Stable isotope ratio ($^{13}C/^{12}C$) mass spectrometry to evaluate carbon sources and sinks: changes and trends during the decomposition of vegetal debris from eucalyptus clone plantations (NW Spain)

New title "Potential biodegradability of eucalyptus litter from northwestern Spanish forests planted with a different clone: F0 or F1 generation"

I. Fernandez[1], and A. Cabaneiro[1]

[1]{Instituto de Investigaciones Agrobiológicas de Galicia, Consejo Superior de Investigaciones Científicas IIAG-CSIC, Santiago de Compostela, Spain}

Correspondence to: I. Fernandez (ifernandez@iiag.csic.es)

Abstract

Vegetal debris is known to participate in key soil processes such as the formation of soil organic matter (OM), also being a potential source of greenhouse gases to the atmosphere. However, its contribution to the isotopic composition of both the soil OM and the atmospheric carbon dioxide is not clear yet. Hence, the main objective of the present research is to understand the isotopic ^{13}C changes and trends that take place during the successive biodegradative stages of decomposing soil organic inputs. By incubating bulk plant tissues for several months under laboratory controlled conditions, the kinetics of the CO_2 releases and shifts in the ^{13}C natural abundance of the solid residues were investigated using litter samples coming from forest plantations with a different clone (Anselmo: F0, 1st clonal generation attained by morphological selection and Odiel: F1, 2nd clonal generation genetically obtained) of *Eucalyptus globulus* Labill. developed over granitic or schistic bedrocks and located in northwestern Spain. Significant isotopic variations with time were observed, probably due to the isotopically heterogeneous composition of these complex substrates in conjunction with the initial selective consumption of more easily degradable ^{13}C-differentiated compounds during the first stages of the biodegradation, while less available or recalcitrant litter components were decomposed at later stages of biodegradation, generating products that have their own specific isotopic signatures. These results, which significantly differ depending on

the type of clone, suggest that caution must be exercised when interpreting carbon isotope studies (at natural abundance levels) since perturbations associated with the quality or chemical composition of the organic debris from different terrestrial ecosystems can have an important effect on the carbon stable isotope dynamics.

1 Introduction

Since *Eucalyptus globulus* Labill. is an allochthonous species in Galicia (NW Spain), with characteristics that certainly differs from those of the autochthonous flora, its massive utilization generated a big controversy about its environmental repercussion, especially on the soil quality, that are not still totally elucidated. As a consequence of this concern, some investigations related to this type of plantations within the humid-temperate zone have been carried out (Jones et al., 1999; Álvarez González et al., 2005; Vega-Nieva et al., 2013), some of them focussing on the effect of this type of vegetation on the soil (Calvo de Anta, 1992; Brañas et al., 2000; Álvarez et al., 2002; Camps Arbestain et al., 2004). These studies are of exceptional interest in Galicia, where forest soils are usually acid and sandy although very rich in organic matter (OM), which content and quality practically determines the soil productivity. Given that the success of a sustainable silviculture is mainly based in effective recycling of nutrients, the role of the soil OM that controls cation and water reservoirs is very important for forest plantations with a longer rotation cycle as compared to agricultural soils with annual crops (González-Prieto et al., 1996; Mutabaruka et al., 2002; Matus et al., 2008). 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.

The notable extension of forest surface dedicated to *E. globulus* silviculture, joined to the most recent tendency to use clonal eucalyptus plants oriented to increase wood productivity supports the interest of investigations that point to evaluating the environmental influence of these forest plantations as well as the role of *E. globulus* debris as a C sink/source during its progressive incorporation into the soil.

Heavy stable isotopes have been frequently used to trace C-flow through the plant-soil system (Van Dam et al., 1997; Fernandez et al., 2006a, 2006b) since isotopic techniques applied to diverse research fields can provide an integrated and quantitative view of chemical, biological and ecological transformations in nature (Boutton et al., 1998; Griffiths et al., 1999). Due to the precision and efficiency of these techniques many studies use the ^{13}C stable isotope to monitor the C cycle in different biochemical processes, such as photosynthetic fixation of atmospheric CO_2, decomposition of complex plant debris, etc (Schleser et al., 1999; Fernández and Cadisch, 2003; Fernández et al., 2003, 2004, 2006a, 2006b).

Therefore, the purpose of this research is to use stable isotope ratio ($^{13}C/^{12}C$) mass spectrometry to obtain direct and updated information about litter decay in *E. globulus* clone plantations from the NW of Spain in order to attain a double objective: i) to elucidate the possible differences between the biodegradability of litter from two different eucalyptus clones and, ii) to evaluate the half-lives or residence times of litter C in this type of ecosystems, whose potential remains unknown for this climatic zone.

2 Material and methods

2.1 Study area and experimental design

A laboratory controlled experience about vegetal debris decomposition was carried out using litter samples collected from a total of 9 eucalyptus clone plantations (*E. globulus*) in the SW of Europe (Galicia, NW of Spain), within the temperate-humid climate zone. Because the establishment of eucalyptus clone plantations is still a recent practice in this region, at the present time this type of eucalyptus forests are usually young, so that in our experimental design the age factor was not considered and the following two pre-established selection criteria were used:

i) clone type (rooted cuttings):
- F0, 1st generation clonal plants (morphologically selected): "Anselmo C-14", or
- F1, 2nd generation clonal plants (genetically obtained): "Odiel"

ii) the type of underlying rock on which the soil was developed:
- granitic material, or
- schistic material

Therefore for the present study, 6 clonal plantations were selected over granitic bedrock, half of them (3 stands) planted with the Anselmo clone and the other half (3 stands) using Odiel clonal plants, in order to compare litter biodegradability for both types of clones under similar growing conditions. To highlight the possible effects associated with a particular parent material, other 3 plantations with Anselmo clonal plants developed over schistic bedrock were also selected and compared with the corresponding plantations developed over granitic parent material. Therefore, in this experimental design, a total of 9 plots have been chosen, in order to obtain litter samples from 3 forest patches for each soil parent material and clone type. A stand trial of approximately 900 m^2 (30 m x 30 m) was established in each selected forest plantation.

