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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)

I. Fernandez and A. Cabaneiro

Instituto de Investigaciones Agrobiológicas de Galicia, Consejo Superior de Investigaciones Científicas IIAG-CSIC, Santiago de Compostela, Spain

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

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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: 1st clonal generation attained by morphological selection and Odiel: 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.

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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 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.

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, b) since isotopic techniques applied to diverse research fields can provide an integrated and quantitative

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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, b).

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:

1. clone type:
   - 1st generation clonal plants (morphologically selected): “Anselmo C-14”, or
   - 2nd generation clonal plants (genetically obtained): “Odiel”

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2. 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 × 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 subsamples 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

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results obtained were expressed as means from at least three replicate determinations on oven dry basis (105 °C).

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 L hermetic glass containers that were maintained at 28 °C and 80 % moisture content for 560 days). The flask atmospheres were periodically renewed (every day, every 2 days or every week, 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: $C_{mineralized}/C_{total} \times 100$).

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):

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

where C_t is the cumulative C release after time t ($g\,kg^{-1}_{d.m.}$), C_o is the labile C pool ($g\,kg^{-1}_{d.m.}$), k is the instantaneous mineralization rate of the labile C pool (d^{-1}), and

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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 % mass 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 analyses (^{13}C)

Litter samples were oven-dried (40 °C) and finely ground (< 100 µm) 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 $^{13}C/^{12}C$ ratio was determined. The ^{13}C natural abundance was assessed using a ThermoFinningan DeltaPlus isotope-ratio mass spectrometer coupled to a Flash EA 1112 elemental analyser. 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 (VPDB, 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).

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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. globulus* 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

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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 in 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).

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

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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 ($150.3 \pm 14{,}7\,g\,C\,kg^{-1}_{d.m.}$) as compared with the other clone ($113.4 \pm 18.3\,g\,C\,kg^{-1}_{d.m.}$) 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

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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 (T_1: $P \leq 0.017$, T_2: $P \leq 0.021$, T_3: $P \leq 0.29$, T_4: $P \leq 0.042$). 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 yr, which corresponds to turnover rates of up to 111 days for the labile compounds and up to more than 23 yr for the more recalcitrant ones, these latter rates being sometimes nearly 100

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times higher than the turnover rates of the labile fraction (e.g., Granitic O3 in Table 3: 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

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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.

4 Conclusions

The genetic diversity between the two studied clonal plants (Anselmo: first generation clonal variety attained by morphological selection and Odiel: 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 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. 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

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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.

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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. Data in bold show mean values of the 3 plots with the same bedrock type and clonal variety.

		Soil					Litter			
	Plot	pH H_2O	pH KCl	C g C $kg^{-1}_{d.s.}$	N g N $kg^{-1}_{d.s.}$	C-to-N	C g C $kg^{-1}_{d.m.}$	N g N $kg^{-1}_{d.m.}$	C-to-N	$\delta^{13}C$ ‰
A	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
N	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
S	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
E	**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
L	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
M	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
O	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
O	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
D	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
I	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
E	**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
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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): $C_{mineralized} = C_o(1 - e^{-kt}) + C_r(1 - e^{-ht})$. Data in bold show mean values of the 3 plots with the same bedrock type and clonal variety.

	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 ($gC\,kg^{-1}_{d.m.}$)	k (d^{-1})	C_r ($gC\,kg^{-1}_{d.m.}$)	$h \times 10^4$ (d^{-1})	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

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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. Data in bold show mean values of the 3 plots with the same bedrock type and clonal variety.

