegradation process, at the beginning ($T_0=0$ days), in the middle ($T_1=36$, $T_2=98$, $T_3=359$ days) and at the end ($T_4=560$ days) of the incubation period, the litter $^{13}\text{C}/^{12}\text{C}$ ratio was determined. The ^{13}C natural abundance was assessed using a ThermoFinnigan DeltaPlus isotope-ratio mass spectrometer coupled to a Flash EA 1112 elemental analyser.”

Adapted text: “To show possible isotopic changes during the biodegradation process, at the beginning ($T_0=0$ days), during ongoing ($T_1=36$, $T_2=98$, $T_3=359$ days), and at the end ($T_4=560$ days) of the incubation period, the litter $^{13}\text{C}/^{12}\text{C}$ ratio was determined. Litter samples were oven-dried (40°C), finely ground ($<100\ \mu\text{m}$) and weighed in tin capsules for isotopic analysis. The ^{13}C natural abundance was assessed by continuous flow isotope-ratio mass spectrometry using a ThermoFinnigan Delta^{plus} mass spectrometer coupled to a FlashEA1112 elemental analyzer through a ConFlo II interface. As part of each analytical batch run, a set of international reference materials (NBS 22, IAEA-CH-6, USGS 24) were analysed and, all through the successive isotopic determinations, the precision (standard deviation) for the analysis of $\delta^{13}\text{C}$ of the laboratory standard (acetanilide, $n=10$) was lower than $\pm 0.102\text{‰}$.”

Comment: “Carbon mineralisation of litter from both clones was similar and therefore it is questionable that this small difference in isotopic composition of evolved CO₂ at the beginning of the incubation has any significance for biogeochemical C cycling.”

Response:

We are not sure we understand this comment. Although in this remark the referee indicates that the “carbon mineralisation of litter from both clones was similar”, when comparing the values of the C mineralization activity for Anselmo and Odiel clonal plantations developed over the same type of parent material (granitic bedrock) included in Table 2, it is discernible that the averaged values presented by Odiel plantations (150.3 g C kg⁻¹_{d.m.} and 29.2% of the total litter C content) exceeds by more than 20% the averaged values exhibited by Anselmo plantations (113.4 g C kg⁻¹_{d.m.} and 22.9% of the total litter C content), these differences being statistically significant for both mineralization indices: the total quantity of C mineralized (P<0.042) and the C mineralization coefficient (P<0.040).

Regarding the comment on the “small difference in isotopic composition”, it is true that the isotopic dissimilarity is not very big as compared with inter-specific variations, which can be of more than 1 delta (e.g. differences of 1.6‰ between *Pinus pinaster* and *Pinus sylvestris* from the same region were found by Fernandez et al., 2005). However, the escalating needs for lowering the uncertainties of existing models of the global C cycle must be also taken into account in order to obtain increasingly accurate estimations. In fact, one of the greatest advantages of modern technologies, such as the use of stable heavy isotopes (¹³C) to trace C fluxes during diverse chemical or biological processes, is that the precision of these new techniques usually allow the quick and early quantification of some ecological differences that are hardly detectable by using other less accurate methods, the notable efficacy of isotopic monitoring (¹³C) for organic matter studies in different ecosystem compartments as well as for the correct and detailed understanding of the C cycle biofunctioning in a variety of environments being widely recognized (Bernoux et al, 1998; Boutton et al, 1998; Griffiths et al, 1999; Preston et al, 2006; Gavrishkova et al., 2011). Moreover, given that nowadays a very large extension of land is dedicated to the cultivation of *Eucalyptus globulus* Labill, not only in the Iberian peninsula (around 1.2·10⁶ ha) but also worldwide (more than 2.3·10⁶ ha), as well as the serious direct implications (at both economic and ecological levels) that could be clearly associated with possible future changes in silvicultural management practices applied to this type of forests, the anticipated scientific demonstration of either the absence (which would also be an interesting outcome, worthy of publication) or, even with more consequences, the existence of any (big or small) impact on the biosphere-atmosphere interactions (and on the corresponding total or partial C transfers that takes place between them) caused by the likely introduction of the new clonal varieties at a global scale (e.g. the eucalyptus clones of F0 or F1 generation included in the present article: "Anselmo" or "Odiel") seems to acquire an increasingly significance with specific influences on the biogeochemical C cycling from a forestry point of view and, due to the extent of these potential implications, it can be considered as extremely important not only for the regional communities involved but also for the society as a whole.