2.2 Field sampling

A representative sample of the litter layer, composed by merging 24 subsamples regularly taken from the whole stand area following a squared pattern (4 rows and 6 columns), was collected from each plot. The litter layer, formed by organic input coming from the vegetal cover was sampled during the winter season, being composed by a mixture of debris at different decomposing stages that were accumulated over time on the soil surface. Simultaneously and following the same pattern, a combined soil sample was also obtained from the upper layer of the A horizon (0-15 cm depth).

2.3 Vegetal debris and soil analysis

After the combined soil samples were mixed, the soil pH and the total soil C and N contents were assessed by the methods described by Fernandez et al. (2012). The same methodology was used to determine total C and N contents of litter samples. All results obtained were expressed as means from at least three replicate determinations on oven dry basis (105ºC).

2.3.1 Litter mineralization under controlled conditions

Long-term aerobic incubations of eucalyptus debris, finely crushed (particle size ≈ 400 µm, Kinematica laboratory grinding mill using sieve with hole Ø 2 mm mesh size), were carried out in laboratory incubators (with natural convection) under conditions for optimal microbial activity (from each plot, ten replicates of 2 g were placed into 4 litre hermetic glass containers that were maintained at 28ºC and 80% moisture content for 560 days). The flask atmospheres were periodically renewed (every day, every 2 days, every week, etc depending on the flask CO_2 concentration) and the C mineralization during the biodegradative processes was monitored by periodically taking a gas sample from each container and by measuring its CO_2 concentration using a multiple infrared gas analyser (7000FM GFC IR ANALYSER, Signal Group

Limited). Potential C mineralization was expressed as grams of CO_2-C evolved per kilogram of dry material (g $C_{mineralized}$ $kg^{-1}_{d.m.}$) and as a percentage of the total litter C (C mineralization coefficient: $C_{mineralized}/C_{total}*100$).

2.3.2 Kinetic modelling

To quantify C mineralization kinetic parameters, the cumulative data on the CO_2 released at different degradation stages along the incubation period were fitted, as described by Cabaneiro et al. 2008, to the double exponential equation proposed by Andrén and Paustian (1987):

$$C_t = C_o\,(1-e^{-kt}) + (C-C_o)(1-e^{-ht}) \qquad (1)$$

where C_t is the cumulative C release after time t (g C $kg^{-1}_{d.m.}$), C_o is the labile C pool (g C $kg^{-1}_{d.m.}$), k is the instantaneous mineralization rate of the labile C pool (d^{-1}), and h is the instantaneous mineralization rate of the recalcitrant C pool (d^{-1}). To avoid parameter estimation errors, Updegraff et al. (1995) convergence criteria were applied.

For all debris samples collected from the different eucalyptus clone plantations, the time required for 50% C loss (half-life) and the turnover rate of both labile (L) and recalcitrant (R) organic fractions of the litter was calculated as *Half-life L*= $0.693/k$ or *Half-life R* = $0.693/h$ and as *Turnover L* = $1/k$ or *Turnover R* = $1/h$, respectively.

2.3.3 Isotopic analysis (^{13}C)

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 $^{13}C/^{12}C$ ratio was determined. Litter samples were oven-dried (40ºC), finely ground (<100 µm) 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 $\delta^{13}C$ of the laboratory standard (acetanilide, n=10) was lower than ± 0.102‰.

The $^{13}C/^{12}C$ ratio or $\delta^{13}C$ signature was obtained according to the following equation, which is based on the deviation of the $^{13}C/^{12}C$ ratio from the reference standard Vienna Pee Dee Belemnite standard:

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1) \times 10^3 \quad (2)$$

where $R = {}^{13}C/{}^{12}C$.

2.3.4 Statistical analyses

Data were statistically analysed by one-way ANOVA and least significant difference (LSD) test at the 95% probability level ($P \leq 0.05$) was applied to the results. Pearson correlation coefficients were calculated to examine relationships between the different variables. All statistical analyses were performed using the computer software package IBM® SPSS® Statistics, version 21.0.0.0 (2012).

3 Results and discussion

All eucalyptus clone plantations selected to carry out this study are developed over acidic soils (pH_{H_2O} ranged from 4.1 to 5.1) with a strong potential acidity (pH_{KCl} ranged from 3.2 to 4.1). The soil OM of these forest plantations exhibits values of the C-to-N ratio between 11 and 22, with soil C and N contents highly scattered (Table 1). As compared with soils, the litter from the two studied clones of *E. globulu*s shows markedly higher values of the total C concentration (with mean values at least five times the total soil C concentration). However, total N concentration in litter samples is closer to total soil N values, resulting in higher eucalyptus debris C-to-N ratios, which present notable differences among plantations, especially in granitic plots (with C-to-N ratios ranging from 45 to 82).