	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

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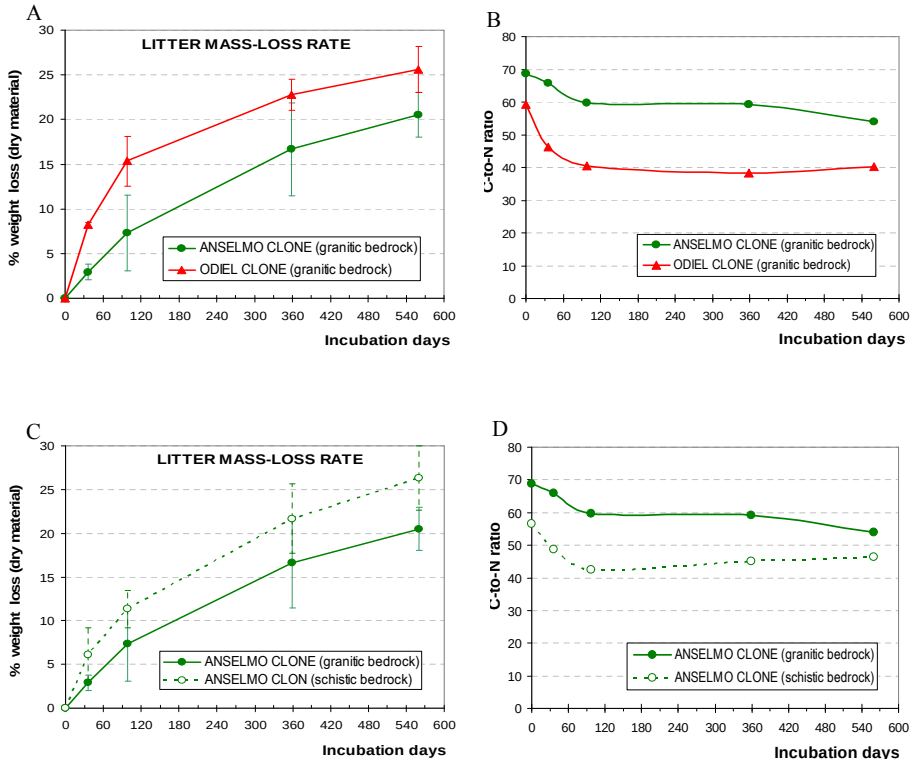

Fig. 1. Evolution of the weight loss proportion and the C-to-N ratio during the biodegradation of eucalyptus litter collected from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic parent material) as a function of the incubation time (vertical bars 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)**.

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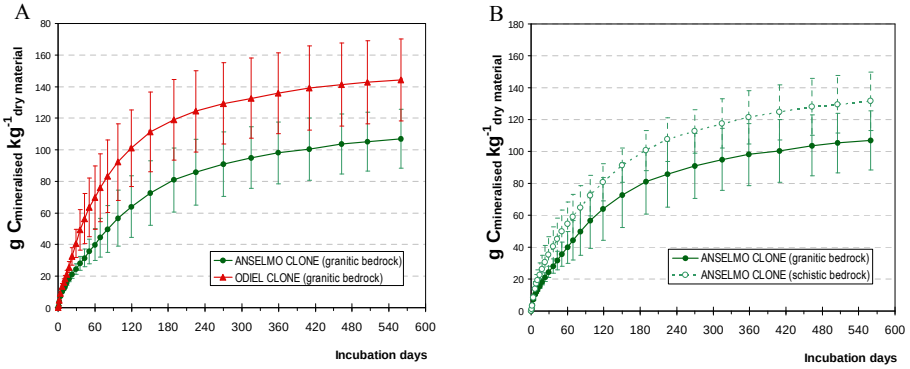

Fig. 2. Cumulative curves of the C mineralization during incubation of eucalyptus litter collected from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic bedrock). Comparison between litter from both clonal varieties **(A)**. Comparison between litter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

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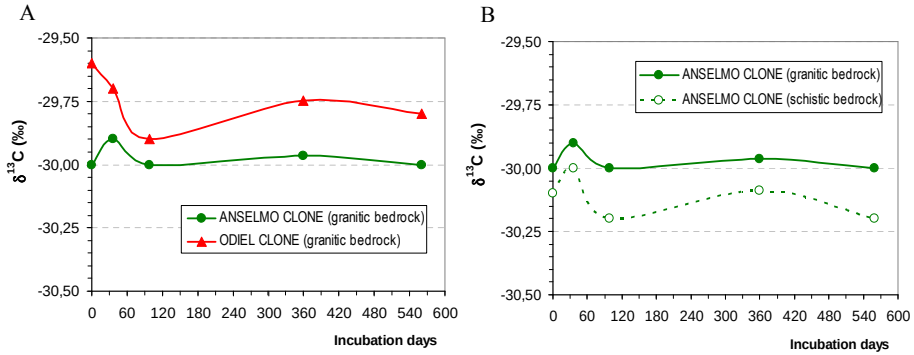

Fig. 3. Evolution of the Isotopic ^{13}C composition during incubation of eucalyptus litter collected from two types of clone plantations (Anselmo or Odiel clonal varieties developed over granitic or schistic bedrock) as a function of the incubation time. Comparison between litter from both clonal varieties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

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