Comment: “Moreover, the authors consider that aboveground litter fall is controlling SOM built up. This may however be far from accurate, as belowground plant litter may be much more important.”

Response:

We absolutely endorse the importance of belowground plant litter and in no way it is intended to compare aboveground and belowground contributions to the SOM built up in any part of this article. Although we do not know from where in the present manuscript it can be deduced the previously mentioned interpretation of the anonymous referee #1 (and we would be very grateful if an indication of the concrete sentence or sentences leading to such deduction is provided), we accordingly tried to specify our hypothesis more explicitly by adding or changing some expressions in two different subdivisions of the paper: in both the “Introduction” and in the “Conclusions” sections. As indicated below:

Former text (Introduction, page 2825, line 16): “Soil OM characteristics in forest ecosystems mainly depend on the vegetal debris coming from the dominant tree species, and for this reason the study of their biochemical characteristics and mineralization kinetics is essential when we want to know the present and future status of nutrient uptake and cycling in a forest ecosystem.”

Adapted text: “Soil OM characteristics in forest ecosystems mainly depend on organic debris (including aboveground and belowground vegetal residues) largely coming from the dominant tree species, and for this reason the study of their biochemical characteristics and mineralization kinetics is essential when we want to know the present and future status of nutrient uptake and cycling in a forest ecosystem.”

Former text (Conclusions, page 2835, line 16): “Even if more detailed research that would involve the study of the biochemical and isotopic composition of live leaves would be convenient, our findings on the isotopic behaviour during the decomposition of debris coming from Galician forest plantations (NW Spain) with two different *E. globulus* clones seem to reveal the existence of possible differences between both eucalyptus clonal plants at photosynthetic levels, affecting their internal chemistry and therefore the C dynamics of decaying litter.”

Adapted text: “Even if more detailed research that would involve the study of the biochemical and isotopic composition of live leaves as well as of other belowground organic inputs directly entering into the underneath soil layers would be convenient, our findings on the isotopic behaviour during the decomposition of debris coming from the duff layer of Galician forest plantations (NW Spain) with two different *E. globulus* clones seem to reveal the existence of possible differences between both eucalyptus clonal plants at photosynthetic levels, affecting their internal chemistry and therefore the C dynamics of decaying litter.”

Comment: “Moreover, litter decomposition was assessed under complete artificial conditions (in the laboratory, without soil and plants). Therefore it is highly questionable is such an incubation will yield any valuable information about degradation in the field, which the authors claim to be important knowledge gap. The degradation behaviour in the field may be better assessed by a litterbag study.”

Response:

We totally agree with the statement of the referee indicating that litter decomposition assessed in the laboratory under artificially controlled conditions will not give the same results than experiments oriented to investigate litter degradation in field conditions that will depend not only on the peculiar characteristics of the litter but also on other particular environmental or ecological factors. However, precisely for that same reason, to study the possible differences in the potential biodegradability of litter from two clonal varieties of the same tree species (in this case *Eucalyptus globulus* Labill.: Anselmo or Odiel clones) and to avoid the unquestionable influences of many external environmental variables that may mask the results on their potential biodegradability it is necessary to accurately control every system setting that may affect the activity of decomposing microorganisms so that the biodegradative process can be basically maintained under equivalent laboratory conditions (temperature: 28°C, moisture content: 80%) for all litter samples (collected from the different locations) in order to be perfectly comparable and to put side by side the potential biodegradability of eucalyptus litter from both types of clone plantations.

The article’s title was also modified (as it was suggested by the anonymous referee #2 too) so as to reflect the research conducted in a simpler and more straightforward way as well as with the purpose of preventing any possible misleading expectation concerning the evaluation of litter decay in the field or under natural environmental conditions, which was never the objective of the experiences described in this manuscript. Thus, the new proposed title is: “Potential biodegradability of eucalyptus litter from northwestern Spanish forests planted with a different clone: F0 or F1 generation”

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