3.1 Biodegradability of eucalyptus debris

Respirometric techniques and incubation procedures for measuring the biological C mineralization are common approaches to assess the potential turnover rate of OM for soils and litter samples (Cabaneiro et al., 2008; Fernandez et al., 2010). Monitoring biodegradation of litter from the two types of eucalyptus clonal plantations studied using long-term aerobic incubations (18 months) under laboratory controlled conditions, allowed not only the continuous determination of the CO_2 released during decomposition but also the tracking of the isotopic ^{13}C dynamics, the quantification of weight losses rates and the progressive changes in the chemical quality of the remaining substrate at different biodegradation stages or incubation times (T_0=0, T_1=36, T_2=98, T_3=359, T_4=560 days of incubation).

When we compare the biodegradability of litter from the two eucalyptus clonal varieties developed over the same bedrock type which in this section trial consisted of granitic parent material, visible weight losses were observed for both clones, with mean decreases of over 20% of the dry material at the end of the incubation (Fig. 1A), these decreases being more pronounced in debris coming from plantations with Odiel clonal plants. As usually reported for plant debris decomposition (Berg et al., 2000), the declines found in our experiment were more pronounced during the first months of incubation, with significant initial differences depending on the type of clone studied (ANOVA, $P<0.014$ and $P<0.021$ at 36 and 98 days respectively) that are not longer statistically significant from the first year of incubation. These weight losses, observed during the decomposition of samples collected from the litter layer (which was composed by a mixture of fallen eucalyptus debris with uneven time periods on the atop soil), are lower than those reported bibliographically by other authors during fresh plant material degradation (Lusk et al., 2001, Garcia-Velazquez et al., 2010, Jacob et al., 2010). Besides the previously mentioned weight losses registered during litter degradation, a change in the chemical composition of the substrate was also observed, as reflected by their C-to-N ratio evolution (Fig. 1B). Thus, Odiel clone debris (that had the highest weight loss rate) presented the lowest C-to-N ratios, with values of this parameter that start to differ from the values exhibited by Anselmo litter samples from the first year of incubation (ANOVA, $P<0.034$ and $P<0.043$ for 359 and 560 days of incubation, respectively). The fact that litter samples exhibiting the lowest C-to-N ratios also showed the highest weight losses during the biodegradation seems to agree with the results found by some authors, who associate litter decay to labile C and N availabilities during the initial decomposition phases (Berg et al., 2007; Berg and McClaugherty, 2008), when labile compounds such as carbohydrates, proteins, and other simple compounds are rapidly degraded by fast growing microorganisms requiring high N concentrations (Fioretto et al., 2005).

To elucidate the effects that the type of parent material under the eucalyptus plantations can produce on the processes involved on litter biodegradation, the results obtained from the 6 Anselmo clone plantations, 3 of them developed over granitic and 3 over schistic bedrock, were compared. These results seem to indicate that litter decay is slightly more active in eucalyptus stands developed over schistic bedrocks than in plantations developed over granitic parent materials, this being reflected by a more pronounced weight loss (Fig. 1C) and a lower C-to-N ratio (Fig. 1D) in schistic plantations. However, these differences associated to the underlying parent material were not statistically significant for none of these two parameters.

3.1.1 Carbon mineralization dynamics

As shown in Table 2, values of the C mineralization coefficient (expressed as % of the total litter C content), obtained at the end of the incubation period for litter from both eucalyptus clones ranged from 20% to barely surpassing 30% in the most active cases. These results are within the range of mineralization coefficients reported by other authors for debris from different tree species (Fernandez et al., 2003). In our study the mean value of the C mineralization activity (expressed as the total quantity of CO_2 evolved per unit weight) positively correlates with both the initial C content of the substrate ($P \leq 0.033$) and the C mineralization coefficient ($P \leq 0.000$). The differences on C mineralization activity between litter samples coming from both eucalyptus clonal plants are clearly illustrated when comparing the C mineralization coefficients presented by debris collected from the plantations developed on granitic rocks exclusively, the values presented by Odiel being significantly higher than those showed by Anselmo plantations (ANOVA, $P \leq 0.040$). A similar behaviour was found when comparing the total quantity of C released as CO_2 during the whole incubation period, that practically in all eucalyptus plantations surpassed 100 g C $kg^{-1}_{d.m.}$, debris from Odiel plantations having again significantly higher activity values (150.3±14,7 g C $kg^{-1}_{d.m.}$) as compared with the other clone (113.4±18.3 g C $kg^{-1}_{d.m.}$) over the same bedrock type (ANOVA, $P \leq 0.042$).

3.1.2 Kinetic modelling and half-life study

Despite the previously mentioned differences on the CO_2 released during the biodegradation of eucalyptus debris collected from Odiel and Anselmo clone plantations, in both cases experimental cumulative data of the CO_2 evolved during the incubation (Fig. 2) significantly fitted to the Eq. (1), first-order double exponential kinetic model proposed by Andrén and Paustian (1987), which supports the hypothesis of two organic pools of different microbial stabilities and mineralization rates, allowing the estimation of the labile and recalcitrant C pools in each substrate (Table 2).

By comparing the CO_2 evolved along the incubation of litter samples from the two eucalyptus clonal varieties over the same parent material “granitic bedrock” (Fig. 2A), from the first month of monitoring substrate biodegradation significantly higher CO_2 releases were observed for debris coming from Odiel as compared with Anselmo plantations ($P \leq 0.029$), the significance of these differences lasting until the end of the experimental period (T1: $P \leq 0.017$, T2: $P \leq 0.021$, T3: $P \leq 0.29$, T4: $P \leq 0.042$). However when comparing the values obtained for all Anselmo clone plantations (Fig. 2B), to elucidate the effects of the underlying parent material (granite/schist), the results indicate that the differences are never statistically significant, although CO_2 released by litter from schistic plots are slightly higher than from granitic ones along the whole incubation period. Determination coefficients (R^2) obtained by using the previously mentioned kinetic model were always higher than 0.99 (Table 2) and the estimated values of labile C pool for the different eucalyptus debris ranged from 38.3 g C $kg^{-1}_{d.m.}$ (for a plot with Anselmo clonal plants over granitic bedrock) to 123.3 g C $kg^{-1}_{d.m.}$ (for a plot with Odiel clonal plants over granitic bedrock), representing from 7.6 to 23.9 % of their total C content, respectively. Thus, as compared with debris from Anselmo clone plantations, significantly bigger sizes of the labile C pools were estimated for Odiel litter samples (ANOVA, $P \leq 0.040$). Besides, the size of the labile C pool of litter samples collected from the 9 studied eucalyptus plantations was found to be positively correlated with both C mineralization indices ($P \leq 0.028$ and $P \leq 0.023$ with the total C mineralized and the C mineralization coefficient, respectively).

As it can be seen in Table 2, the instantaneous mineralization rate of the labile fraction (*k*) is between 50 and 100 times higher than that of the most recalcitrant pool (*h*), without any statistically significant difference between Amselmo and Odiel debris or between granitic and schistic plots. Both *k* and *h* instantaneous mineralization rates show a very strong positive intercorrelation ($P \leq 0.000$) and their values point to very different turnover rates for the labile or the recalcitrant fractions (Table 3). Thus, half-life values obtained for the labile fraction of eucalyptus litter ranges from 26 to 77 days, whereas half-life times for the recalcitrant pool vary from approximately 5 to 16 years, which corresponds to turnover rates of up to 111 days for the labile compounds and up to more than 23 years for the more recalcitrant ones, these latter rates being sometimes nearly 100 times higher than the turnover rates of the labile fraction (see Table 3, O3: Odiel clone over granitic bedrock).

3.2 Isotopic ^{13}C evolution during eucalyptus litter decay

The ^{13}C natural abundance values of eucalyptus debris, obtained according Eq. (2), exhibited by both type of clonal plantations (Table 1) are within the range reported for C3 vegetation (Fernandez et al., 2003, 2005, Cabaneiro et al., 2009). The ^{13}C isotopic composition was found to be slightly different for the two eucalyptus clones, debris from Odiel clonal plantations being significantly more ^{13}C enriched than debris from Anselmo clones (ANOVA, $P \leq 0.046$) with variations that ranged from -30.3‰ (Anselmo plot) to -29.5‰ (Odiel plot).

Isotopic differences between Anselmo and Odiel clones on the temporal evolution during debris biodegradation were also revealed when the behaviour of the $^{13}C/^{12}C$ ratios of the litter samples collected from both types of eucalyptus plantations over granitic bedrocks (Fig. 3A) were compared along the incubation period, which suggests different ^{13}C fractionation dynamics that maybe associated to a dissimilar distribution of some biochemical compounds in both substrates. Thus, during the biodegradation of Odiel debris, visible ^{13}C depletion was observed during early decomposition stages, suggesting a selective mineralization of relatively enriched ^{13}C compounds, such as proteins, lipids, sugars, starch and other carbohydrates or biomolecules included in the non-ADF fraction described by Fernandez et al. (2003) for decomposing plant materials. On the other hand, organic debris collected from Anselmo clone plantations showed slight and brief initial increases of their ^{13}C contents, probably due in this case to the preferential mineralization of some easily degradable biomolecules relatively depleted in this carbon isotope, (e.g. amino acids can be significantly ^{13}C depleted according to Borland et al., 1994). As a consequence of these contrasting tendencies, a quick reduction of the initial isotopic differences between litter samples from Odiel and Anselmo plantations was observed, these differences becoming statistically insignificant since the first month of incubation. Once this early stage of high mineralization activity has elapsed, another phase of litter biodegradation with minor ^{13}C variations begins and the isotopic composition of the substrate remains practically unaltered until the end of the decomposition experiment. Once more, it can be highlighted that the underlying bedrock did not seem to have a remarkable influence on the isotopic dynamics during litter decay (Fig. 3B), since not significant differences were found between debris collected from Anselmo clone plantations growing either on granitic or on schistic stands, although the litter from the latter is slightly depleted in this carbon isotope as compared to the litter coming from Anselmo plantations over granitic bedrocks.

3.3 Factors influencing eucalyptus litter biodegradation

Taken as a whole, after a general scrutiny of all these results, the integrated outcomes of the research including all the different parameters studied during eucalyptus litter decomposition support the hypothesis of the existence of some biochemical heterogeneity between the litter collected from the duff layer of the two studied types of *Eucalyptus globulus* clonal plantations (with F0 or F1 clonal plants) and allows to deduce that, for these kind of forests from northwestern Spain, the biodegradability of the aboveground litter seems to be strongly influenced by the following two key factors:

i) The litter decaying stage. The results of eucalyptus litter decomposition illustrates that two differentiated decaying phases during the biodegradative process can be clearly described: a relatively brief more active initial phase (first weeks/months), when the greatest weight losses, CO_2 releases, or isotopic ^{13}C shifts occurs, and a later or delayed second less active phase, when all these variables show a posterior progressive stabilization and remain practically constant until the end of the incubation. Two or more decomposing phases during litter decay have already been reported by many authors for different tree species (Melillo et al., 1989; Aber et al., 1990; Guillon et al., 1993; Coûteaux et al., 1995; Rovira and Rovira, 2010; Castellanos-Barliza and León, 2011; Patricio et al., 2012).

ii) The clonal origin or intrinsic characteristics of the litter. Some dissimilarities in the promptness or slowness of the degradative process can be also distinguished between both types of aboveground residues collected either from the F0 (1st generation) or from the F1 (2nd generation) eucalyptus clonal plantations.

More to the point, the influence of these above mentioned two factors appear to be firstly related with the initial chemical composition or quality of the litter (N content, C-to-N ratio, and ^{13}C signature) mainly determined by its genetic origin that seems to have a certain influence on its biodegradability and on its C mineralization kinetics. However, although to a lesser extent, it seems to be also moderately affected by the underlying bedrock type.

4 Conclusions

The genetic diversity between the two studied clonal plants (Anselmo: F0, first generation clonal variety attained by morphological selection and Odiel: F1, second generation clonal variety genetically obtained) are reflected not only into a different production of vegetal biomass but also into a higher lability of forestal debris coming from Odiel plantations, with lower C-to-N ratios and higher C mineralization coefficients, as compared to litter tissues collected from Anselmo plots. 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. The different isotopic ^{13}C behaviour at early stages of litter decay found for both eucalyptus clonal varieties exhibits evidences of a different proportion of some labile compounds associated to the genotypic characteristics of each type of clone plantation. Thus, microbial fractionation of ^{13}C during detritus decomposition can not be neglected when attempting to evaluate the isotopic aspects of the C cycle comprehension and the quantification of ^{13}C discrimination have to be taken into consideration in order to obtain more reliable estimates of the contribution of decaying vegetal debris to soil OM buildup in each specific ecological context. This has direct implications in studies of soil OM dynamics using isotopic (^{13}C) techniques, particularly to avoid errors in appraising the contribution of eucalyptus litter decay to the global C balance.

Acknowledgements

This research was conducted as a part of the project AGL2010-22308-C02-02 financed by the Spanish Government (Ministerio de Ciencia e Innovación). We thank the departments of "Ingeniería Agroforestal" and "Producción Vegetal" of the University of Santiago de Compostela for their invaluable assistance in plot selection. We also thank the Scientific Research Support Services of the University of A Coruña for the isotopic ^{13}C analyses. ENCE is gratefully acknowledged for allowing us to use their clone plantations. Finally, we thank Ana Argibay, Daniel Caride and María García for their technical assistance in the laboratory and fieldwork, as well as SILVANUS, GIT. Forestry Consulting SL, NORFOR and Dirección Xeral de Montes (Consellería do Medio Rural, Xunta de Galicia) for showing interest in this research.

References

Aber, J., Melillo, J., and McClaugherty, C.: Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can. J. Bot., 68, 2201-2208,1990.

Alvarez, E., Monterroso, C., and Fernández Marcos, M. L.: Aluminium fractionation in Galician (NW Spain) forest soils as related to vegetation and parent material. Forest Ecol..Manag., 166, 193-206, 2002.

Alvarez Gonzalez, J. G., Balboa Murias, M. A., Merino, A., and Rodríguez Soalleiro, R.: Estimación de la biomasa arbórea de Eucalyptus globulus y Pinus pinaster en Galicia. Recursos Rurais, 1, 21-30, 2005.

Andrén, O. and Paustian, K.: Barley straw decomposition in the field a comparison of models. Ecology, 68, 1190-1200, 1987.

Berg, B. and McClaugherty, C.: Plant litter. Decomposition, Humus Formation, Carbon Sequestration, 2nd ed. Ed. Springer Verlag, Heidelberg, Berlin, 2008.

Berg, B., Meentemeyer, V., and Johansson, M. B.: Litter decomposition in a climatic transect of Norway spruce forests-climate and lignin control o f mass-loss rates. Can. J. Forest Res. 30, 1136-1147, 2000.

Berg, B., Steffen, K. T., and McClaugherty, C. Litter decomposition rate is dependent on litter Mn concentrations. Biogeochemistry, 82, 29-39, 2007.

Borland, A. M., Griffiths, H, Broadmeadow, M. S. J., Fordham, M. C., and Maxwell C.: Carbon-isotope composition of biochemical fractions and the regulation of carbon balance in leaves of the C_3-crassulacean acid metabolism intermediate *Clusia minor* L. Growing in Trinidad., Plant Physiol., 106, 493-501, 1994.

Boutton, T. W., Archer, S. R., Midwood, A. J., Zitzer, S. F., and Bol, R.: $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. Geoderma, 82, 5-41, 1998.

Brañas, J., González-Río, F., and Merino, A.: Contenido y distribución de nutrientes en plantaciones de Eucalyptus globulus del noroeste de la península ibérica. Forest systems, 9(2), 317-335, 2000.

Cabaneiro, A., Fernandez, I., Pérez-Ventura, L., and Carballas, T.: Soil CO_2 Emissions from Northern Andean Páramo Ecosystems: Effects of Fallow Agriculture. Environ. Sci. Technol., 42, 1408-1415, 2008.

Cabaneiro A. and Fernandez I. Testemuño Isotópico (^{13}C) do Cambio Global en Galicia, in: Evidencias e Impactos do Cambio Climático en Galicia, edited by Pérez Muñuzuri, V., Fernandez Cañamero, M., and Gomez Gesteira, J. L., Consellería de Medio Ambiente e Desenvolvemento Sostible, Xunta de Galicia, Santiago de Compostela, 229-245, 2009.

Calvo de Anta, R.: El Eucalipto en Galicia. Sus relaciones con el medio natural. Ed. Universidad de Santiago de Compostela, 1992.

Camps Arbestain, M., Mourenza, C., Alvarez, E., and Macías, F.: Influence of parent material and soil type on the root chemistry of forest species grown on acid soils. Forest Ecol. Manag., 193, 307-320, 2004.

Castellanos-Barliza, J. and León, J. D.: Descomposición de hojarasca y liberación de nutrientes en plantaciones de Accacia mangium (Mimosaceae) establecidas en suelos degradados de Colombia. Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744), 59, 113-128, 2011.

Coûteaux, M. M., Bottner, P., and Berg, B.: Litter decomposition, climate and litter quality. Tree, 10, 63-66, 1995.

Dewar, R.C. and Cannell, M. G. R.: Carbon sequestration in the trees, products and soils of forest plantations: an analysis using UK examples. Tree Physiol. II, 49-71, 1992.

Ehleringer, J. R., Buchmann, N., and Flanagan, L. B.: Carbon isotope ratios in belowground carbon cycle processes. Ecol. Appl., 10, 412-422, 2000.

Fernández, I., Cabaneiro, A., and Carballas, T.: Thermal resistance to high temperatures of the different soil organic fractions from soils under pine forests. Geoderma, 104, 281-298, 2001.

Fernandez, I. and Cadisch, G.: Discrimination against ^{13}C during degradation of simple and complex substrates by two white rot fungi. Rapid Commun. Mass Spectrom., 17, 2614-2620, 2003.

Fernandez, I., Mahieu, N., and Cadisch, G.: Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochem. Cy., 17, 1075-1085, 2003.

Fernandez, I., Cabaneiro, A., and González-Prieto, S. J.: Use of ^{13}C to monitor soil organic matter transformations caused by a simulated forest fire. Rapid Commun. Mass Spectrom., 18, 435-442, 2004.

Fernandez, I., González-Prieto, S.J., and Cabaneiro, A.: ^{13}C-isotopic fingerprint of *Pinus pinaster* Ait. and *Pinus sylvestris* L. wood related to the quality of standing tree mass in forests from NW Spain. Rapid Commun. Mass Spectrom., 19, 3199-3206, 2005.

Fernández, I., Cabaneiro, A., and González-Prieto, S. J.: Partitioning CO_2 Effluxes from an Atlantic Pine Forest Soil between Endogenous Soil Organic Matter and Recently Incorporated ^{13}C-Enriched Plant Material. Environ. Sci. Technol., 40, 2552-2558, 2006a.

Fernandez, I., Pérez-Ventura, L., González-Prieto, S. J., and Cabaneiro, A.: Soil $\delta^{13}C$ and $\delta^{15}N$ as a good indicator for predicting the site index of Galician pine forests (*P. pinaster* Ait. and *P. sylvestris* L.). Forest systems, 15(1), 3-13, 2006b.

Fernández, I., Carrasco, B., and Cabaneiro, A.: Comparing the potential carbon mineralization activity of the soil organic matter under tow braoadleaf autochthonous tree species from the NW of Spain (*Quercus robur* L., *Betula alba* L.). Forestry Ideas, 16, 258-265, 2010.

Fernández, I., Carrasco, B., and Cabaneiro, A.: Evolution of soil organic matter composition and edaphic carbon effluxes following oak forest clearing for pasture: climate change implications. Eur. J. Forest Res., 131, 1681-1693, 2012.

Fioretto, A., Di Nardo, C., Papa, S., and Fuggi, A. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biol. Biochem., 37, 1083-1091, 2005.

García-Velásquez, L. M., Ríos-Quintana, A., and Molina-Rico, L.: Structure, plant composition and leaf litter decomposition in soil, at two sites of an andean cloud forest (reforested and in spontaneous succession) in Peñas Blancas, Calarcá (Quindío), Colombia. Actual Biol., 32(93, 147-164, 2010

Gillon, D., Joffre, R., and Ibrahima, A.: Inital litter properties and decay rate: a microcosm experiment on Mediterranean species. Can. J. Bot., 72, 946-954, 1993..

González-Prieto, S. J., Cabaneiro, A., Villar, M. C., Carballas, M., and Carballas T.: Effect of soil characteristics on N nimeralization capacity in 112 native and agricultural soils from the northwest of Spain. Biol. Fertil. Soils, 22, 252-260, 1996.

Griffiths, H., Borland, A., Gillon, J., Harwood, K., Maxwell, K., and Wilson, J.: Stable isotopes reveal exchanges between soil, plants and the atmosphere, in: Physiological Plant Ecology, edited by: Press, M. C., Scholes, J, D., and Barker, M. G., Blackwell Science, Oxford, 415- 441, 1999.

Jacob, M., Viedenz, K., Polle, A., and Thomas F. M.: Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (Fagus sylvatica). Oecologia, 164, 1083-1094, 2010.

Jones, H.E., Madeira, M., Herraez, L., Dighton, J., Fabiâo, A., González-Rio, F., Fernandez Marcos, M., Gomez, C., Tomé, M., Feith, H., Magalhâes, M.C., and Howson, G.: The effect of organic-matter management on the productivity of Eucalyptus globulus stands in Spain and Portugal: tree growth and harvest residue decomposition in relation to site and treatment. Forest Ecol. Manag., 122, 73-96, 1999.

Lusk, C. H., Donoso, C., Jiménez, M., Moya, C., Oyarce, G., Reinoso, R., Saldaña, A., Villegas, P., and Matus, F.: Descomposición de hojarasca de Pinus radiata y tres especies arbóreas nativas. Revista Chilena de Historia Natural, 74, 705-710, 2001.

Matus, F. J., Lusk, C. H., and Maire, C. R.: Effects of soil texture, carbon input rates, and litter quality on free organic matter and nitrogen mineralization in Chilean rain forest and agricultural soils. Commun. Soil Sci. Plant Anal , 39, 187-201, 2008.

Melillo, J. M., Aber, J. D., Linkins, A. E., Ricca, A., Fry, B., and Nadelhoffer, K. J.: Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant Soil, 115, 180-198, 1989.

Mutabaruka, R., Mutabaruka, C., and Fernandez I.: Diversity of arbuscular micorrhizal fungi associated to tree species in semiarid areas of Machakos, Kenya. Arid Land Res. Manag., 16, 385-390, 2002.

Patricio, M. S., Nunes, L. F., and Pereira, E. L.: Litterfall and litter decomposition in chestnut high forest stands in northern Portugal. Forest Systems, 21, 259-271, 2012.

Rovira, P. and Rovira, R.: Fitting litter decomposition datasets to mathematical curves: Towards a generalised exponential approach. Geoderma, 155, 329-343, 2010.

Schleser, G. H., Frielingsdorf, J., and Blair, A.: Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol., 158, 121-130, 1999.

Updegraff K., Pastor J., Bridgham S.D., and Johnston. C. A. 1995. Environmental and substrate quality controls over carbon and nitrogen mineralization in northern wetlands. Ecol. Appl., 5, 151-163, 1995.

Van Dam, D., Veldkamp, E., and Van Bremen, N.: Soil organic carbon dynamics: variability with depth in forested and deforested soils under pasture in Costa Rica. Biogeochemistry, 39, 343-375, 1997.

Vega-Nieva, D. J., Tomé, M., Tomé J., Fontes, L., Soares, P., Ortiz, L., Basurco, F., and Rodríguez-Soalleiro, R.: Developing a general method for the estimation of the fertility rating parameter of the 3-PG model: application in *Eucalyptus globulus* plantations in northwestern Spain. Can. J. Forest Res., 43, 627-636, 2013.

Table 1. Main chemical characteristics and isotopic ^{13}C composition (mean ± standard deviation*)* of both the upper 0-15 cm of soils and litter collected from eucalyptus clone (Anselmo and Odiel) plantations.

Site	Plot	Soil pH H_2O	Soil pH KCl	Soil C $gC\ kg^{-1}_{d.s.}$	Soil N $gN\ kg^{-1}_{d.s.}$	Soil C-to-N	Litter C $gC\ kg^{-1}_{d.m.}$	Litter N $gN\ kg^{-1}_{d.m.}$	Litter C-to-N	Litter $\delta^{13}C$ ‰
ANSELMO	Granitic A1	4.5±0.0	3.8±0.0	83.1± 0.6	5.5±0.0	15	463.6±22.5	10.3±0.3	45	-30.3±0.0
	Granitic A2	4.1±0.0	3.2±0.0	137.3± 2.0	7.3±0.0	19	516.1±24.3	6.3±0.2	82	-29.9±0.0
	Granitic A3	4.7±0.0	3.8±0.0	76.3± 2.5	5.8±0.3	13	500.7±19.8	6.4±0.1	79	-29.8±0.0
	Mean value	**4.4**±0.3	**3.6**±0.4	**98.9**±33.4	**6.2**±0.9	**16**±3	**493.5±27.0**	**7.6**±2.3	**65**±21	**-30.0**±0.3
	Schistic A1	5.1±0.0	4.1±0.0	20.2± 0.4	0.9±0.1	22	523.3±18.6	9.7±0.4	54	-30.1±0.0
	Schistic A2	4.8±0.0	4.0±0.0	57.9± 0.9	3.6±0.1	16	498.0±10.6	9.0±0.0	55	-30.1±0.0
	Schistic A3	4.6±0.0	3.7±0.0	60.1± 2.7	3.7±0.3	16	490.8±18.1	8.1±0.1	61	-30.2±0.0
	Mean value	**4.8**±0.3	**3.9**±0.2	**46.1**±22.4	**2.8**±1.6	**18**±4	**504.0±17.1**	**8.9**±0.8	**56**±4	**-30.1**±0.1
ODIEL	Granitic O1	4.7±0.0	3.9±0.0	47.6± 0.4	3.3±0.0	15	511.2±10.4	9.6±0.0	53	-29.6±0.0
	Granitic O2	5.0±0.0	4.1±0.0	47.6± 0.9	3.5±0.1	11	515.3±19.3	10.4±0.0	50	-29.8±0.0
	Granitic O3	4.6±0.0	3.7±0.0	109.6± 1.2	6.5±0.0	17	517.2±12.8	6.9±0.4	75	-29.5±0.0
	Mean value	**4.8**±0.2	**3.9**±0.2	**68.3**±35.8	**4.4**±1.8	**14**±3	**514.6**± 3.1	**9.0**±1.8	**59**±14	**-29.6**±0.2

Table 2. Total C mineralized, C mineralization coefficients and kinetic parameters obtained after 560 days of incubation of litter collected from different eucalyptus clone (Anselmo and Odiel) plantations by using a first-order kinetic model based on the double exponential equation proposed by Andrén and Paustian (1987): $C_{mineralized} = C_o\,(1-e^{-kt}) + C_r(1-e^{-ht})$.

	Potential C mineralization mean±standard deviation		**Kinetic parameters** Estimated value±standard asymptotic error				
	C mineralized ($g\ kg^{-1}_{d.m.}$)	**C mineralization coefficient** (%)	C_o ($g\ C\ kg^{-1}_{d.m.}$)	***k*** (d^{-1})	$\mathbf{C_r}$ ($g\ C\ kg^{-1}_{d.m.}$)	$\boldsymbol{h}\mathbf{x10^4}$ (d^{-1})	$\mathbf{R^2}$
ANSELMO CLONE							
Granitic A1	106.3 ±8.4	22.9±1.8	71.8±5.1	0.012±0.001	391.8±27.6	1.8±0.3	0.995
Granitic A2	134.2±19.7	26.0±3.8	107.3±5.3	0.010±0.001	408.8±29.6	1.2±0.3	0.998
Granitic A3	99.6 ±3.3	19.9±0.7	38.3±2.9	0.018±0.002	462.4±22.7	2.7±0.2	0.994
Mean value	**113.4**	**22.9**	**72.5**	**0.013**	**421.0**	**1.9**	
SD	18.3	3.1	34.5	0.004	36.9	0.7	
Schistic A1	160.9±6.3	30.7±1.2	77.1±3.5	0.027±0.002	446.2±22.1	4.1±0.2	0.995
Schistic A2	123.9±6.7	24.9±1.3	91.0±7.0	0.009±0.001	407.0±17.6	1.6±0.4	0.997
Schistic A3	133.2±0.7	27.2±0.1	87.4±2.4	0.014±0.001	403.4±20.5	2.2±0.1	0.999
Mean value	**139.3**	**27.6**	**85.2**	**0.017**	**418.9**	**2.6**	
SD	19.2	3.0	7.2	0.009	23.7	1.3	
ODIEL CLONE							
Granitic O1	145.9±7.8	28.6±1.5	102.8±3.7	0.015±0.001	408.4±14.1	2.2±0.2	0.998
Granitic O2	166.8±2.7	32.4±0.5	123.3±3.3	0.015±0.001	392.0±22.6	2.2±0.2	0.999
Granitic O3	138.3±8.9	26.7±1.7	107.8±1.8	0.014±0.000	409.4±14.6	1.4±0.1	0.999
Mean value	**150.3**	**29.2**	**111.3**	**0.015**	**403.3**	**1.9**	
SD	14.7	2.9	10.7	0.001	9.8	0.5	

C_o, carbon of the labile pool

k, instantaneous mineralization rate of the labile carbon pool

C_r, carbon of the recalcitrant pool

h, instantaneous mineralization rate of the recalcitrant carbon pool

R^2, determination coefficient

Table 3. Values of the time required for 50% mass loss (half-life) and turnover rates of both labile (L) and recalcitrant (R) organic fractions of litter collected from different eucalyptus clone (Anselmo and Odiel) plantations estimated by applying a first-order kinetic model based on the double exponential equation* proposed by Andrén and Paustian (1987) to the cumulative quantity of C mineralized during 560 days of substrate incubation under laboratory controlled conditions and calculated as *Half-life L*= 0.693/*k* or *Turnover L* = 1/*k*, for the labile pool and as *Half-life R* = 0.693/*h* or *Turnover R* = 1/*h* for the recalcitrant reservoir, respectively.

	Labile fraction		Recalcitrant fraction	
	Half-life L (months)	***Turnover L*** (months)	***Half-life R*** (months)	***Turnover R*** (months)
ANSELMO CLONE				
Granitic A1	1.9	2.8	128.3	185.2
Granitic A2	2.3	3.3	192.5	277.8
Granitic A3	1.3	1.9	85.6	123.5
Mean value	**1.8**	**2.7**	**135.5**	**195.5**
SD	0.5	0.7	57.8	77.7
Schistic A1	0.9	1.2	56.3	81.3
Schistic A2	2.6	3.7	144.4	208.3
Schistic A3	1.7	2.4	105.0	151.5
Mean value	**1.7**	**2.4**	**101.9**	**147.0**
SD	0.9	1.3	44.1	63.6
ODIEL CLONE				
Granitic O1	1.5	2.2	105.0	151.5
Granitic O2	1.5	2.2	105.0	151.5
Granitic O3	1.7	2.4	165.0	238.1
Mean value	**1.6**	**2.3**	**125.0**	**180.4**
SD	0.1	0.1	34.6	50.0

* $C_{mineralized} = C_o (1-e^{-kt}) + C_r(1-e^{-ht})$

C_o, carbon of the labile pool

C_r, carbon of the recalcitrant pool

k, instantaneous mineralization rate of the labile carbon pool

h, instantaneous mineralization rate of the recalcitrant carbon pool

Figure Captions

Figure 1. Evolution of the weight loss proportion and the C-to-N ratio during the biodegradation of eucalyptus litter collected from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic parent material) as a function of the incubation time (vertical bars are ± 1 standard deviation). Weight loss comparison between both clonal varieties (A). Comparison of the C-to-N ratio between both clones (B). Weight loss of litter for both bedrock types (C). Litter C-to-N ratio for both bedrock types (D).

Figure 2. Cumulative curves of the C mineralization during incubation of eucalyptus litter collected from from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic bedrock). Comparison between litter from both clonal varieties (A). Comparison between litter from granitic and schistic plantations (B). Vertical bars are ± 1 standard deviation.

Figure 3. Evolution of the Isotopic ^{13}C composition during incubation of eucalyptus litter collected from from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic bedrock) as a function of the incubation time. Comparison between litter from both clonal varieties (A). Comparison between litter from granitic and schistic plantations (B).